MASARYK UNIVERSITY

FACULTY OF MEDICINE

DEPARTMENT OF BURNS AND PLASTIC SURGERY

UNIVERSITY HOSPITAL BRNO

Habilitation thesis

Multi-modal perspectives on pressure ulcers

(Collection of previously published scholarly works)

MUDr. Alica Hokynková, Ph.D.

Brno 2025

I declare that I have prepared my habilitation thesis independently using the sources listed in the reference list. I would like to acknowledge the assistance of various tools and platforms in the writing and refinement of this habilitation thesis. The AI-based language model ChatGPT was used to assist in structuring, rewording, and improving the clarity of scientific content while ensuring coherence throughout the manuscript. Additionally, DeepL Translator, DeepL Writer and QuillBot were utilized for accurate and high-quality translations between languages to maintain precision in technical terminology and for paraphrasing and grammar refinement, contributing to the overall readability and fluency of the text. Despite the use of these tools, the responsibility for the scientific content, data interpretation, and conclusions drawn in this thesis remains entirely my own.

……

signature of the author

Acknowledgements

My thanks go first to the chief surgeon, Ladislav Březina, M.D., who guided my first surgical steps in medicine. I would like to thank also the heads of the Department of Burns and Plastic Surgery of the University Hospital in Brno, Prof. Pavel Brychta, M.D., CSc. and Assoc. Prof. Libor Streit, M.D., Ph.D., for the opportunity to devote myself to the topic of hard-to heal wounds, especially pressure ulcers.

This habilitation thesis combines clinical and surgical insights with several scientific disciplines, so I would like to thank all the specialists who have collaborated with me. I would like to thank Prof. Andrea Pokorná, Ph.Dr., PhD., Vice-Dean, Head of the Department of Health Sciences and President of the European Pressure Ulcer Advisory Panel, for several years of close collaboration on all joint projects and publications related to prevention, treatment and the use of new technologies in the field of pressure ulcers.

I would like to thank Prof. Petr Babula, Pharm.Dr, Ph.D., Prof. Marie Nováková, M.D., Ph.D. and Tibor Stračina, M.D., Ph.D. from the Department of Physiology and RNDr. Hana Paulová, CSc. from the Department of Biochemistry, Faculty of Medicine, Masaryk University, Brno, for their cooperation in animal experiments and for their scientific work and support dedicated to nutritional and biochemical parameters and unexplored issue of oxidative stress parameters in the field of pressure ulcers. I would also like to thank my collaborators from Brno University of Technology, Assoc. Prof. Ing. Jan Podroužek, Ph.D. and Ing. Rostislav Krč, Ph.D. for their cooperation and new insight into the world of medicine through the eyes of specialists in the realm of artificial intelligence. Furthermore, I would like to thank the illustrator and visual designer Radek Zmítek for the unique and beautiful animations of the pressure ulcers and their localisation published in this habilitation thesis.

Finally, I would like to thank my parents, my partner Petr and my lovely daughter Adélka, for supporting me all the time and giving me a space for professional growth in the area of medical and scientific research.

Content

Foreword 6

1. PRESSURE ULCERS 8

1.1 Definition and incidence 8

1.2 Pathophysiology of pressure ulcers formation 9

1.3 Risk factors 10

1.4 Pressure ulcer assessment risk score scales 15

1.5 Pressure ulcers risk areas 17

1.6 Classification 20

1.7 Treatment of PUs 25

1.7.1. Preventive measures 25

1.7.2. Promoting wound healing, conservative therapy 26

1.7.3. Surgical therapy 27

1.7.3.1 Types of reconstruction 28

1.7.3.2 Selection of flap plasty according to localization 28

1.7.3.3 Post-operative complications 31

1.8 Summary 33

1.9 Articles related to this issue 33

2. NUTRITIONAL PERSPECTIVE 45

2.1 Pressure ulcer surgical management in critically ill patients 45

2.2 Pressure ulcer surgical management in long-term pressure ulcers 46

2.3 Summary 49

2.4 Articles related to this issue 51

3. OXIDATIVE STRESS PERSPECTIVE 68

3.1 Oxidative stress and wound healing 68

3.2 Oxidative stress and pressure ulcer surgical management 70

3.3 Summary 71

3.4 Articles related to this issue 72

4. ARTIFICIAL INTELLIGENCE PERSPECTIVE 99

4.1 Definition of artificial intelligence 99

4.2 Artificial intelligence in healthcare and medicine 100

4.3 Clinical medical databases 108

4.4 Artificial intelligence and pressure ulcers 111

4.5 Summary 112

4.6 Article related to this issue 113
5. FUTURE PERSPECTIVE 127
6. CONCLUSIONs 129
7. List of references 131
8. List of abbreviations 144
9. List of pictures 145
10. List of tables 145
11. List of diagrams 145
12. List of appendices 146

Foreword

This habilitation thesis presents the issue of decubital lesions from a multi-modal perspective. Pressure ulcers are a serious health problem for critically ill patients and, in the long term, especially for patients who are restricted in movement for various reasons. These wounds are very often described as non-healing, hard-to-heal or chronic wounds. They represent a significant health burden for the affected person, both in terms of physical disability, psychological discomfort and limitations in social contacts. The complex therapy of patients suffering from deep category and eventually from multiple pressure ulcers is a challenge for medical, non-medical and nursing staff and has a significant negative socioeconomic impact on the healthcare system.

Prevention, conservative and surgical therapy are the pillars of the complex medical care of these defects. In clinical practice, there are several preventive measures aimed at reducing and/or better eliminating risk factors for pressure ulcers. The basis for targeted conservative or surgical therapy is, among other things, the classification of pressure ulcers, the correct interpretation of which is still not easy for many medical and non-medical healthcare professionals.

All abovementioned issues are still the subject of increasing interest in various studies carried out by modern medicine, which I also aim to achieve. Over the course of almost fifteen years, I have tried to understand and grasp the issue of pressure ulcers from different perspectives.

To understand the difficult issue of chronic or hard-to-heal wounds, which is what pressure ulcers really are, it is first necessary to understand the basic phases of skin wound healing. Therefore, I started my scientific career by focusing on a specific part of the nutrition (various ratios of n-3 and n-6 polyunsaturated fatty acids) and its effects on skin wound healing in animal experiments. This study inspired me to combine nutritional and specific biochemical approach with my area of expertise, pressure ulcers, in daily clinical practice. We have managed to set up regular monitoring of nutritional parameters, individual nutritional support in the form of sipping, enteral and parenteral nutrition both in

hospitalized patients and in the phase of ambulatory patients' preparation before planned surgical therapy and pressure ulcer reconstruction.

The scientific focus continued with further research into wound healing in the form of oxidative stress parameters in pressure ulcers. This was the aim of a grant project focusing on different types of pressure ulcer debridement (water jet *vs.* sharp necrectomy), where oxidative stress parameters were measured locally in wounds and systemically in blood and urine.

My other area of interest is the potential application of artificial intelligence methods to pressure ulcers, which in some areas of medicine offer new possibilities for the diagnosis, treatment and prevention of a wide range of diseases. We first started using artificial intelligence in pressure ulcer prevention to determine the best Machine Learning method to identify risk factors in intensive care units based on data from the MIMIC IV database. A link with an artificial intelligence specialist led to further collaboration on a mathematical model of the "active wound surface", which recounts 3D missing tissue mass into 2D "active wound surface", which will be my next area of expertise.

All the mentioned areas of interest to me as a plastic surgeon are focused on positive influencing the healing process of these difficult wounds. The aim is also to identify and to eliminate the risk factors for post-operative complications, which, even with a properly performed surgical reconstruction, remain much higher than in other elective surgical procedures.

Effectively tackling the complex issue of pressure ulcers demands a multidisciplinary approach, emphasizing the collaboration between clinical practice and scientific research. Integrating the expertise of healthcare professionals and researchers fosters innovation and enhances patient care outcomes.

1. PRESSURE ULCERS

Pressure ulcers (PUs) are a significant healthcare challenge, particularly in immobile and critically ill patients. They result from prolonged pressure, shear forces, and microvascular dysfunction, leading to progressive tissue damage. The incidence of PUs varies across different patient populations, with those in intensive care units, long-term care facilities, and individuals with neurological impairments being at the highest risk. Identifying risk factors is essential for early intervention and prevention, as intrinsic factors such as malnutrition, neuropathy, and vascular disease, along with extrinsic factors like pressure and friction, contribute to ulcer formation.

Standardized risk assessment tools help clinicians stratify patients based on their susceptibility to PUs, enabling the implementation of targeted preventive measures. Specific anatomical locations, particularly bony prominences, are most vulnerable to pressure-related injury, requiring careful monitoring and protective strategies. International classification systems provide a framework for staging PUs based on severity, guiding treatment decisions and long-term management. Understanding the pathophysiology, assessment methods, and classification of PUs is essential for developing effective preventive and therapeutic strategies. This chapter provides an overview of these key aspects, serving as a foundation for comprehensive pressure ulcer management.

1.1 Definition and incidence

Pressure ulcers (PUs) are referred to in many ways: as pressure wounds, bed sores or pressure sores, and as new as pressure injuries (PIs). The issue of pressure ulcers dates back to the 16th century, when the French surgeon Ambrose Paré described the case of a French aristocrat who was successfully treated for a pressure ulcer. [1]

According to the third and most recent edition (2019) of the European Pressure Ulcer Advisory Panel's (EPUAP) International Guideline for Prevention and Treatment of Pressure Ulcers/Injuries, PUs are defined as localized damage to the skin and/or adjacent tissues caused by pressure or a combination of pressure and shear forces.[2] A new edition of these guidelines is expected to be released this year, providing updated recommendations based on the latest research and clinical advancements.

Other organisations involved in the prevention and treatment of PUs at international and supranational levels are NPIAP (National Pressure Injury Advisory Panel) and PPPIA (Pan Pacific Injury Alliance). Due to the specific nature of the issue of PUs, Evidence Based Medicine has issued Recommendations for the Prevention and Treatment of Pressure ulcers, which have been published in the Czech Republic under the title CPG (Clinical Practice Guidelines – Prevention and Treatment of Pressure ulcers, adopted CPG of the European Pressure Ulcer Advisory Panel, National Pressure Injury Advisory Panel and Pan Pacific Pressure Injury Alliance). [3] The issue of PUs in the Czech Republic is covered by the portal *www.dekubity.eu.*

The incidence of PUs in healthcare facilities worldwide ranges from 0% to 72.5%.[4–7] The wide dispersion of statistical indicators of incidence and prevalence is mainly due to the different methodologies of data collection and analysis.[8,9] It is estimated that approximately 10% of patients in hospitals and 5% of patients in community care suffer from PUs and 72% of all PUs cases occur in people over 65 years of age.[10–12] Incidence rates of PUs are recorded in the adverse event reporting system for a specific reporting period. In 2019, a diagnosis of L89* was reported in 30,590 patients, representing 287 cases per 100,000 population. The majority of patients were reported as PUs of category II (26,1 %) or category III (23,9 %). [13] During the decade spanning 2010-2019, 264,442 patient records containing the diagnoses L89.0 - L89.9 were identified (an average of 26,442 patients per year). Between 2010 and 2019, there has been a 40% increase in the number of occurrences.[14] Analyses of national health registries showed that the prevalence of PUs before the start of the COVID-19 pandemic and during the 2020 pandemic was higher in patients hospitalized with SARS-CoV-2 infection.[15]

1.2 Pathophysiology of pressure ulcers formation

The fundamental process underlying the development of PUs involves the application of pressure from internal anatomical structures, such as bones and tendons, or a combination of pressure and shear forces resulting from movement of the body, against an external surface, such as a medical device or pad. Individuals who experience reduced sensitivity, limited mobility, or altered cognitive function may be unable to adequately perceive and respond to these mechanisms of action.[16] Damage to surrounding tissues (skin, subcutaneous tissue, muscles, tendons, bones) can occur due to tissue ischemia when

pressure is applied for an extended period of time that surpasses the individual's physiological capability and the resistance of the deformed tissues. [17,18] According to Kottner, empirical evidence suggests two basic pathophysiological mechanisms of pressure ulcers. [19] The first one is a reduction in soft tissue perfusion, which is caused by the deformation of soft tissue. In terms of pathophysiology, there is an external pressure that is higher than the filling pressure of the arterial capillaries, which is approximately 32 mmHg, and a higher outflow pressure from the venous capillaries, which is approximately 8-12 mmHg. This causes the blood flow in the area to either slow down or stop completely, which in turn leads to hypoxia in the tissue. [20] Nutrient availability is reduced, waste products from the tissues accumulate and acidification occurs. Reperfusion injury is a type of tissue damage that can occur when blood flow is restored after a period of ischaemia. [21,22] The second pathogenic process is soft tissue deformation above a particular tolerance threshold, which leads to direct deformation and injury to cells through structural breakdown of the cytoskeleton and plasma membrane. [17,23] A theoretical model of PU has been created by Defloor [24], and it consists of four fundamental components: compressive pressures, shear forces, tissue tolerance to pressure (mechanical stress and/or wetness), and tissue tolerance to oxygen (low blood pressure, poor oxygenation). According to Slomka and Gefen's research, the interplay of these elements in an enhanced degree can lead to the creation of a PU in less than an hour of prolonged pressure on sensitive tissues. [25,26] These facts are central to preventive measures, especially for critically ill patients in intensive care units.

The pathogenesis of PUs is known to progress from the tissue depths, i.e., starting closest to the bony prominence and progressing upwards towards the skin. However, there are also studies suggesting that tissue deformation and ischaemia could originate at the level of the epidermis or dermis. [27] However, PUs have to be distinguished from skin lesions such as dermatitis associated with incontinence, intertrigo, etc., since these nosological entities are not classified as PU. [27,28] The prevention of pressure ulcers has recently focused on the microclimate in the affected area. [19]

1.3 Risk factors

The primary goal of preventive interventions for PUs is the reduction or removal of any risk factors that may be present. In 2003, Lyder enumerated more than one hundred different

risk factors that were discovered via research. [29] According to Michel et al.'s research, the risk factors for PUs may be divided into two categories: external and internal.[30]

External risk factors

Pressure (intensity, duration and gradient)

The rate of PUs formation depends on the applied pressure, its duration and gradient. Body tissues have different resistance and tolerance to the applied pressure. Muscle tissue is more susceptible to compression than skin; therefore, ischaemia and necrotising of deep muscle fibres may occur even in an intact skin. [31]The formation of PU is contingent upon various factors, as outlined below, and may occur within a timeframe ranging from hours (in patients admitted to intensive care units) to days. The pressure gradient resulting from bone prominences pressing against a solid surface takes on a conical or V-shaped form, with the highest-pressure gradient occurring at the apex. The aforementioned location is the primary site for ischemia and is highly susceptible to tissue necrosis, particularly in muscle and adipose tissue. The Pressure Mapping System (PMS) is capable of providing an objective assessment of pressure by measuring the pressure distribution across the human body. This is achieved through the use of the Body Pressure Measurement System (BPMS). [32]

Skin shear forces and friction

The shear forces involved in the formation of PUs are forces that act in an oblique direction at the level of the subcutaneous tissue. This mechanism is particularly applicable in persons slumping in a semi-sitting position or in recumbent patients and is caused by the pulling of the body at points where the skin is in contact with a solid surface, but at the same time there is a pull in the underlying tissues. Shear forces occur particularly in areas of higher skin moisture, which may 'stick' to the underlay or a hard surface. The key to prevention is to eliminate moisture by using adherent coverings that can minimize the risk of friction and shear forces.

Skin maceration

Minematsu et al. [33] have defined skin maceration as a condition in which the skin barrier is disrupted, resulting in reduced tolerance to irritants and force. This is caused by structural

changes in the intercellular lipid layers and junctions between keratinocytes in the epidermis. Skin lesions are believed to be triggered by the invasion of irritants and reduced intercellular junctions, which lead to an inflammatory response. Maceration in perineal lesions can also be caused by incontinence of urine and stool. There are several studies [34–37], that have investigated the interaction of digestive enzymes (trypsin, alpha-chymotrypsin, lipase) and intestinal bacterial flora and maceration on the development of skin lesions associated with faecal incontinence. Proteases have been found to increase the transdermal penetration of macromolecules, suggesting that proteases themselves can invade and digest skin tissue. [35] In the pathogenesis of decubital lesions, maceration is implicated mainly as a factor of shear forces on the skin and dermal plexus.

Internal risk factors

Immobility due to impaired mental and/or motor function

It has long been acknowledged that the aforementioned pressure and shear forces, which are frequently associated with decreased activity and mobility, are the leading cause of pressure ulcers. [11] Limited patient mobility and reduced physical activity are considered significant risk factors for developing PUs ulcers. [38,39] These risks are already well known, and nursing and medical staff are striving to eliminate them through various tools as part of preventive measures. Immobile patients are bedridden or wheelchair-bound, with a predisposition to pressure lesions depending on body position. The causes of immobility or reduced activity of patients are numerous. In acute care, these include patients dependent on artificial pulmonary ventilation who, even with preserved sensation, cannot reflect the pain caused by pressure. Conversely, many neurological disorders (spinal cord damage due to trauma, tumour or congenital defect, multiple sclerosis, etc.) are often accompanied by impaired sensitivity, where the body's response to applied pressure is not possible due to the absence of pain. Pressure ulcers associated with reduced mobility or activity are also encountered in patients with psychiatric diagnoses such as dementia, Parkinson's disease [40], depression [41] or as a result of the use of fixation splints in psychotic patients. [42]

Malnutrition

Malnutrition is a prevalent occurrence in patients with PUs and is regarded as a significant risk factor. [43] The severity level is contingent upon the patient's general state (e.g., critically ill, presence of multiple long-term non-healing PUs, etc.). The consistent monitoring of

nutritional factors, with particular emphasis on total protein, albumin, and prealbumin, is a crucial component in the prevention and conservative treatment of PUs. Adequate replacement of deficient nutritional components, such as vitamins and trace elements, is also necessary before and after surgical reconstruction. Based on the findings of Litchford et al, the underlying principle of the 2019 Nutritional Guidelines for the Prevention and Management of Pressure Ulcers is to increase energy expenditure and correct protein intake in people who are vulnerable to PUs due to malnutrition or at risk of malnutrition. [44] In a study conducted by Reed, a sample size of 2771 individuals was analysed. The results show a significant correlation between low albumin levels (less than 30 g/l) and the likelihood of developing PUs. [45]

Urinary or stool incontinence, skin condition

Incontinence is strongly associated with an increased likelihood of developing PUs. [2] The prevalence and severity of PUs acquired during hospitalization was found to be higher in patients with incontinence compared to patients who are continent. [46,47] Kayser et al. in their recent study described up to 5.8 times higher risk of sacral PUs progression in patients with incontinence to category III and IV. [48] The occurrence of incontinence results in skin maceration, rendering it more vulnerable to mechanical stress and deformation, thereby increasing the friction between the skin and the pad. Both factors play a significant role in the development of PUs. [49] According to a comprehensive study conducted by Koloms et al., incontinence has been identified as a major risk factor for PUs. The study revealed that a significant proportion (72.6%) of patients with PUs acquired during hospitalization or in hospital settings had either urinary, faecal incontinence, or a combination of both. [50]

Reduced blood flow or low blood pressure

Impaired oxygenation and tissue perfusion are recognized as significant risk factors for PUs and are prevalent among critically ill patients. Frequently, these patients require mechanical ventilation and circulatory support through vasopressors to attain hemodynamic stability. [51] The aforementioned fact leads to an adverse effect of hypoxia and ischemia on both the skin and underlying tissues. In their publication, Cox and Schallom [52] proposed a theoretical framework for the advancement of PUs in patients who are critically ill. This framework outlines the interconnections between factors that hinder oxygenation and perfusion, such as mechanical ventilation, hypotension, cardiovascular

disease, diabetes mellitus, and vasopressors. Anaemia is a notable factor that can hinder proper nutrition and sufficient oxygenation of tissues. This can lead to delayed wound healing as a result of reduced nutrient supply and ineffective removal of waste products from tissues.

Neuropathy

Sensory neuropathy increases the prevalence of PUs in the heel area, especially in elderly hospitalized patients, and is considered a critical risk factor in this regard. Sensory neurites play a key role in the microcirculation in the skin and adjacent area at the time of pressure. [53]

Mental state or lack of motivation for rehabilitation

Psychological deprivation, sedative effects of drugs, and alteration of psychological state may result in lack of motivation for active physiotherapy. These facts result in the persistence of immobility, which also leads to prolonged pressure on the sites of bony prominences and is therefore another risk factor for the development of decubital lesions.

Age and other factors

The higher age is a contributing factor in the development of PUs. The ageing process leads to an increase in the fragility of blood vessels and connective tissue, while fat and muscle mass decrease. This reduction in fat and muscle mass results in a reduced ability to dissipate pressure. [54,55] There is also flattening of the dermo-epidermal junction and other age-related changes in the skin, such as decreased skin elasticity, thinning of the fat layers, accentuated sarcopenia of the muscles, and low intradermal vascular oxygenation and perfusion. [56–58] Other risk factors described above are also present to a greater extent in older patients, so there is a potentiation of the effects of risk factors in older individuals. Other risk factors include morbid obesity, associated comorbidities, especially diabetes mellitus, cardiac decompensation, smoking, atherosclerosis etc.

The objective of preventive measures is to recognize the risk factors and evaluate the individual's susceptibility to developing PUs in each patient. *Diagram 1* illustrates the

various factors that can influence an individual's susceptibility to developing PUs.

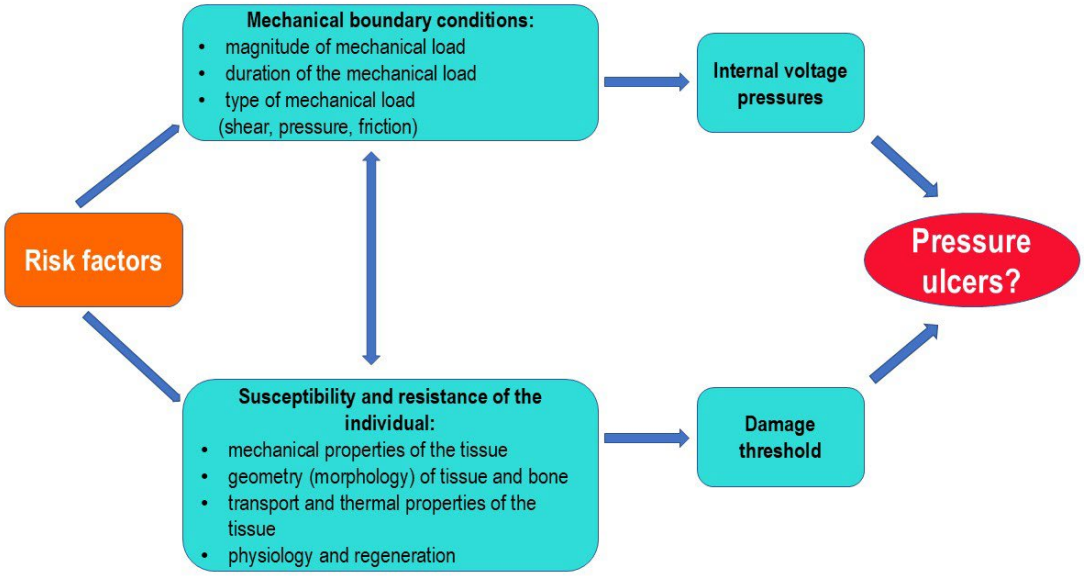


Diagram 1: Factors influencing the susceptibility (tendency) of an individual to develop PUs. Modified and adapted from [59]

1.4 Pressure ulcer assessment risk score scales

To reduce these risk factors and take preventive actions, a scoring system has been created based on the risk variables to estimate the degree of risk of developing PUs. An increased degree of prophylaxis is ensured by constantly adhering to complete anti pressure ulcer measures starting on the first day of admission thanks to patient risk assessment, which enables patients with a potentially high risk of developing PU to be selected. [60] Worldwide, about 40 scoring systems have been established to predict the risk of PU. [61] In clinical practice, the most commonly used scales are the Braden, Waterloo and Norton. [62,63] The Braden scale was first introduced in 1987, [64] and is one of the most widely used in the world, both in acute care beds and in long-term care facilities. [65]

The Braden scale includes six items: sensitivity, moisture, activity, mobility, nutrition, and friction and shear force. The six items are rated on a scale of 1 (most affected) to 4 (least affected). The remaining item, friction and shear, is rated on a scale of 1 (problem) to 3 (no problem). The exact Braden scoring system is described in *Table 1.*

Item	1 point	2 points	3 points	4 points
Sensory perception	Completely limited	Very limited	Slightly limited	No impairment
Moisture	Constantly moist	Very moist	Occasionally moist	Rarely moist
Activity	Bedfast	Chair-fast	Walks occasionally	Walks frequently
Mobility	Completely immobile	Very limited	Slightly limited	No limitation
Nutrition	Very poor	Probably inadequate	Adequate	Excellent
Friction/shear	Problem	Potential problem	No apparent problem	
Braden scale risk of pressure ulcer:				**Total points** **mild risk** **15-18 points** **moderate risk** **12-14 points** **severe risk** **≤ 11 points**

Table 1: Braden scale risk of pressure ulcer. Modified and adapted from [65]

The Norton Scale [66] was designed in 1962 to assess the risk of PUs in elderly patients and identified the main risk factors that should be included in the scale: physical condition, mental status, activity, mobility and incontinence; all items are described in the *Table 2*. All items are rated on a scale from 1 (worst condition) to 4 (best condition). A total score of less than 10 predicts a high risk of PUs and maximum intervention in the application of preventive measures. [65,67] This scale system is the most frequently used in healthcare facilities in the Czech Republic.

Item	1 point	2 points	3 points	4 points
Physical condition	Very bad	Fair	Poor	Good
Mental condition	Stuporous	Confused	Apathetic	Alert
Activity	Bedfast	Chair-fast	Walks with help	Ambulant
Mobility	Immobile	Very limited	Slightly impaired	Full
Incontinence	Urinary and faecal	Predominantly urinary	Occasional	None
Norton scale risk of pressure ulcer:			**low risk** **medium risk** **high risk** **very high risk**	**Total points** **>18 points** **14-18 points** **10-14 points** **<10 points**

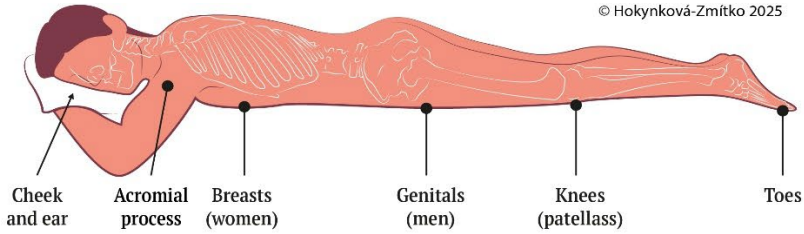
Table 2: Norton scale risk of pressure ulcer. Modified and adapted from [65]

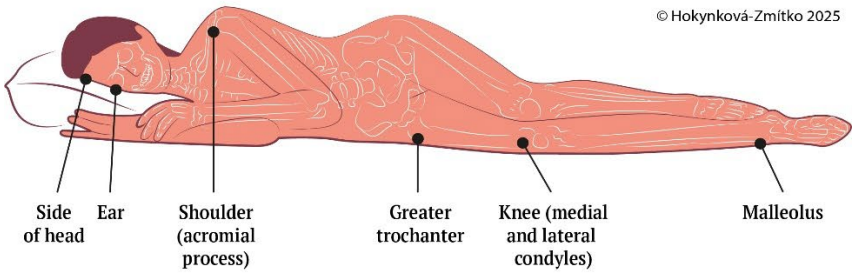
Waterloo scale [68] was introduced in 1985 and includes the following items: body build/weight for height, skin type/visual risk area, sex/age, malnutrition screening tool, continence, mobility, and special risks (malnutrition, neurological deficit, major surgery or trauma, medications). Each category has its own score. A total score of 10-14 points indicates that the patient is at risk for PUs, 15-19 points indicate high risk, and 20 or more points indicate very high risk for PUs. [69] Quantitative risk assessment and prediction of risk factors in selected patient groups have been the subject of research at many levels, in recent years especially at the level of artificial intelligence (AI). [70–72]

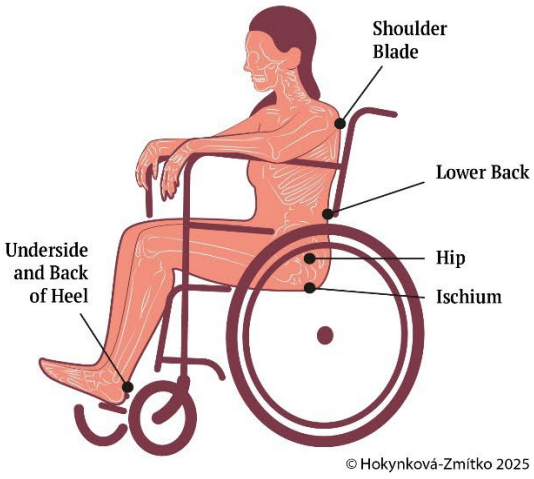
1.5 Pressure ulcers risk areas

The predisposition of the development of PUs determines the risk area for their formation depending on the position of the body. In the supine position, the predilection localization is the occipital, scapular, elbow, sacral, and heel area. In the side position, the ears,

shoulders, trochanter, knees, and ankles are the sites of predisposition. In the prone position, the forehead, cheeks, chin, breasts, genital(male), knees, and toes. The sitting position predisposes to PUs especially in the spinous processes, sacral area, ischial area, and heels. These risk areas are due to the action of pressure points between the prominent skeletal prominence and the pad, or under the shearing forces in different body positions. In the context of adverse event reporting, the location of PUs is marked in a pictogram – the so-called Margoles map. [73] In clinical practice, we most often encounter of PUs in the sacral, trochanteric and ischial regions. The most frequent sites of PUs depending on body position are shown in *Figure1*.







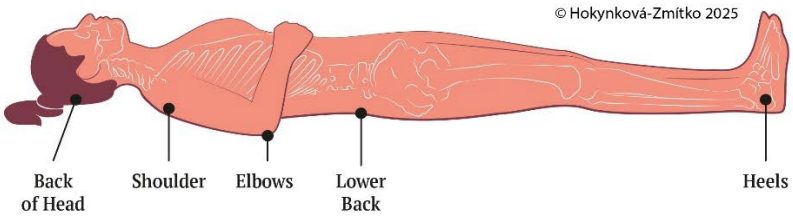


Figure 1: Pressure ulcers risk areas according to body position

1.6 Classification

A diagnosis of pressure ulcer should only be made if it is highly likely that the skin and/or tissue damage is due to prolonged pressure or pressure associated with shear forces. [74] The misdiagnosis and misclassification of PUs is the cause of the underreporting of these adverse events in the Central Adverse Event Reporting System and thus the cause of the very wide variance in the incidence of PUs worldwide in different healthcare settings.

National Pressure Injury Advisory Panel (NPIAP) provides a precise classification of PUs into the following categories. [75]

PU category I

Category I of PUs is classified as superficial and reversible PUs. Clinically, it manifests as persistent redness or erythema on the epidermis, even after the pressure has been removed. It is an erythema on intact, undamaged epidermis that does not blanch (see *Figure 2*). In patients with dark skin, these alterations may be more subtle and difficult to detect. Nonetheless, the skin may exhibit altered sensitivity, temperature, or firmness in comparison to healthy skin.

Figure 2: Category I of pressure ulcer – non-blanchable erythema

PU category II

Category II of PUs is also classified as superficial and reversible PUs. Macroscopically, there is already a partial loss of skin cover at the site of applied pressure at the level of the dermis. It manifests itself as excoriations with a pink or red base or as intact or perforated blisters filled with serous fluid or blood (see *Figure 3*). This entity must be distinguished from dermatitis due to urinary or faecal incontinence. After the pressure has subsided, PUs of category II heal spontaneously. If the pressure persists at the site, depending on the duration and intensity of the pressure, the PUs may deepen into the deeper categories listed below.

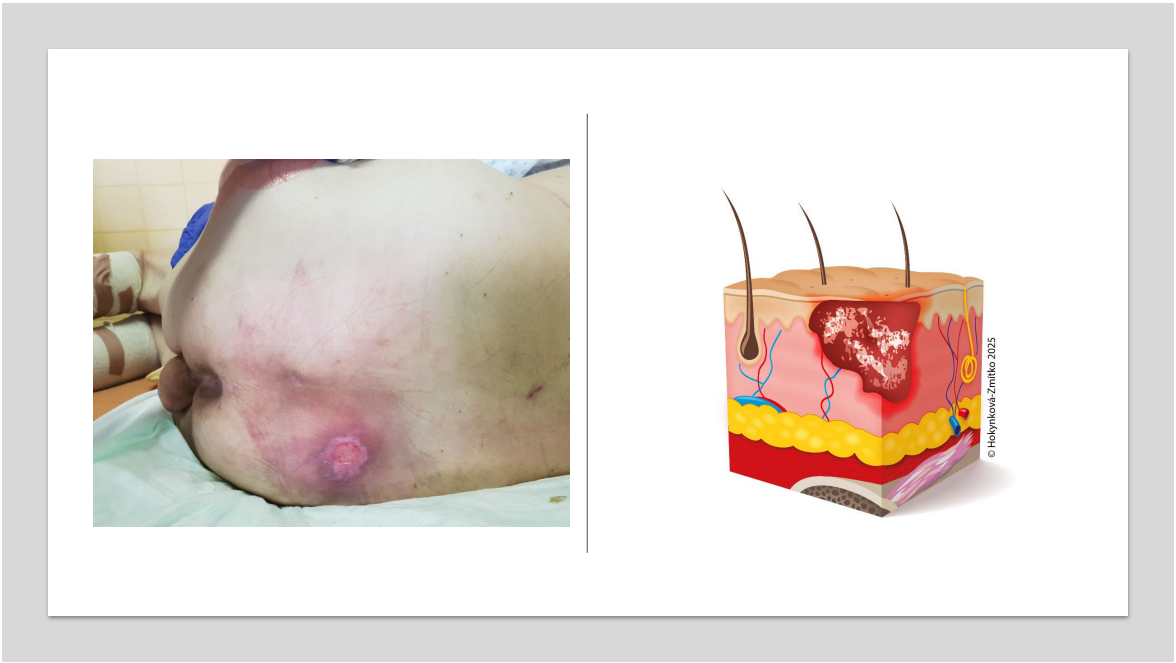


Figure 3: Category II of pressure ulcer

PU category III

Category III of PUs is classified as deep PUs, in which there is complete loss of skin. It may take the form of flat and shallow ulcers at the level of the skin and subcutaneous tissue (see *Figure 4*) with rolled edges, without damage to muscles, tendons and bones. This category is already indicated for surgical treatment, but may spontaneously resolve with conservative treatment, but at a longer interval (several months).

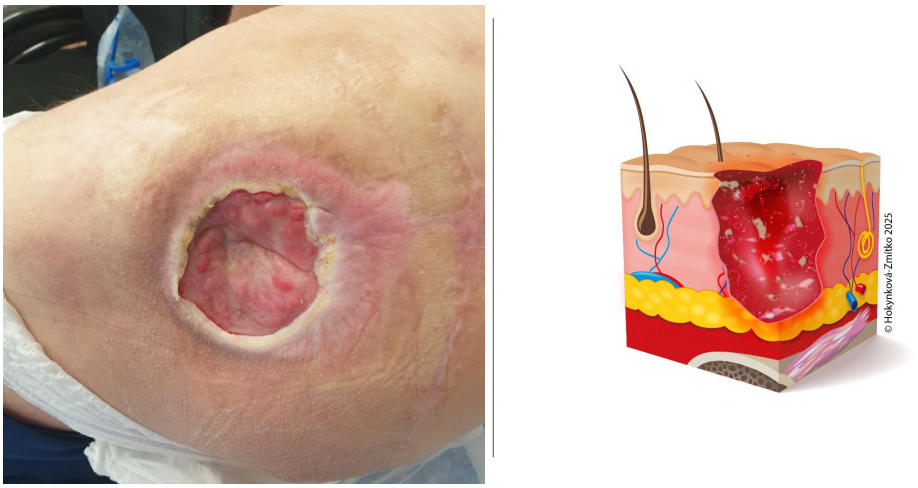


Figure 4: Category III of pressure ulcer

PU category IV

Category IV of PUs represents a more serious nosological unit in which, in addition to skin and subcutaneous tissue, there is also damage to muscles, fascia, tendons, ligaments, cartilage, and skeleton (see *Figure 5*). Clinically, it takes the form of various deep and extensive ulcers, pockets, pseudocysts, sinuses, tunnels, or communicating fistulas. Surgical therapy is usually indicated for this category.

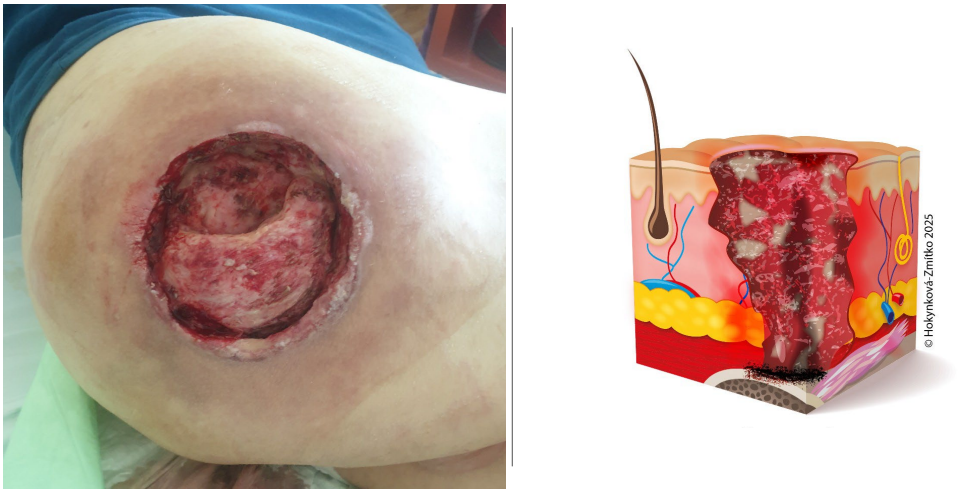


Figure 5: Category IV of pressure ulcer

Unstageable category of PU

The non-classifiable category of PU includes PUs in which the level of compressive damage to adjacent tissues cannot be determined. An adhering eschar, a stiff slough or necrosis (see *Figure 6*) at the site of pressure load makes it impossible to determine the depth of tissue involvement. Removal and detachment of avital tissues by conservative or, as a rule, surgical techniques then allow reclassification of the PU into the first four categories with the possibility of adequate therapy.

Figure 6: Unstageable category of pressure ulcer

Deep tissue injury

Suspected deep tissue injury is associated with a specific type of PU, which clinically manifests purple discoloured or dark brown intact skin in the form of blood-filled blisters, which again make it impossible to determine the depth of tissue damage (see *Figure 7*).

Figure 7: Deep tissue injury

Specific, additional definitions of PUs

Pressure ulcers related to the use of medical devices occur in different locations of the human body depending on the type of diagnostic or therapeutic medical device or apparatus used that causes a PU (endotracheal cannula, indwelling urinary catheter, nasogastric tube etc.).

Mucosal membrane PUs occur at the site of mucous membranes, which have a different anatomical structure compared to the skin and cannot be classified according to the above system.

1.7 Treatment of PUs

The treatment of PUs requires a multidisciplinary and multimodal approach, as these chronic wounds are often difficult to heal and carry a high risk of complications, including infections, prolonged hospitalization, and recurrence. Successful management depends on a combination of preventive measures, conservative therapy, and surgical interventions, tailored to the patient's specific needs and overall health status.

This chapter provides a structured overview of these key therapeutic strategies. The goal of PUs treatment is not only wound healing but also functional restoration and long-term prevention. By integrating preventive, conservative, and surgical approaches, clinicians can achieve successful outcomes, minimizing complications and improving the patient's quality of life.

1.7.1. Preventive measures

Pressure ulcers are referred to as preventable skin and tissue damage, with the identification of risk factors and the overall condition of the patient as well as multidisciplinary cooperation (nutritionist, physiotherapist, general nurse, physicians of various specialties - internal, surgical, orthopaedic, etc.) being the keys to prevention. [76]

Complex therapy for PUs is based on preventing its occurrence through a series of preventive measures on multiple levels: [17]

- Systematic staff education
- Assessment of risk factors
- Assessment of the risk of PUs
- Identification and uniform method of labelling patients at risk of PUs
- Educating hospitalized patients and family members about the risk of PUs and related circumstances, how to prevent it and about measures to reduce the risk of PUs
- Local prevention
- Positioning (patient handling, frequency of positioning etc.)
- Use of anti-pressure ulcers aids (special mattresses, seat cushions etc.)
- Nutrition
- Early mobilisation

Individualized preventive measures are designed and implemented according to the requirements of each patient, considering his or her risk factors, the anticipated impact of pressure points based on the patient's position, and other parameters.

These precautions should be taken for all patients at risk of developing PU, not only in hospitals and operating rooms, but also in the patient's residence. It is crucial to educate and motivate the patient and his/her caregivers to comply with these preventive measures; social services such as peer mentoring (for example organisation CZEPA in the Czech Republic, *https://czepa.cz/*) are useful in this regard to assist wheelchair users in returning to an active lifestyle.

1.7.2. Promoting wound healing, conservative therapy

Wound bed preparation is a clinical concept involving a systematic and holistic approach to wound assessment and management that promotes normal wound healing. [77] PUs are a challenging form of wound that typically does not heal or is difficult to heal or heals slowly. The objective of conservative therapy is to remove avital tissue fragments (cellular debris, plaques), eradicate bacterial load, and promote vascularization of the underlying tissue in order to stimulate the growth of granulation tissue. [78]

Wound base preparation includes the following main aspects of wound care (abbreviated as TIMERS) [79] :

- ***T – Tissue management***
- ***I – Infection and inflammation control***
- ***M – Moisture balance***
- ***E – Epithelial edge advancement***
- ***R – Repair and regeneration***
- ***S – Social factors and factors related to the individual***

Conservative therapy should be based on the current state of the wound, which evolves according to the *wound healing continuum* from necrosis to sloughing, granulating to epithelializing wound. [60] Therefore, different forms of conservative therapy are used

depending on the localization, depth of involvement, bacterial colonization, nature of the defect, but also on the current stage of healing of the wound. Solutions with antiseptics, hydrogels, hydrocolloids, enzymatic products capable of lysing avital tissue, alginates, polyurethanes with silicone and silver, and various other products and forms of moist wound healing are used. Conservative therapy of long-term healing wounds should include peri wound skin management. Preventive measures include the use of polymer, silicone sheeting, or film spray in peri wound skin care.

Particularly for deep categories of PUs, negative pressure wound therapy (NPWT) is often used to promote healing by applying controlled continuous or intermittent sub-atmospheric pressure (80-125 mm Hg) to the open wound and has recently been used extensively for the temporary treatment of traumatic wounds and to treat difficult wounds such as pressure ulcers. [80,81] NPWT promotes the growth of granulation tissue, vascularization, reduces the bacterial load of the wound, etc. Some forms of NPWT have an installation mode that allows the injection of antiseptic or antibiotic directly into the wound bed. NPWT is often used in the period after debridement, extirpation of pseudocysts or necrectomy of decubitus wounds before planned flap reconstruction.

1.7.3. Surgical therapy

The basic procedure prior to planned reconstructive surgery of PUs is the removal of all avital parts and tissues. [82] Types of debridement include enzymatic debridement, ultrasonic debridement (thermal and biological effects), sharp debridement (removal of all devitalized tissue), autolytic debridement (hydrogels, hydrocolloids), and other forms. [83] Invasive debridement and removal of PUs pseudocysts is performed using a sharp debridement (scalpel, scissors, electrocautery) or a water jet. [84] After the preparation of the bed of the deep PUs defect, the reconstruction is proceeded to one session, or in our workplace, often postponed (usually one week after debridement), i.e., in two stages. [85]

The choice of the reconstructive procedure is guided by nature, depth, extent, localization of the defect, the quality of the adjacent tissues (presence of scars from previous reconstructions in case of recurrence), the overall condition and mobility of the patient, as well as the experience and preferences of the plastic surgeon. The reconstructive ladder is slightly modified in case of closure of deep category of PUs. [86]

1.7.3.1 Types of reconstruction

Direct suture is rarely used for small PUs and ideally for mobile patients with preserved sensation. The resulting scar tissue is usually localized over a fixed prominence and is less resistant than the surrounding fat or muscle tissue and therefore has a higher risk of recurrence of PUs.

Autotransplantation using skin grafts is also rarely performed because of the need of minimal polymicrobial wound contamination and the higher fragility of skin grafts in healing compared with other reconstructive techniques. [86] This type of closure can be used in shallow flat defects, also optimally in mobile patients, in whom it is assumed that the applied tissue pressure will not persist after reconstruction with this technique.

The gold standard in reconstruction of deep PUs is local **fasciocutaneous (FC) or musculocutaneous (MC) flaps**, which use adjacent tissue containing subcutaneous skin and fascia or subcutaneous skin and muscle. The basic idea is to reconstruct the missing volume with high quality, well vascularised tissue.

Rare types of PUs reconstruction include very challenging procedures such as fillet flaps from an amputated lower limb to reconstruct a very large defect that cannot be reconstructed with local resources. Methods using tissue expansion and free flaps are also reported, however, their employment in clinical practice is limited. The most challenging technique for reconstruction of large and very often osteomyelitis-complicated PUs is hemicorporectomy, which is associated with very high perioperative and postoperative mortality.

1.7.3.2 Selection of flap plasty according to localization

The most used flaps in deep **sacral PUs** are rotational or VY shift fasciocutaneous flaps, among muscle flaps it is mainly the musculocutaneous flap of the gluteus maximus muscle in the form of VY displacement. The two types may combine with each other and may be unilateral or bilateral. An overview of the basic flap reconstruction procedures in sacral PUs is given in *Table 3*.

Type of used tissue	Type of flap plasty
Fasciocutaneous flaps	Rotation gluteal FC flap
	VY shift unilateral
	VY shift bilateral
Musculocutaneous flaps	Gluteus maximus muscle
	VY with gluteus maximus muscle
	S-GAP flap (Superior gluteal artery perforator flap)
	Superior gluteal artery island flap

Table 3: Flap plasties in reconstruction of sacral PUs. Modified, translated and adapted from [85]

Trochanter PU is another frequent localization PUs. In this instance, the MTFL (tensor fasciae latae muscle) - MC flap is the most prevalent. Rotational flaps (anterior and lateral thigh flaps), transposition flaps from the anterior thigh surface to the upper pedicle, and others, may also be used. [85] *Table 4* provides a summary of the most frequently utilized flaps in reconstruction of trochanteric PUs.

Type of used tissue	Type of flap plasty
Fasciocutaneous flaps	ALT flap (anterolateral thigh flap)
	Random thigh flap
	Bipedicled thigh flap
Musculocutaneous flaps	MTFL (tensor fasciae latae muscle) flap - Rotation - VY shift
	Gluteus maximus muscle
	Vastus lateralis muscle Rectus femoris muscle

Table 4: Flap plasties in reconstruction of trochanter PUs. Modified, translated and adapted from [85]

The FC dorsal thigh flap, medially or laterally pedicled, is most used for reconstruction of **ischial PUs**. Especially in recurrent forms, volume supplementation by insertion of well-vascularized muscle tissue, e.g., hamstring flap, is advantageous. At our department, the so-called "turn over flap" is also newly introduced: the long head of biceps femoris muscle, in which the volume of the missing tissue is supplemented with the distal portion of the long head of biceps femoris muscle and its "turning" by 180 degrees and fixated into the defect above the ischial tuber. [87] *Table 5* provides an overview of the individual flap plasties used in the reconstruction of ischial PUs.

Type of used tissue	Type of flap plasty
Fasciocutaneous flaps	Medial thigh flap
	Lateral thigh flap
	Dorsal distal gluteal flap I-GAP (Inferior gluteal artery perforator flap)
Muskulocutaneous flaps	Hamstring flap: Biceps femoris muscle-long head Turn over flap of biceps femoris muscle Semitendinosus muscle Semimembranosus muscle
	MTFL (Tensor fasciae latae muscle)
	Gluteus maximus muscle-pars inferior

Table 5: Flap plasties in reconstruction of ischial PUs. Modified, translated and adapted from [85]

1.7.3.3 Post-operative complications

In general surgery, the Clavien-Dindo classification (see *Table 6*) is used to objectify postoperative complications, which was developed based on a study of a cohort of 6336 patients. [88,89] Lindqvist et al. first used this classification of post-operative complications in study on 118 patients after pressure ulcer surgery, with findings of 43.2% of minor post-operative complications and rare occurrence of serios Clavien-Dindo III or IV complications. [90] Flap-related complications are described in 25% in Lindqvist study and in 21% in Biglari et al. study [91] on 352 spinal cord patients undergoing pressure ulcer surgery. The most common complication was suture dehiscence, followed by infection and hematoma in Lindqvist study with no difference in complication rates or types between different flaps. [90] Keys et al. defined as a predictors of failure after flap coverage of pressure ulcers low serum albumin, young age and also previous pressure ulcer surgery. [92] The above complications can be assessed as acute or subacute a few weeks after surgery. However, one of the late and serious complications is the recurrence of PU after flap plasty. McCranie

et al. analyses of a national database with 4796 patients included show the recurrence rate of PUs to be 73.6%. [93] The recurrence rate in certain localizations in study of Paker et al. in 39 patients was 82.1 % in sacral, 20.5% in ischial, 15.4 % in trochanteric ulcers, and 2.6% in other locations. [94] Scarred terrain, previously used local tissue material, complications in terms of osteomyelitis, and a multiple PUs may lead to the conclusion of inoperability in recurrent PUs. This status may have serious consequences for the patient's overall health. Assessing risk factors for pressure ulcers recurrence is critical for surgical planning. By understanding the characteristics that contribute to recurrence, clinicians can optimize preoperative care, and plastic surgeons may select the individualised surgical approach that is least likely to require reoperation.

Grades
No complication
Grade I
E.g., post-operative need of antiemetics, analgesics, diuretics, electrolytes
Grade II
Per- or post-operative need of drugs other than above, e.g., blood transfusion, antibiotics
Grade III
Need of surgical intervention
IIIa intervention not under general anaesthesia
IIIb intervention under general anaesthesia
Grade IV
Life-threatening complication requiring intensive care unit management
IVa single organ dysfunction
IVb multiorgan dysfunction
Grade V
Death of patient

Table 6: Post-operative complications by Clavien-Dindo. Modified and adapted from[90].

1.8 Summary

This chapter summarizes the general knowledge about PUs, including their complex therapy, which must be multidisciplinary and properly managed at all levels of the health service. PUs are very challenging, especially because of the considerable variability of patients, their comorbidities, the length of their immobilization, the local wound condition, the compliance and motivation of patients and their caregivers, and many other factors affecting the course of treatment and healing of the wound itself. Reconstructive techniques applied in the surgical treatment of deep categories of PUs stand at the top of the ladder of comprehensive therapy. However, even properly performed flap reconstruction leads to a very high risk of postoperative complications, including a high risk of PUs recurrence. These findings led me to consider the possibility of eliminating risk factors at the level of patient preparation prior to planned reconstructive surgery. We wrote a guide for patients and physicians preparing patients for surgery called *"Guide to the Surgical Treatment of Pressure ulcers. Selected advice for patients, physicians, medical staff and non-professional caregivers" ("Průvodce chirurgickou léčbou dekubitů. Vybrané rady pro pacienty, lékaře, zdravotnický personál a laické pečovatele "),* see *Appendix 1.* We have also tried to raise awareness of PUs at the level of medical education by using electronic scripts on the topic of *„Complex therapy of pressure ulcers" („Komplexní léčba dekubitů")*, and I have also participated in the revision of the Czech translation of several chapters of the clinical guidelines *„Prevention and treatment of pressure ulcers/injuries: Clinical Practice Guideline" („Klinické doporučené postupy – Prevence a léčba dekubitů").* Articles related to the topic of surgical treatment of PUs were published in Supplements dealing with pressure ulcers in the journal *„Česká a slovenská neurologie a neurochirurgie".*

1.9 Articles related to this issue

Hokynková, A., Šín, P., Černoch, F., Nováková, M., & Babula, P. (2017). Využití lalokových plastik v operační léčbě dekubitů. *Česká a slovenská neurologie a neurochirurgie*, Vol. 80/113 (Suppl 1), p. 41-44.

Hokynková, A., Šín, P., Adlerová, T., & Černoch, F. (2022). Transplantace kůže v chirurgické léčbě dekubitů. *Česká a Slovenská Neurologie a Neurochirurgie*, Vol. 85/118, (Suppl 1), p. 12-14.

Šín, P., Holoubek, J., Hokynková, A., & Lipový, B. (2019). Multidisciplinary approach in surgical pressure ulcer therapy after spinal cord injury. *Česká a Slovenská Neurologie a Neurochirurgie*. Vol. 82/115, (Suppl 1), p. 52-55.

doi: 10.14735/amcsnn2017541

Využití lalokových plastik v operační léčbě dekubitů

Employment of Flap Surgery in Pressure Ulcers Surgical Treatment

Souhrn

Cíl: Cílem příspěvku je popsat operační léčbu dekubitů, která zahrnuje především pečlivou nekrektomii s kompletní exstirpací pseudocysty dekubitu, odstraněním píštělí a event. resekcí osteolytického nebo osteomyelitického ložiska. *Soubor a metodika:* Prezentován bude přístup k operační léčbě dekubitů vč. obrazové dokumentace, a to na konkrétním případu pacienta (nar. 1974, paraplegik) s dekubitem, který byl ošetřen lalokovou plastikou. *Závěr:* Chirurgická léčba dekubitů zejména v první fázi vyžaduje multidisciplinární přístup. Poté je individuálně načasován a naplánován typ lalokové plastiky s ohledem na komorbidity pacienta v závislosti na velikosti, hloubce a lokalizaci defektu. Antidekubitní prevence ve všech fázích léčby – předoperačně, perioperačně i pooperačně – významně snižuje riziko akutních i pozdních komplikací a recidivy dekubitů.

Abstract

Aim: Aim of the paper is to describe surgical treatment of pressure ulcers, which includes primarily careful necrectomy with complete extirpation of ulcer's pseudocyst, removing of fistulas and eventual resection of osteolytic or osteomyelitic centre. *Material and methods:* This case report presents surgical procedure of pressure ulcer treatment, including photo documentation, in a paraplegic patient (born 1974) with pressure ulcer treated by flap surgery. *Conclusion:* Surgical treatment of pressure ulcer requires multidisciplinary approach, especially at the treatment onset. Type as well as timing of flap surgery is planned individually according to patient's comorbidities, based on size, depth and localisation of defect. Antidecubital prevention in all phases of treatment – before, during and after the surgery – significantly decreases risk of acute and late complications and pressure ulcers recurrence.

Autoři deklarují, že v souvislosti s předmětem studie nemají žádné komerční zájmy.
The authors declare they have no potential conflicts of interest concerning drugs, products, or services used in the study.
Redakční rada potvrzuje, že rukopis práce splnil ICMJE kritéria pro publikace zasílané do biomedicínských časopisů.
The Editorial Board declares that the manuscript met the ICMJE "uniform requirements" for biomedical papers.

A. Hokynková[1], P. Šín[1], F. Černoch[1], M. Nováková[2], P. Babula[2]

[1] Klinika popálenin a rekonstrukční chirurgie LF MU a FN Brno
[2] Fyziologický ústav, LF MU, Brno

✉
MUDr. Alica Hokynková
Klinika popálenin a rekonstrukční chirurgie
LF MU a FN Brno
Jihlavská 20
625 00 Brno
e-mail: alicah@post.cz

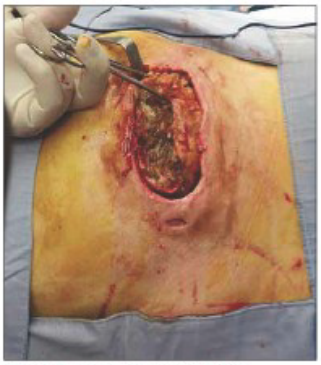
Přijato k recenzi: 28. 5. 2017
Přijato do tisku: 27. 6. 2017

Klíčová slova
nekrektomie – dekubitus – lalokové plastiky – léčba

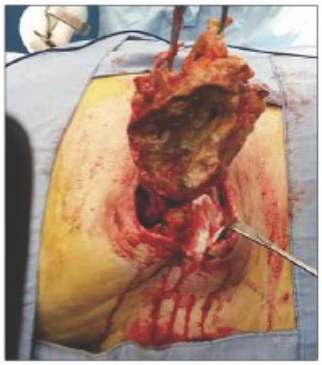
Key words
necrectomy – pressure ulcer – flap surgery – treatment

Úvod

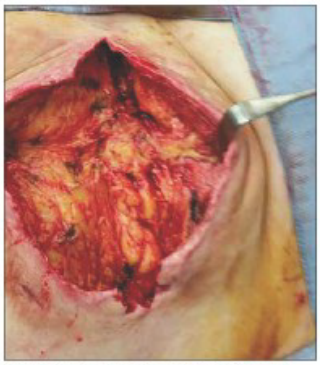
Dekubitus je lokalizované poškození kůže a/nebo podkožní tkáně, obvykle nad kostním výčnělkem, které vzniká v důsledku tlaku nebo tlaku v kombinaci se střižným efektem [1]. Vytvoření dekubitálního vředu, stejně tak jako jeho velikost, hloubka a délka hojení je podmíněno mnoha faktory (sepse, deplece bílkovin, věk, přidružené nemoci, stav imunitního systému, poškození CNS – kóma, míšní léze – spastické plegie atd.). Lokalizace dekubitů je určena místem, kde dochází k vytvoření tlaku mezi kostí a podložkou. Na naší klinice se setkáváme nejčastěji s dekubity ischiadickými, sakrálními a trochanterickými.



Obr. 1. Trochanterický dekubitus s pseudocystou.
Fig. 1. Trochanteric decubitus (pressure ulcer) with pseudocyst.



Obr. 2. Exstirpace pseudocysty dekubitu.
Fig. 2. Extirpation of pseudocyst of decubitus (pressure ulcer).



Obr. 3. Obraz dekubitu po nekrektomii.
Fig. 3. Image of decubitus (pressure ulcer) after necrectomy.

Dekubity se velmi často, jak vyplývá z analýzy Národního registru hospitalizací (NRHOSP), vyskytují zejména u pacientů s poškozením míchy různého původu (traumatického, nádorového, zánětlivého), u pacientů se sklerózou multiplex, po cévních mozkových příhodách se vzniklou poruchou hybnosti, u různých psychiatrických onemocnění a dále u pacientů s některými metabolickými poruchami [2].

Klinický obraz dekubitů může mít různou podobu: od zarudnutí, drobných exkoriací, puchýřů, píštělí, suchých nebo vlhkých nekróz kůže, až po postižení podkožní tkáně, svalů nebo i kostí ve formě lýzy nebo osteomyelitidy (mezinárodní EPUAP/NPUAP klasifikační systém dekubitů) [3]. Konzervativní terapie se využívá zejména u dekubitů I. a II. kategorie. Dle klasifikace EPUAP/NPUAP je přítomnost nekrózy diagnostickým kritériem pro III. a IV. stadium dekubitů. Nekrotická tkáň v ráně blokuje hojení a tvorbu granulační tkáně, je zdrojem infekce, zápachu, představuje pro ránu závažnou biozátěž [4]. Pečlivá nekrektomie je tedy základ operační léčby.

Metodika

Na našem pracovišti Kliniky popálenin a rekonstrukční chirurgie LF MU a FN Brně (dále jen KPRCH FN Brno) je operační léčba dekubitů poměrně častou intervencí (cca 100 operačních výkonů ročně) [5]. Následně bude popsán proces léčby u jednoho zajímavého případu (muž, narozen 1974, paraplegik (po motonehodě) se dvěma ischiadickými a sakrálním dekubitem). Obecně je před plánovaným operačním řešením dekubitů nutné provést interní předoperační vyšetření. Zahrnuje laboratorní odběry (krevní obraz, krevní skupinu, základní koagulační parametry, biochemický panel vč. CRP, celkové bílkoviny a albuminu), RTG oblasti s dekubitem (nejčastěji RTG pánve k vyloučení osteomyelitických procesů přilehlé kosti), případně fistulografii a stěr z rány a nosu k posouzení mikrobiálního osídlení, event. zavedení bariérového režimu při MRSA pozitivitě [6].

Operační léčba dekubitů probíhá zpravidla dvouetapově. V první fázi provádíme ostrou nekrektomii s kompletní exstirpací pseudocysty dekubitu (jedná se o případ jiného pacienta – ilustrativní účel) vč. všech píštělí, chobotů a lytického povrchu kosti (obr. 1–3) [7]. V některých případech může dojít až k vytvoření osteomyelitického ložiska v kosti, které je nutné – ve spolupráci s ortopedem – resekovat (nejčastěji se jedná o hlavici femuru). Po nekrektomii dekubitu aplikujeme několik dnů konzervativní nebo podtlakovou terapii ran (Negative Pressure Wound Therapy; NPWT) ke snížení bakteriálního osídlení rány a k podpoře granulací.

Ve druhé etapě přistupujeme k uzávěru dekubitů. Typ plánované lalokové plastiky se odvíjí nejen od lokalizace a hloubky dekubitu, ale také od základní diagnózy, kterou pacient trpí. Musíme rozlišit, zda se jedná o chodícího pacienta, u kterého je předpoklad, že tlak způsobující tlakovou lézi byl přechodný a daná oblast již nebude do budoucna tomuto tlaku vystavena – v takových případech dáváme přednost krytí defektů fasciokutánními laloky. Pokud se jedná o pacienta paraplegického (jakým je námi prezentovaný případ), u kterého je oblast s dekubitem pravidelně vystavována tlaku (např. oblast sedacích hrbolů při sezení na invalidní prominenci), volíme k vyplnění tzv. mrtvého prostoru dutiny dobře prokrvenou svalovou tkáň muskulokutánních laloků [8]. Například u ischiadických dekubitů se můžeme někdy setkat s relativním dostatkem měkkých tkání po nekrektomii. V těchto případech je vhodnější dekortikovat část přilehlé kůže a vyhnout se vytvoření sutury přímo nad defektem z důvodu horšího prokrvení vaziva v místě jizvy, což zvyšuje riziko případné recidivy léze. Podobně dochází k vytvoření nestabilní jizvy při konzervativním hojení hlubokých dekubitů per secundam intentionem [9]. Výjimečně využívanými laloky jsou tzv. fillet flap (z amputované končetiny) nebo mikrochirurgický přenos volného tkáňového celku (nejčastěji m. latissimus dorsi). Zde je na místě zdůraznit nutnost antidekubitních opatření v průběhu jakéhokoli operačního výkonu s použitím antidekubitních pomůcek jako prevence prohloubení stávajících či vytvoření nových dekubitů [10].

Typy uzávěrů u jednotlivých dekubitů

Sakrální dekubity se nejčastěji vyskytují u pacientů ležících na zádech a mají různou podobu a velikost. V případech sakrálních dekubitů můžeme použít fasciokutánní lalok rotační nebo VY posun jednostranný či oboustranný, muskulokutánní lalok m. glu-

Tab. 3. Přehled lalokových plastik (trochanterický dekubitus).

Podle typu tkáně	Podle typu posunu
fasciokutánní laloky	ALT lalok (anterolateral thigh flap)
	random thigh flap
	bipedicled thigh flap
muskulokutánní laloky	MTFL (musculus tensor fasciae latae) lalok*:
	· rotační
	· VY posun
	m. gluteus maximus
	m. vastus lateralis m. rectus femoris

*Nejčastěji prováděn na pracovišti autorů.

ischiadicum, pak se v rámci nekrektomie provádí i ischiektomie. Uzávěr tohoto typu dekubitu provádíme často fasciokutáním lalokem z dorzální strany stehna stopkovaným mediálně nebo laterálně [12] anebo muskulokutánním lalokem, kdy transponujeme do dutiny sval – dolní porce m. gluteus maximus (obr. 6) či dlouhá hlava m. biceps femoris [13] nebo m. semitendinosus anebo m. semimembranosus, tzv. hamstring flap. Sekundární defekty řešíme místním posunem, Z-plastikou, přímou suturou nebo kožním transplantátem. Přehled lalokových plastik k rekonstrukci ischiadického dekubitu prezentuje tab. 2.

Trochanterické dekubity

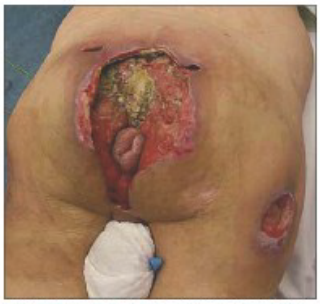
Trochanterické dekubity jsou lokalizovány v oblasti proximálních částí laterálních ploch stehen v místě tlaku podložky na oblast trochanteru kosti stehenní. Mohou mít opět charakter píštělí, chobotů, stenozujících vředů, velmi často i s kalcifikacemi v jizevnaté tkáni. V případě postižení kosti je nutno resekovat i část femuru, v závažnějších případech provést i exartikulaci končetiny. K vykrytí defektu se nejvíce užívá MTFL (Musculus Tensor Fasciae Latae), muskulokutánní lalok. Dále je možné využití rotačního laloku z přední a laterální plochy stehna na laterální stopce, transpoziční lalok z přední plochy stehna na horní stopce atd. Přehled lalokových plastik k rekonstrukci trochanterických dekubitů uvádí tab. 3.

Rozsáhlejší dekubity mají charakter kombinovaných dekubitů (obr. 7, jedná se o případ jiného pacienta – ilustrativní účel), např. sakrogluteální, u kterých využíváme kombinace jednotlivých lalokových plastik, místních posunů nebo jiných technik.

Per- i pooperačně je nezbytný komplexní přístup: cílená ATB terapie, pravidelné poradenství nutričního terapeuta, dle krevních ztrát podání krevních derivátů atd. Po uzávěru lalokovou plastikou vždy využíváme podtlakových drénů, které ponecháváme nejméně 5 dnů. V pooperačním období je velmi důležitá prevence recidivy dekubitů s využitím pravidelného polohování, antidekubitních pomůcek a odlehčením operované oblasti po dobu min. 6 týdnů. S výhodou lze jako preventivní postup využít spolupráci s rehabilitačním lékařem a např. pressure mapping [14].

Závěr

Operační léčba dekubitů zahrnuje nekrektomii, exstirpaci pseudocysty s vhodně zvolenou lalokovou plastikou a nutnost multioborové spolupráce (ortoped, chirurg, gynekolog, rehabilitační lékař, vhodně edukovaný nelékařský zdravotnický personál). Pečlivá příprava pacienta, využívání vhodné antidekubitní prevence v celém průběhu hospitalizace, ale i perioperačně významně snižují riziko komplikací a recidivy. Po vyčerpání operačních možností zůstávají i v dnešní době některé dekubity s ohledem na celkový stav pacienta inoperabilní.



Obr. 7. Rozsáhlý sakrogluteální dekubitus.
Fig. 7. Extensive sacrogluteral decubitus (pressure ulcer).

Literatura

1. European Press ure Ulcer Advisory Panel and National Pressure Ulcer Advisory Panel. Treatment of pres sure ulcers: Quick Reference Guide. Washington DC: National Pres sure Ulcer Advisory Panel 2009.

2. Pokorná A, Benešová K, Mužík J, et al. Sledování dekubitálních lézí u pacientů s neurologickým onemocněním – analýza Národního registru hospitalizovaných. Cesk Slov Neurol N 2016;79/112(Suppl 1):S8–14. doi: 10.14735/amcsnn2016514.

3. Haesler E, ed. National Pressure Ulcer Advisory Panel, European Pressure Ulcer Advisory Panel and Pan Pacific Pressure Injury Alliance. Prevention and Treatment of Pressure Ulcers: Quick Reference Guide. Perth, Australia: Cambridge Media 2014.

4. Stryja J. Význam a možnosti chirurgického débridementu dekubitů. Cesk Slov Neurol N 2016;79/112(Suppl 1): S25–7. doi: 10.14735/amcsnn2016525.

5. Hokynková A, Flamoli M, Černoch F. Operační léčba dekubitů. Geriatrie a Gerontologie 2014;3(3):123–7.

6. Siegel JD, Rhinehart E, Jackson M, et al, the Healthcare Infection Control Practices Advisory Committee Guideline for isolation precautions: preventing transmission of infectious agents in healthcare settings, June 2007. [online]. Available from URL: http://www.cdc.gov/ncidod/dhqp/pdf/guidelines/Isolation2007.pdf.

7. Riebeková V, Válka J, Franců M. Trendy soudobé chirurgie. Dekubity. Praha: Galén 2000.

8. Thiessen FE, Andrades P, Blondeel P, et al. Flap surgery for pressure sores: Should the underlying muscle be transferred or not? J Plast Reconstr Aesthet Surg 2011;64(1):84–90. doi: 10.1016/j.bjps.2010.03.049.

9. Bauer J, Phillips LG. MOC-PSSM CME article: Pressure sores. Plast Reconstr Surg 2008;121(Suppl 1):1–10. doi: 10.1097/01.prs.0000294671.05159.27.

10. Franců M, Hodová S, et al. Perioperační péče o pacienta v rekonstrukční chirurgii a léčbě popálenin, Brno: NCONZO 2011:125–32.

11. Hurbungs A, Ramkalawan H. Sacral pressure sore reconstruction: the pedicled superior gluteal artery perforator flap. S Afr J Surg 2012;50(1):6–8.

12. Lin H, Hou C, Chen A, et al. Long-term outcome of using posterior-thigh fasciocutaneous flaps for the treatment of ischial pressure sores. J Reconstr Microsurg 2010;26(6):355–8. doi: 10.1055/s-0030-1249318.

13. Bertheuil N, Huguier V, Allilet S, et al. Biceps femoris flap for closure of ischial pressure ulcers. Eur J Plast Surg 2013;6(10):639–44. doi: 10.1007/s00238-013-0862-z.

14. Vašíčková L, Sieglová J, Mašek M. Význam tlakové mapy (pressure mapping system) pro pacienty s mobilitou na vozíku. Cesk Slov Neurol N 2016;79/112(Suppl 1): S15–9. doi: 10.14735/amcsnn2016515.

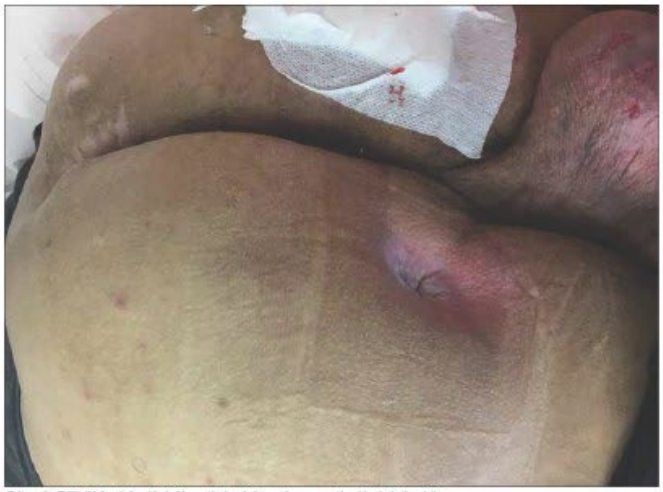
Tab. 1. Přehled lalokových plastik (sakrální dekubitus).

Podle typu tkáně	Podle typu posunu
fasciokutánní laloky	rotační gluteální FC lalok*
	VY posun jednostranný
	VY posun oboustranný
muskulokutánní laloky	m. gluteus maximus
	VY s m. gluteus maximus*
	s-GAP lalok (superior gluteal artery perforator flap)
	superior gluteal artery ostrůvkový lalok (island flap)

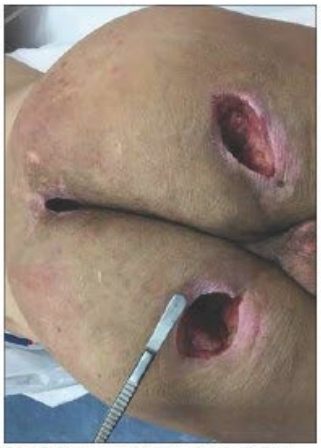
*Nejčastěji prováděn na pracovišti autorů.

teus maximus s cévní stopkou na a. glutea superior [11]. Přehled lalokových plastik k rekonstrukci sakrálního dekubitu uvádí tab. 1.

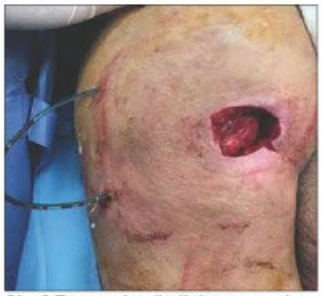
Ischiadické dekubity jsou nejčastější u pacientů připoutaných na invalidní vozík a velmi hojně se vyskytují ve formě píštělí. V takovém případě může být na kůži patrný defekt jen několik mm, jako je tomu u našeho pacienta (obr. 4, 5), ale v podkoží se zpravidla nachází několikacentimetrová kapsa (přičemž píštěle mohou komunikovat s jinými orgány). Z toho důvodu je nezbytné důsledné dovyšetření terénu defektu i okolní tkáně. Pokud je lytický tuber



Obr. 4. Píštěl ischiadického dekubitu vlevo, sakrální dekubitus.
Fig. 4. Fistula of the left ischidaic (sciatic) pressure ulcer, sacral decubitus (pressure ulcer).



Obr. 5. Stav po nekrektomii sakrálního a obou ischiadických dekubitů.
Fig. 5. Condition after necrectomy of sacrall and both sciatic decubitus (pressure ulcers).



Obr. 6. Transpozice distální porce m. gluteus maximus nad tuber ischiadicum.
Fig. 6. Transposition of the distal portion of gluteus maximus over the tuber ischiadicum.

Tab. 2. Přehled lalokových plastik (ischiadický dekubitus).

Podle typu tkáně	Podle typu posunu
fasciokutánní laloky	mediálně stopkovaný dorzální stehenní lalok (medial thigh flap)*
	laterálně stopkovaný dorzální stehenní lalok (lateral thigh flap)*
	dolní dorzální gluteální lalok i-GAP lalok (inferior gluteal artery perforator flap)
muskulokutánní laloky	Hamstring flap:* • dlouhá hlava m. biceps femoris • m. semitendinosus • m. semimembranosus
	MTFL (musculus tensor fasciae latae)
	m. gluteus maximus-pars inferior

*Nejčastěji prováděn na pracovišti autorů.

doi: 10.48095/cccsnn2022S12

Skin grafting in surgical treatment of pressure ulcers

Transplantace kůže v chirurgické léčbě dekubitů

The Editorial Board declares that the manuscript met the ICMJE "uniform requirements" for biomedical papers.
Redakční rada potvrzuje, že rukopis práce splnil ICMJE kritéria pro publikace zasílané do biomedicínských časopisů.

A. Hokynková, P. Šín, T. Adlerová, F. Černoch

Department of Burns and Plastic Surgery, Faculty of Medicine, Masaryk University and University Hospital Brno

✉
Petr Šín, MD, PhD.
Department of Burns and Plastic Surgery
Faculty of Medicine,
Masaryk University
and University Hospital
Jihlavská 20
625 00 Brno
e-mail: p.sin@seznam.cz

Summary
Optimal treatment of deep category of pressure ulcers/injuries (category III and IV) is represented by numerous surgical procedures accompanied with conservative therapy and preventative measures. Skin grafting represents a reconstructive option in plastic surgery with very specific indications in pressure ulcer's surgical therapy. The aim of this article is to clarify the indications for plastic surgery interventions and to describe possible disadvantages of skin grafting in pressure ulcers reconstruction. One of the specific indications is presented as case report of 89-years-old women with unstageable pressure ulcer in heel area that was surgically reconstructed by skin grafting.

Souhrn
Základem léčby hlubokých dekubitů (III. a IV. kategorie) jsou různé chirurgické techniky provázené konzervativní terapií a preventivními opatřeními. Transplantace kůže představuje jednu z možností v chirurgické léčbě dekubitů, má však své specifické indikace. Cílem tohoto článku je tyto indikace v rámci plastické chirurgie objasnit a popsat nevýhody transplantace kůže v rekonstrukci dekubitů. Jedna z těchto indikací je prezentována v kazuistice 89leté pacientky s neklasifikovatelným dekubitem paty, který byl chirurgicky rekonstruován pomocí transplantace kůže.

Key words
pressure ulcer – pressure injury – reconstructive surgical procedures – skin grafting – case report

Klíčová slova
decubitus – proleženina – chirurgické rekonstrukční techniky – transplantace kůže – kazuistika

Introduction
Optimal treatment of deep category of pressure ulcers (PUs)/injuries (category III and IV) is represented by numerous surgical procedures accompanied with conservative therapy and preventive care. Surgical treatment of these types of PUs usually consists of two steps: debridement and proper reconstructive surgery of the wound [1]. Generally, well known reconstructive ladder – wound closure from the easiest reconstructive technique to the most demanding one, is usually used in surgical coverage of most majority wounds in plastic surgery [2–5]. However, in such specific type of defects as the PUs really are, the reconstructive ladder is quite modified. Healing by secondary intention can be expected primarily in minor defects. It is applicable also in bigger defects, in case when surgical treatment is not recommended for some reasons (poor general health status, inability to undergo general anaesthesia etc.). In these cases, conservative therapy can lead to healing up of these defects, but it may take several months. Second stair in the reconstructive ladder is primary closure of the defects. Nevertheless, direct suture in PUs surgical therapy is recommended only in certain indications [6], especially in small sized defect. Disadvantage of this technique is represented by the fact that resulting scar is localised above the bony prominence. Connective tissue of the scar is less resistant against the pressure than other tissues (fascia, muscle etc.) According to our experience, there is a higher risk of dehiscence development [7] and recurrence of PUs using direct suture in comparison with reconstructions using flap closure technique. Tissue expansion [8] and free flap transfer are on the top of the reconstructive ladder techniques, but they are used in PUs reconstruc-

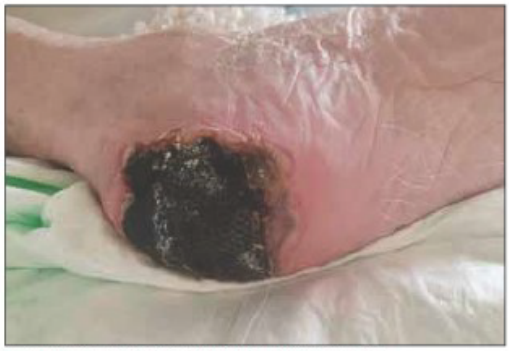


Fig. 1. Unstageable pressure ulcer in left heel area.
Obr. 1. Neklasifikovatelný dekubitus levé paty.

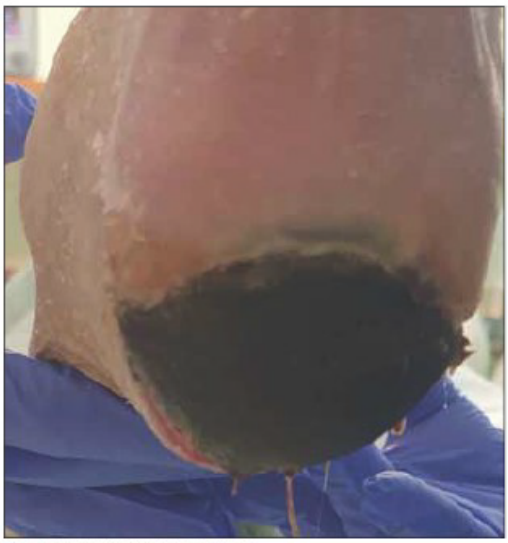


Fig. 2. Detailed picture of eschara.
Obr. 2. Detailní obraz eschary.

tive procedures only rarely. Therefore, fasciocutaneous or musculocutaneous flaps [9], that are widely used in surgical treatment of PUs, are rightly signed as a workhorse in reconstructions of Pus [10].

Skin grafting technique description and indications in PUs therapy

Third stair in reconstructive ladder in plastic surgery belongs to skin grafting (SG). This method is generally used in burns, extensive soft tissue defects, or specific defects localised in the head or another area. Skin grafting can be performed in a form of split-thickness skin graft (STSG) that contains the epidermis and a part of the dermis, or in a form of full-thickness skin graft (FTSG) containing the epidermis and the entire dermis. STSG is usually harvested by airdermatom from the ventral thigh area and then meshed in ratio of 1 : 1.5, 1.2, 1 : 3. FTSG is often harvested from the retroauricular, supraclavicular or groin area and is perforated only. FTSG is performed in areas where the scar contraction is expected or in reconstruction of head area. Fixation to the defects is performed by skin stapler or by non-resorbable filament. Basic healing up is around 7–10 days and continues with scar maturation. This method has some disadvantages, therefore its using in reconstruction of PUs is very restricted. PUs are classified as complicated healing wounds or non-healing wounds. These types of wounds are burdened almost always by polymicrobial wound bed colonisation, often with resistant strains (Methicillin resistant Staphylococcus aureus etc.). Skin grafting as a method of choice has to be taken in consideration only in case when the wound bed is minimally colonised by bacteria. Local bacterial infection leads to damage of skin graft. On the other hand, the donor site has to be protected from transposed infection from the wound bed of PUs. STSG is very thin (0.008–0.75mm) [11]) and unpliable and has to be placed on the shallow defects with well-granulating tissue refined of avital tissue and slough [12]. Skin grafting can be used only in category III, not in category IV of PUs, where bone is exposed or affected by lysis or osteomyelitis. Another disadvantage of this reconstructive method is the fragility of the skin graft. It must be considered that in patient suffering from PU, the main cause-pressure between bony prominences and the base, as well as friction shear, will continue. Therefore, SG is not recommended as a method of choice in PUs reconstruction. However, in certain indications this technique may be very useful. It includes reconstructions of small or moderate PUs in head area, incidence of which was rising up during COVID-19 pandemic, especially in patients ventilated in the prone position. Head area is well-vascularised, thus providing adequate wound bed for skin grafting. SG can be considered as a reconstructive option in medical-device-related PUs, especially in reconstruction of larger defects of mucosal membrane PUs [13], according to localisation, size or wound bed. Other indication is reconstruction of extensive soft tissue defects, for example positional trauma, where local flaps are insufficient for covering. Another possibility to use this technique is PUs localised in lower limb, especially in heel area, as described below in the case report.

Case report

Eighty-nine years old woman with severe comorbidities (hypertension, diabetes mellitus, post stroke syndrom, immobility etc.) was admitted for PU localised on the left heel, classified as hospital acquired PUs. The PUs was clinically expressed as black thick eschar on the surface with slushy smelling and movable base accompanied by marginal erythema of surrounding skin (Fig. 1, 2). The size of this unstageable PU was 8 × 7 cm with unknown depth. It was indicated for surgical treatment due to suspicion of local infection of soft tissue. The X-ray was performed to rule out osteomyelititis or osteolysis of calcaneus. Sharp debridement was performed in spinal anaesthesia with removing all the avital tissue and slough, including necrotic parts of plantar fascia and insertion of flexor digitorum brevis et longus muscle. After the surgery, PU was classified to a category IV – exposed, but stiff calcaneal bone in size 2 × 1 cm. Negative pressure wound therapy (NPWT) using hydrochlorohexidin dressing supplying foam was used to support growth of granulation tissue, especially above the exposed bone part (Fig. 3). Due to worsening of general health status (hypertension decompensation, infection in

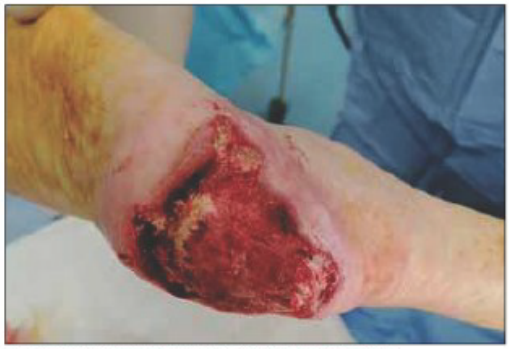


Fig. 3. Granulation tissue above the calcaneal bone.
Obr. 3. Granulační tkáň and patní kostí.

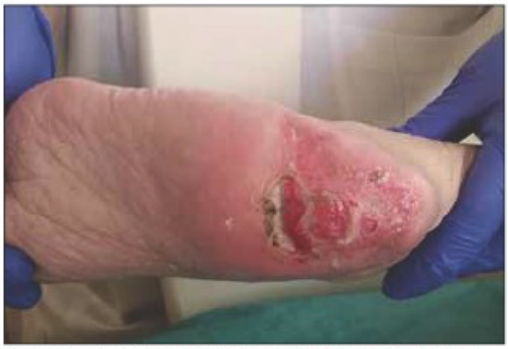


Fig. 4. The wound in left heel area – status 2 months after the split-thickness skin graft.
Obr. 4. Zbytkový defect v oblasti levé paty – 2 měsíce po rekonstrukci dermoepidermálním kožním štěpem.

the urinary tract and the inability to undergo general anaesthesia) reconstruction using flap reconstructive surgery was not indicated. Therefore, conservative therapy (wet dressing) was running with regularly monitoring of bacterial wound bed contamination (*Proteus mirabilis, Prevotella melanogenica, Enterobacter cloace* ESBL-extended spectrum beta-lactamase, *Pseudomonas aeruginosa*) and accurate antibiotics therapy for next 2 months. The granulation tissue in the wound bed grew up and covered exposed bone. Wound was microscopically sterile before reconstructive surgery. Due to general health status, SG in local anaesthesia was performed. STSG was harvested from ventral part of the left thigh, meshed in ratio 1 : 1.5 and fixed into the wound with bolus of gauze. Secondary local infection of the donor site occurred (and treated by conservative therapy) postoperatively. Skin graft was healed up in majority of the wound in 14 days, but two months after the surgery small rest defect remains (Fig. 4.) treated by conservative therapy. Diagnostic and treatment timeline is shown in the Tab. 1.

Conclusion

Skin grafting is not a method of choice in reconstruction of pressure ulcers, but it still has its own place in pressure ulcer's surgery treatment in specific indications.

Conflict of interest

The authors declare they have no potential conflicts of interest concerning drugs, products, or services used in the study.

Tab. 1. Diagnostic and treatment timeline (2021–2022).

Procedure	Timeline 2021–2022				
	September	October	November	December	January
admission	×				
SWAB	××	××	××	××	×
X-RAY	×				
NPWT	×	×			
debridement	×				
skingrafting			×		
nutritional support	×	×	×	×	×
preventive measures	×	×	×	×	×
discharge					×

* NPWT in continual regimen with negative pressure –125 mmHg was regularly changed every 4 days
NPWT – negative pressure wound therapy

References

1. Hokynková A, Šín P, Černoch F et al. Employment of flap surgery in pressure ulcers surgical treatment. Cesk Slov Neurol N 2017; 80 (Suppl): S41–S44. doi: 10.14735/amcsnn2017S41.
2. Wong CJ, Niranjan N. Reconstructive stages as an alternative to the reconstructive ladder. Plast Reconstr Surg 2008; 121(5): 362e–363e. doi: 10.1097/PRS.0b013e31816b1171.
3. Mardini S, Wei FC, Salgado CJ et al. Reconstruction of the reconstructive ladder. Plast Reconstr Surg 2005; 115(7): 2174. doi: 10.1097/01.prs.0000165497.92397.be.
4. Gottlieb LJ, Krieger LM. From the reconstructive ladder to the reconstructive elevator. Plast Reconstr Surg 1994; 93(7): 1503–1504. doi: 10.1097/00006534-199406000-00027.
5. Rubayi R, Wagenheim BR, Mcleland A. Reconstructive plastic surgery of pressure ulcers. Berlin: Springer 2015.
6. Bergstrom N, Bennett MA, Carlson CE et al. Treatment of pressure ulcers. Clinical practice guideline #15. Rockville, MD: US Dept of Health and Human Services', Public Health Service, Agency for Health Care Policy and Research, 1994.
7. Anthony JP, Huntsman WT, Mathes SJ. Changing trends in the management of pelvic pressure ulcers: a 12-year review. Decubitus 1992; 5(3): 44–51.
8. Esposito G, Di Caprio G, Ziccardi P et al. Tissue expansion in the treatment of pressure ulcers. Plast Reconstr Surg 1991; 87(3): 501–508. doi: 10.1097/00006534-199103000-00018.
9. Černoch F, Jelínková Z, Rotschein P. Reconstruction of recurrent ischiadic pressure ulcer using turnover hamstring muscle flap. Cesk Slov Neurol N 2019; 82 (Suppl): S15–S18. doi: 10.14735/amcsnn2019S15.
10. Chen YC, Huang EY, Lin PY. Comparison of gluteal perforator flaps and gluteal fasciocutaneous rotation flaps for reconstruction of sacral pressure sores. J Plast Reconstr Aesthet Surg 2014; 67(3): 377–382. doi: 10.1016/j.bjps.2013.12.029.
11. Gupta DK. Thin and ultra thin split thickness skin grafts (STSG-UT, STSG-T): In: Microskin grafting for vitiligo. London: Springer 2009: 15–18. doi: 10.1007/978-1-84882-605-2_5.
12. Sørensen JL, Jørgensen B, Gottrup F. Surgical treatment of pressure ulcers. Am J Surg 2004; 188 (1A Suppl): 42–51. doi: 10.1016/S0002-9610(03)00290-3.
13. Hess CT. Classification of pressure injuries. Adv Skin Wound Care 2020; 33(10): 558–559. doi: 10.1097/01.ASW.0000697324.90597.6d.

doi: 10.14735/amcsnn2019552

Multidisciplinary approach in surgical pressure ulcer therapy after spinal cord injury

Multidisciplinární přístup v chirurgické léčbě dekubitů u pacientů s míšní lézí

The authors declare they have no potential conflicts of interest concerning drugs, products, or services used in the study.
Autoři deklarují, že v souvislosti s předmětem studie nemají žádné komerční zájmy.
The Editorial Board declares that the manuscript met the ICMJE "uniform requirements" for biomedical papers.
Redakční rada potvrzuje, že rukopis práce splnil ICMJE kritéria pro publikace zasílané do biomedicínských časopisů.

P. Šín[1,2], J. Holoubek[1,2], A. Hokynková[1,2], B. Lipový[1,2]

[1] Department of Burns and Plastic Surgery, University Hospital Brno, Czech Republic
[2] Faculty of Medicine, Masaryk University, Brno, Czech Republic

Alica Hokynková, MD
Department of Burns and Plastic Surgery
University Hospital Brno
Jihlavská 20
625 00, Brno
Czech Republic
e-mail: alicah@post.cz

Accepted for review: 30. 6. 2019
Accepted for print: 4. 7. 2019

Abstract
Surgical treatment of extensive pressure ulcers in patients with spinal cord injury still represent a major challenge and often neglected part of care with which many experts is confronted daily. Treatment of these patients often requires extensive multidisciplinary approach across, not only, surgical disciplines. Close cooperation with a nutritionist, microbiologist and other surgical specialists is often the only way to successfully manage the problem. The aim of this publication is to briefly summarize the basic parameters in multidisciplinary collaboration in the treatment of large pressure ulcers in patients with spinal cord lesions. We then briefly demonstrate the above mentioned issues on a series of case reports resulting from our daily practice and then discuss the role of individual surgical specializations, the importance of nutritional support and the solution of infectious complications.

Souhrn
Chirurgická léčba rozsáhlých dekubitů u pacientů s traumatickou míšní lézí stále představuje velkou výzvu a často opomíjenou kapitolu, s níž je celá řada odborností denně konfrontována. Léčba pacientů často vyžaduje multidisciplinární spolupráci napříč nejen chirurgickými obory. Právě úzká spolupráce s výživovým specialistou, mikrobiologem a pracovníky z chirurgických oborů je mnohdy jedinou možností k úspěšné terapii. Cílem příspěvku je ve stručnosti shrnout základní parametry multidisciplinární spolupráce při léčbě rozsáhlých dekubitů u pacientů s míšní lézí. Výše zmíněný přehled v krátkosti demonstrujeme na sérii kazuistik z naší každodenní praxe. V diskuzi se zabýváme rolí jednotlivých chirurgických specializací, významem nutriční podpory a řešením infekčních komplikací.

Key words
pressure ulcers – spinal cord injury – infection – nutrition – multidisciplinary approach

Klíčová slova
dekubity – míšní poranění – infekce – výživa – multidisciplinární spolupráce

Introduction

Pressure ulcers after spinal cord injury (SCI) represent a difficult problem leading to repeated hospitalizations, multiple operations and potentially devastating complications. This problem presents a challenge for patients, nurses and doctors [1]. Patients with SCI and its associated comorbidities are among the highest risk population for developing pressure ulcers. The incidence of pressure ulcers in the SCI population is 25–66% [2,3].

In this publication we describe the need for extensive multidisciplinary approach in surgical treatment of pressure ulcers in SCI patients. In the individual chapters we describe the importance of nutritional support, microbiological surveillance and interdisciplinary cooperation within the surgical closure of the defect. We then demonstrate the discussed topics with several examples based on our clinical practice.

Case series

Case report 1

A 68-year-old paraplegic patient was admitted to our clinic in septic condition with an

extensive pressure ulcer in the sacral region (Fig. 1). Multiple necrectomy of the whole region was performed and intensive antibiotic therapy was introduced (further adjusted according to the results of cultivation and antibiotic sensitivity). Due to repeated contamination of the wound with stools (within closed distance of anus) and mainly the presence of the posterior wall of the rectum just below the bottom of the defect, it was decided that a sigmoidostomy would be created by a general surgeon. The condition was further complicated by the development of the ileus state, with the necessity of surgical revision due to rotation and malposition of intestinal loops. Derotation, desuflasion and re-suture were performed during revision surgery. Following the surgery, the post-operative period was uneventful. The closure of the pressure ulcer in such a stressful situation and under these conditions would be a high risk, especially in cases of wound healing in fields that have been re-operated several times. By this point, the patient was in malnutrition, hypoproteinemia and all his reserves were exhausted. Therefore, a conservative approach and intensive correction of all macro- and micronutrients was priority. Surgical closure of the wound was postponed for several weeks after the patient's complete realimentation. The final wound closure was performed one month later. The entire defect was covered by a large fasciocutaneous flap from the left half of the sacral/gluteal region (Fig. 2). The whole hospitalization period was managed under precise microbiological surveillance and targeted antibiotic therapy.

Case report 2

As our second case, we present a 43-year-old paraplegic man, who was admitted to our clinic with large infected pressure ulcers in the sacral and right trochanteric region (Fig. 3). Both defects were severely contaminated by multiresistant strains of *Pseudomonas aeruginosa* and *Acinetobacter baumanii*, which required prolonged and challenging antibiotic therapy with close cooperation with the Department of Clinical Microbiology. Preoperative examination and CT scans showed extensive osteomyelitis in the right proximal femur. In cooperation with an orthopedic surgeon, we performed radical resection of the proximal femur and subsequently radical necrectomy of both defects. At first, we decided to close the smaller and more superficial defect in the sacral region with a V-Y fasciocutaneous flap. In the trochanteric region, the defect was large and deep so we decided to apply NPWT (Negative Pressure Wound Therapy) for promoting granulation and remediation of infection. We sustained the vacuum assisted closure therapy for several weeks, together with intense nutrition support. Finally, after 1 month we could close the defect with a large transposition flap (Fig. 4). Further healing and the post-operative period were without any complication.

Discussion

University Hospital Brno provides one of four SCI in the Czech Republic. As a part of this service, a multidisciplinary surgical team was established for the surgical management of pressure ulcers arising in this patient group. Despite the growing possibilities of conservative therapy, the extensive pressure ulcers of grades III and IV can only be closed by surgical excision and reconstruction. Due to the fact that such an intervention is generally an elective surgical procedure, patient's overall condition and chronic medical comorbidities (e.g. diabetes mellitus, hypertension, malnutrition, and anemia) have to be compensate by the multidisciplinary team before a patient is considered a good candidate for surgery [1]. If the ulcer or ulcers are in close proximity to the anus, clinical judgment should address the need for bowel diversion by colostomy. The presence of stool significantly compromises wound healing and makes its toilet virtually impossible. Furthermore, the close proximity of the rectum to the defect may complicate the surgical procedure and its possible perforation may result in fatal complications [4]. The same applies to the perineal region – the creation of a temporary or permanent urinary diversion should be considered here. The presence of an orthopedic surgeon is often required due to osteomyelitis, which will also have a significant impact on further surgical plans. Often, a radical osteotomy is required before final wound closure [5]. Only after proper radical surgical debridement, including of bone structures and conceiving a proper surgical field, the defect can be closed by a plastic surgeon. Surgical management of pressure ulcer closure involves

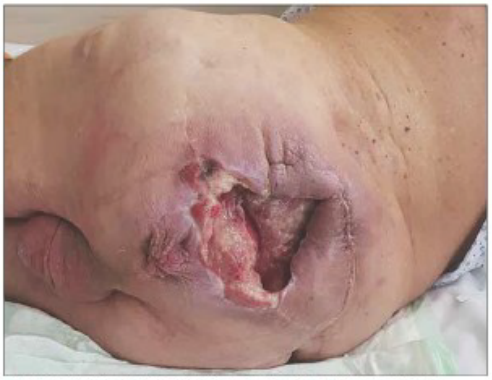


Fig. 1. Large and deep pressure ulcer in whole sacral region.
Obr. 1. Rozsáhlý dekubitus postihující prakticky celou oblast sakra a hýždí.

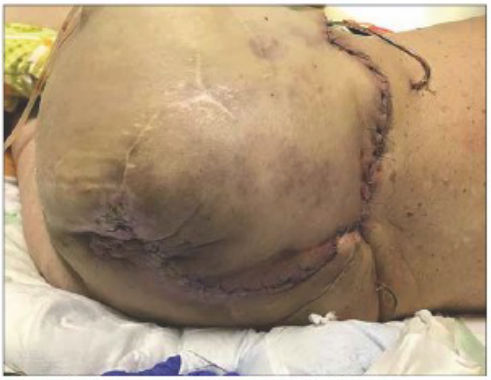


Fig. 2. Defect after closure with large transposition flap.
Obr. 2. Defekt po definitivním uzávěru transpozičním lalokem.

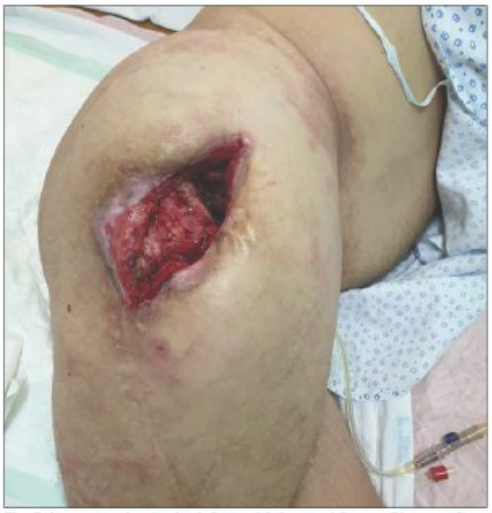


Fig. 3. Large trochanteric defect with luxated femur. Picture after osteotomy of proximal femur, including caput femoris.

Obr. 3. Rozsáhlý trochanterický dekubitus s dominující luxovanou kyčlí. Obrázek již po provedené osteotomii hlavy a proximální části femuru.

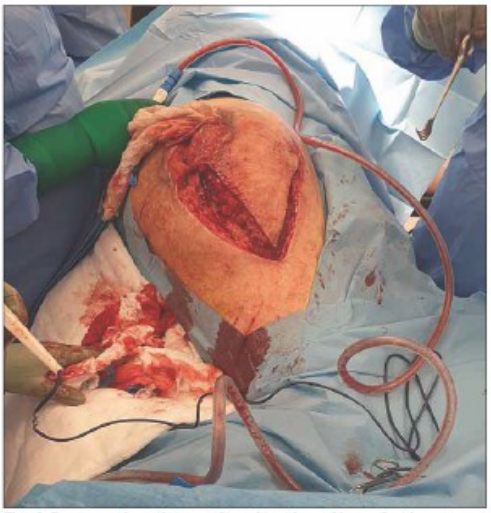


Fig. 4. Peroperation picture after elevation of large fasciocutaneous flap for closure of trochanteric defect.

Obr. 4. Peroperační snímek elevace fasciokutánního laloku k uzávěru trochanterického dekubitu.

a spectrum of options, from simple debridement with direct closure, to skin grafting, fasciocutaneous flaps, myocutaneous flaps or even free flaps [1]. The choice of flap reconstruction always depends on a number of factors such as anatomical location, reoperated terrain, general condition and surgeon's preference [4]. Considering the spectrum of patients with often neglected chronic wounds and a very poor overall condition, it is clear that the resulting surgical effect is burdened with a corresponding percentage of complications and recurrences. Scientific sources show different percentages of complications. For example, Sameem et al reports complications ranging from 9–20%, depending on the type of flap chosen. The most frequent complications in their study were flap necrosis (partial), wound dehiscence, infection and recurrence of pressure ulcer [6]. The ideal nutritional intake continues to be discussed to this day. Energy, protein, arginine, and micronutrients are all essential for proper wound healing. Proteins play the most important role in the healing process and are essential in the fibroblast proliferation process, angiogenesis, collagen synthesis and overall in tissue reparation [7]. In 2009, National Pressure Ulcer Advisory Panel introduced general guidelines for the treatment and prevention of pressure ulcers, these were updated in 2014 [8]. For pressure ulcer stage III/IV, recommended protein intake goes up to 2 g/kg. The key role of protein intake is well demonstrated in a study presented by Crowe et al [9]. The group receiving higher protein (1.8 g protein per kg body weight) demonstrated nearly a two-fold greater rate of healing than those randomized to lower protein intake (1.2 g protein per kg body weight). The role of carbohydrates, fats and micronutrients is equally important. To prevent protein-energy malnutrition and improve wound healing, the diet should be adequate in energy in the form of carbohydrates, fat and protein. Infection of a pressure ulcer may result in soft tissue and bone infections: cellulitis, abscess formation, bursitis, and osteomyelitis of bone underlying the wound bed [10]. In SCI patients, it is one of the most common causes of bacteremia [11]. Control of wound colonization and infection should always be one of the biggest priorities in treatment protocol [1]. Precise microbiological surveillance should be strictly conducted in all patients, along with targeted antibiotic administration based on sensitivity. This is the only way to prevent an increase in antibiotic resistance and at the same time to successfully eradicate pathogens in an effort to prevent further dissemination [12].

Conclusion

Surgical treatment of pressure ulcers in patients after SCI is often a very complicated process involving many aspects of surgery. Today, correct and effective treatment is possible due to large collaboration in a multidisciplinary team. It is the presence of a diverse range of experts, not only from the surgical field, that has contributed to the development in this often neglected issue.

References

1. Kruger EA, Pires M, Ngann Y et al. Comprehensive management of pressure ulcers in spinal cord injury: current concepts and future trends. J Spinal Cord Med 2013; 36(6): 572–585. doi: 10.1179/2045772313Y.0000000093.

2. Fuhrer M, Garber S, Rintala D et al. Pressure ulcers in community-resident persons with spinal cord injury: prevalence and risk factors. Arch Phys Med Rehabil 1993; 74(11): 1172–1177.

3. Regan M, Teasell R, Dalton L et al. A systematic review of therapeutic interventions for pressure ulcers after spinal cord injury. Arch Phys Med Rehabil 2009; 90(2): 213–231. doi: 10.1016/j.apmr.2008.08.212.

4. Rubayi S, Chandrasekhar B. Trunk, abdomen, and pressure sore reconstruction. Plast Reconstr Surg 2011; 128(3): 201e–215e. doi: 10.1097/PRS.0b013e31822214c1.
5. Thomson C, Choudry M, White C et al. Multi-disciplinary management of complex pressure sore reconstruction: 5-year review of experience in a spinal injuries centre. Ann R Coll Surg Engl 2017; 99(2): 169–174. doi: 10.1308/rcsann.2016.0227.
6. Sameem M, Au M, Wood T et al. A systematic review of complication and recurrence rates of musculocutaneous, fasciocutaneous, and perforator-based flaps for treatment of pressure sores. Plast Reconstr Surg 2012; 130(1): 67e–77e. doi: 10.1097/PRS.0b013e318254b19f.
7. Antalová N, Pokorná A, Hokynková A et al. Factors influencing recurrence of the pressure ulcers after plastic surgery – retrospective analysis. Cesk Slov Neurol N 2018; 81/114: S23. doi: 10.14735/amcsnn2018S23.
8. Dunk A, Carville K. The international clinical practice guideline for prevention and treatment of pressure ulcers/injuries. J Adv Nurs 2016; 72(2): 243–244. doi: 10.1111/jan.12614.
9. Crowe T, Brockbank C. Nutrition therapy in the prevention and treatment of pressure ulcers. Wound Practice 2009; 17(2): 90–98.
10. Dana A, Bauman W. Bacteriology of pressure ulcers in individuals with spinal cord injury: what we know and what we should know. J Spinal Cord Med 2015; 38(2): 147–160. doi: 10.1179/2045772314Y.0000000234.
11. Wall B, Mangold T, Huch K et al. Bacteremia in the chronic spinal cord injury population: risk factors for mortality. J Spinal Cord Med 2003; 26(3): 248–253. doi: 10.1080/10790268.2003.11753692.
12. Salcido R, Popescu A, Potter A et al. Pressure ulcers and wound care. [online]. Available from URL: http://www.emedicine.medscape.com/article/31984-overview.

2. NUTRITIONAL PERSPECTIVE

Nutrition is an essential component in the prevention and management of PUs, since it has a significant impact on the wound healing process and overall patient outcomes. As a result of the fact that malnutrition is a well-documented risk factor for the development and duration of PUs, nutritional screening and correction are essential components of comprehensive therapy. Nutrition is a complex and wide-ranging topic that should be handled by nutritionists. The purpose of this chapter is to discuss the fundamental parts of the issue of PUs and nutrition through the eyes of a plastic surgeon in clinical practice. This chapter focuses on PUs in critically ill patients as well as chronic PUs caused by long-term movement disability, typically as a result of a spinal cord injury or disorder.

2.1 Pressure ulcer surgical management in critically ill patients

PU prevalence has been found to be around 25% among adult patients in intensive care units (ICUs) across the world, with 60% of these cases being acquired in the ICU. [95] It has been shown that the prevalence of malnutrition in critically ill patients can vary anywhere from 38 to 78 %. [96] The general opinion is that people with a duration of stay of more than 48 hours are at risk of malnutrition and should be referred for nutrition assessment and management. [97,98] A few studies refer to the level of BMI (body mass index) in relation to the development of PUs. The highest risk is associated with underweight patients (BMI <18.5 kg/m2) and in obese patients (BMI ≥ 30 kg/m2) and the lowest risk is for overweight patients (BMI up to 27.5 kg/m2). [99]

Skin failure is a risk factor for critically ill patients, particularly those who have spent an extended period in the ICU, because of prolonged immobility, mechanical ventilation, acute respiratory distress syndrome, COVID-19, sepsis, multiorgan system dysfunction, vasopressor use, and treatment with extracorporeal membrane oxygenation. The compounded factors of circulatory collapse, metabolite accumulation, compromised lymphatic drainage, patient comorbidities, and ischaemia via capillary blockage in critically ill patients result in poor perfusion leading to skin disintegration. [100] Surgical management of PUs in critically ill patients must be carefully considered, not only in relation to the patient's overall health, but also in relation to the next local wound healing and/or planned surgical reconstruction. According to our clinical experience, debridement performed in the deep category of PUs often leads to deepening of necrosis in patients with severe

malnutrition. The exception is case of "septic" PU, in which a sepsis has the origin in PU. These PUs frequently present with a clinical appearance of firm necrosis (unstageable category of PU), accompanied by a cavity filled with pus beneath the necrotic tissue that requires surgical intervention. The result of study Küçükdemirci et al. shows that critically ill patients with PUs are more likely to get blood stream infections if they have low albumin levels, kidney failure, and stay in the ICU for a long time during sepsis events. [101]

In other cases, it is beneficial to continue with conservative local wound therapy and with complex therapy in critically ill patient, including preventive measures and positioning of the patients and nutritional assessment, evaluation of malnutrition and tailored nutritional treatment. Surgical therapy is planned after stabilisation of the patient's general health and nutritional status, i.e., in the phase of transition from catabolism to anabolism. This can eliminate postoperative complications, as described in the subchapter *1.7.3.3 „Post-surgical complications ".*

2.2 Pressure ulcer surgical management in long-term pressure ulcers

Long-term PUs of different categories or multiple PUs are very common in patients with reduced mobility and are associated with varying degrees of malnutrition. Acute and chronic infections, wounds, and hypermetabolic states significantly elevate the body's requirements for energy and protein. PUs imposes a significant burden on patients, presenting as chronic wounds often accompanied by a persistent state of inflammation. This inflammatory response not only exacerbates tissue damage but also contributes to the deterioration of nutritional status, leading to malnutrition. The local condition of the wound also contributes to malnutrition. High amounts of exudation, especially in people with long-term PUs, can also cause a lot of protein loss and put the patient at risk for an imbalance of fluids and electrolytes. It is thought that a person with a PU of category IV (i.e., a pressure injury with full thickness tissue loss and exposed bone, tendon, or muscle) could lose 90–100 g of protein every day in fluid. However, many people get less than this amount of protein every day. [102] Malnutrition further impairs the healing of PUs by delaying tissue repair and regeneration while weakening the body's immune defence, increasing susceptibility to infections. [103,104] All above mentioned factors perpetuate a vicious cycle, where poor healing and recurrent infections worsen the patient's overall condition. In such

complex and challenging cases, surgical intervention may serve as the final solution to break this cycle, enabling effective wound closure and restoring the patient's quality of life. Collagen synthesis/deposition and epithelial resurfacing are essential for successful healing, and both procedures heavily rely on sufficient nutritional storage and support. Vitamins, minerals, proteins, carbs, and trace elements are all important in these processes. [105] The Wound Healing Society (WHS) guidelines for the treatment of pressure ulcers-2023 update emphasize the need for nutritional examination and establishing parameters for the diagnosis of malnutrition in patients already in the outpatient setting: *„Assessment of pre-albumin level (reflecting recent protein consumption) and serum albumin level (reflecting long-term protein consumption) is useful to identify outpatient ambulatory patients who are malnourished. These markers are not very effective in hospitalised or ill patients where levels of serum albumin and pre-albumin are influenced by hydration status, presence of infection, or acute stress and thus may not reflect actual nutritional deficit. No individual clinical parameter accurately and consistently identifies adult malnutrition. The Academy of Nutrition and Dietetics and the American Society for Parenteral and Enteral Nutrition suggest that presence of two or more of the following six parameters are strongly diagnostic of malnutrition: insufficient energy intake, weight loss, loss of muscle mass, loss of subcutaneous fat, localised or generalised fluid accumulation that may mask weight loss, and decreased functional status measured by hand grip. Other laboratory values associated with inflammation such as C-reactive protein, white blood cell count or blood glucose may be helpful in determining if malnutrition is related to starvation, chronic disease or acute disease/injury."* [105]

Proper nutrition not only supplies the body with essential nutrients and energy but also supports cell metabolism, enhances tissue repair, accelerates wound healing, and lowers the risk of infection. [106,107] The negative impact of malnutrition on pressure ulcer healing can be seen clinically in the basic characteristics of the wound. It can lead to stagnation of wound healing or deepening of the wound, as we can assess in the daily monitoring of wound status using TIMERS aspects (detailed in subchapter *1.7.2 „Promoting Wound Healing, Conservative Therapy"*). These negative aspects of the wound healing are correlated with lower levels of pre-albumin, albumin, and total protein in the blood; grey or more fragile granulation tissue; no vital signs at the wound surface after debridement as opposed to pink granulation or edge re-epithelialisation of the wound as the levels of these

nutritional parameters increase. Performing the reconstructive procedure in patients with low nutritional factors significantly increases the risk of the postoperative complications, such as wound dehiscence, partial or complete flap necrosis, floating flap plasty, or PU recurrence at the reconstruction site. Therefore, it is essential for the general practitioner, referring physician, or nutritionist perform a nutritional evaluation and therapy prior to elective PUs surgery, preferably during the preoperative phase. All these findings led us to create our internal guidelines used in the Department of Burns and Plastic Surgery with the establishment of certain levels of haematological, biochemical, and nutritional parameters to allow PU surgery (not applicable to septic PUs): *haemoglobin >100 g/L, CRP <50mG/L, total protein >60 g/L, albumin >30 g/L, prealbumin>0.15 g/L.* This and an additional pre-operative evaluation are detailed in *the "Guide to the Surgical Treatment of Pressure ulcers. Selected advice for patients, physicians, medical staff and non-professional caregivers" ("Průvodce chirurgickou léčbou dekubitů. Vybrané rady pro pacienty, lékaře, zdravotnický personál a laické pečovatele "),* see *Appendix 1.* Using only serum visceral protein levels, such as albumin and prealbumin, to assess nutritional status in patients with existing inflammation can be misleading. Pro-inflammatory cytokines shift hepatic protein synthesis toward acute-phase reactants, reducing albumin and prealbumin production. Hypoalbuminemia, therefore, cannot be considered a reliable marker of malnutrition in acute inflamed states or in persistent inflammation, as seen in chronic critical illness. [108] During the hospitalization, we set the following internal guidelines: recording weight, height, BMI, and regular monitoring of nutritional parameters twice a week (total protein, albumin, prealbumin, extended iontogram, urea, creatinine, glucose, orosomucoids will be considered for the future, etc.). Nutritional therapist then calculates the patient's daily caloric intake, assesses his nutritional intake status according to the levels of nutritional parameters. Then, setting of high protein diet, sipping, protein supplementation, etc. is done by surgeon. In case of severe degrees of malnutrition, consultation of a nutritionist with setting of enteral and parenteral individual therapy is performed. Adequate protein intake is essential for wound healing, as it supports tissue repair and regeneration. Providing a balanced supply of calories, protein, vitamins, and trace elements helps patients manage the postoperative stress response more effectively, accelerates tissue repair and healing, and minimizes the risk of complications. [109,110] Therefore, surgical treatment (except „septic" PU) and especially reconstructive surgery are planned after

stabilization of nutritional parameters to eliminate the risk of postoperative complications. Continued nutritional support plays a vital role in the postoperative recovery phase, helping to enhance patient outcomes. Beyond promoting faster wound healing, sustained nutritional support strengthens the immune system and reduces the risk of infections, underscoring its importance throughout the recovery process. [111,112] Stabilization of nutritional parameters can also lead to healing up of superficial or small deep category of PUs.

2.3 Summary

The lack of established guidelines for timing surgical management of pressure ulcers based on nutritional status or objectively measured nutritional parameters represents a significant gap in clinical practice. Malnutrition is a well-recognized factor that impairs wound healing and increases the risk of postoperative complications, yet there are no universally accepted protocols defining when a patient is nutritionally optimized for surgery. This omission hinders the standardization of care and the ability to achieve optimal surgical outcomes.

The current literature provides little guidance in this regard. Reports of randomized clinical trials addressing the surgical treatment of PUs are virtually non-existent. [105] A Cochrane review from 2016 identified no published or unpublished reports of randomized clinical trials, nor any registered studies investigating the role of reconstructive surgery in PU management. [113] This lack of evidence underscores the pressing need for comprehensive research to establish evidence-based guidelines.

To address this critical need, our institution has developed internal guidelines grounded in years of clinical experience. These guidelines provide indicative thresholds for key nutritional parameters, including prealbumin, albumin, and total protein levels, serving as essential tools to assess a patient's readiness for surgical intervention. While these thresholds act as foundational benchmarks, they are not definitive criteria and should be interpreted in conjunction with the broader clinical picture of each patient.

The primary aim of these internal protocols is to ensure that patients undergoing surgical treatment are nutritionally stabilized, thus minimizing the risk of complications such as wound dehiscence, flap necrosis, or pressure ulcer recurrence. However, we recognize that

these guidelines are based on observational data rather than robust statistical analysis and they should be viewed as a practical framework rather than a universally applicable standard.

Further efforts are necessary to refine these parameters and validate them through evidence-based studies. By integrating nutritional optimization into preoperative planning, clinicians can break the vicious cycle of malnutrition, chronic inflammation, and delayed healing, paving the way for successful surgical interventions and improved patient quality of life.

In the scientific exploration of the relationship between nutrition and wound healing, chronic wounds like pressure ulcers present unique challenges, particularly in experimental models. Developing an animal model that fully replicates the complexity of chronic wounds, including the prolonged inflammatory response and multifaceted healing obstacles, is nearly impossible. This limitation has led us to focus on acute wound models to study the fundamental mechanisms of wound healing and the effects of nutritional interventions.

As part of our research efforts, we conducted a study titled *Fatty Acid Supplementation Affects Skin Wound Healing in a Rat Model*, published in *Nutrients*. This work investigated the impact of dietary fatty acid supplementation on acute wound healing using a rat model. By manipulating the ratios of n-3 and n-6 polyunsaturated fatty acids in the diet, we aimed to elucidate their roles in modulating inflammation, tissue repair, and overall healing processes. The results provided valuable insights into how dietary interventions could influence wound healing, laying a foundation for understanding how similar strategies might be applied to chronic wounds in clinical settings.

While findings from acute wound models cannot be directly extrapolated to chronic wounds like PUs, they provide a crucial starting point for developing targeted nutritional therapies. These models allow us to explore the biochemical and cellular pathways involved in wound healing and evaluate the potential of specific nutrients to enhance these processes. Building on these foundational studies, future research can aim to translate these insights into practical clinical applications for improving the management of hard-to-heal wounds.

2.4 Articles related to this issue

Hokynková, A., Wilhelm, Z., Nováková, M., Babula, P., Stračina, T., Paulová, H., Hlaváčová, M., & Sedláčková, M. (2018). Wound healing effects after application of polyunsaturated fatty acids in rat. *Česká a slovenská neurologie a neurochirurgie*, Vol. 81/114 (Suppl 1), p. 21-31.

Hokynková, A., Nováková, M., Babula, P., Sedláčková, M., Paulová, H., Hlaváčová, M., Charwátová, D., & Stračina, T. (2022). Fatty acid supplementation affects skin wound healing in a rat model. *Nutrients*, *14*(11), 2245.

doi: 10.14735/amcsnn2018529

Změny v kvalitě hojení ran po podání nenasycených mastných kyselin u potkana

Wound healing effects after application of polyunsaturated fatty acids in rat

Souhrn

Úvod: Ačkoli mechanizmus vlivu nenasycených mastných kyselin na hojení ran nebyl doposud zcela objasněn, je jisté, že nutriční stav pacienta hojení kožní rány zásadně ovlivňuje. *Cíl:* Cílem bylo zjistit, jaký vliv na rychlost a kvalitu hojení kožní rány bude mít krátkodobé podání omega-3 a omega-6 nenasycených mastných kyselin. *Materiál a metodika:* Mladým potkanům kmene Wistar bylo orogastrickou sondou podáváno buď přesně definované množství 20% tukových emulzí (experimentální skupiny E a F s různým poměrem omega-3 : omega-6 : omega-9 nenasycených mastných kyselin) nebo vody (skupina C, kontrolní). Po týdnu aplikace byla zvířatům na hřbetu vytvořena kožní rána a týden bylo pokračováno s dietou. Při ukončení pokusu byla odebrána krev a vzorky rány. Sledovány byly změny v plazmatických hladinách mastných kyselin, 4-hydroxy-2-nonenalu a míra oxidačního stresu jak v plazmě, tak v ráně. Rychlost a kvalita hojení byly posouzeny pomocí digitální planimetrie a histologicky. Výsledky byly statisticky zpracovány neparametrickými testy. *Výsledky:* Byly zjištěny signifikantní změny u zvířat v experimentálních skupinách v hladinách mastných kyselin v plazmě a dále vyšší schopnost tkáně a plazmy vyrovnat se s oxidačním stresem. *Závěr:* Krátkodobé podání určitého poměru nenasycených mastných kyselin má pozitivní vliv na hojení ran.

Abstract

Introduction: Although mechanisms of polyunsaturated fatty acids influence on skin wound healing have not been fully elucidated yet, it is undisputable that nutritional state affects it profoundly. *Aim:* The study was focused on speed and quality of wound healing influenced by short-lasting oral administration of omega-3 and omega-6 polyunsaturated fatty acids. *Materials and methods:* Young Wistar rats received by orogastric tube either 20% fat emulsion (experimental groups E and F differed by ratio between omega-3 : omega-6 : omega-9 polyunsaturated fatty acids) or water (control group C). After 7 days, skin wound on back was performed and emulsion or water application continued for another week. Blood and tissue samples were obtained during experiment termination. Plasmatic levels of fatty acids, 4-hydroxy-2-nonenal and oxidative stress both in plasma and in wound tissue were examined. Speed and quality of wound healing were assessed by digital planimetry and histological examination. Results were statistically evaluated by non-parametric tests. *Results:* Significant changes of fatty acids plasmatic levels were observed in animals in experimental groups as well as better ability of their plasma and tissues to cope with oxidative stress. *Conclusion:* Short-lasting administration of certain ratio of polyunsaturated fatty acids positively affects skin wound healing.

Poděkování

Autoři děkují p. Jaroslavu Nádeníčkovi za technickou pomoc. Tato studie byla součástí projektu Masarykovy Univerzity „Kardiovaskulární systém napříč obory – od molekulární diagnostiky po klinická vyšetření" MUNI/A/1157/2017 a projektu „Studium molekulární podstaty vybraných patologických stavů a onemocnění" MUNI/A/0910/2017 s podporou Specifického výzkumného univerzitního grantu poskytovaného MŠMT ČR v roce 2018.

Autoři deklarují, že v souvislosti s předmětem studie nemají žádné komerční zájmy.
The authors declare they have no potential conflicts of interest concerning drugs, products, or services used in the study.
Redakční rada potvrzuje, že rukopis práce splnil ICMJE kritéria pro publikace zasílané do biomedicínských časopisů.
The Editorial Board declares that the manuscript met the ICMJE "uniform requirements" for biomedical papers.

A. Hokynková[1], † Z. Wilhelm[2], M. Nováková[2], P. Babula[2], T. Stračina[2], H. Paulová[3], M. Hlaváčová[3], M. Sedláčková[4]

[1] Klinika popálenin a plastické chirurgie LF MU a FN Brno
[2] Ústav fyziologie, LF MU a FN Brno
[3] Ústav biochemie, LF MU a FN Brno
[4] Ústav histologie a embryologie, LF MU a FN Brno

✉ **Alica Hokynková, MD**
Klinika popálenin a plastické chirurgie LF MU a FN Brno
Jihlavská 20
625 00 Brno
e-mail: alicah@post.cz

Přijato k recenzi: 26. 6. 2018
Přijato do tisku: 14. 8. 2018

Klíčová slova

nenasycené mastné kyseliny – hojení ran – antioxidační kapacita – 4-HNE – 4-hydroxy-2-nonenal

Key words

polyunsaturated fatty acids – wound healing – anti-oxidative capacity – 4-HNE – 4-hydroxy-2-nonenal

Úvod

Přestože se s problematikou hojení ran setkáváme v chirurgické praxi denně, incidence a prevalence ran a hojivých procesů není dobře standardizována a monitorována a existuje jen málo celostátních dat týkajících se péče o rány. Hojení ran jak akutních, tak chronických (dekubity, syndrom diabetické nohy, ulcus cruris, atd.), rány po plánovaných operacích i speciální typy ran, jako jsou popáleniny, představují velkou ekonomickou zátěž zdravotního systému, a to zejména u hospitalizovaných pacientů [1].

Rychlost a kvalita hojení kožní rány závisí nejen na velikosti, hloubce a typu rány, ale také na komorbiditách pacienta, věku a jeho compliance. Nutriční stav by měl být jedním ze sledovaných parametrů při evaluaci pacienta s jakýmkoli typem rány. Jednotlivé složky výživy – bílkoviny [2,3], vitaminy [4], stopové prvky a mastné kyseliny (MK) [5,6] již byly předmětem řady studií zaměřených na hojení ran, nicméně ovlivnění rychlosti a kvality hojení kožní rány po podání nenasycených MK nebylo prozatím zcela objasněno.

Cílem prezentované experimentální studie bylo zjistit, zda se krátkodobé podání omega-3 a omega-6 nenasycených MK v různém poměru odrazí v kvalitě hojení kožní rány u laboratorního potkana.

Materiál a metodika

Experiment byl prováděn v souladu s doporučením European Community Guide for the Care and Use of Laboratory Animals, projekt pokusů byl schválen Komisí pro ochranu zvířat Masarykovy Univerzity a komisí Ministerstva zemědělství ČR.

Do experimentu bylo zařazeno 30 potkanů kmene Wistar (stáří 3 týdny, hmotnost 160,4 ± 12,64 g). Zvířata byla náhodně rozdělena do 3 skupin (n = 10), kontrolní skupina C, experimentální skupiny E a F. Po týdenní přípravě (návyk na úchop rukou a aplikace sondou) byla zvířatům další týden (preoperační fáze) 1× denně pomocí orogastrické sondy aplikována buď 20% tuková emulze v dávce 0,2 g/kg (v poměru omega-3 : omega-6 : omega-9 MK 1 : 1 : 1,5 ve skupině E a v poměru 3 : 1 : 5 ve skupině F) nebo aqua pro injectione za stejných podmínek o stejném objemu skupině kontrolní. Poté byla zvířatům v i.p. anestezii ketaminem (100 mg/kg) a xylazinem (10 mg/kg) provedena kruhovitá excize kůže o průměru 2 cm v plné tloušťce na hřbetu a ponechána k hojení per secundam intentionem. V průběhu dalšího týdne (pooperační fáze) bylo pokračováno v aplikaci speciální diety. Experiment byl ukončen 21. den, kdy byla zvířatům v celkové i.p. anestezii odebrána krev pomocí intrakardiální punkce a vzorky tkáně z oblasti rány.

Z krve byl stanoven krevní obraz, hladiny MK v plazmě ve fosfolipidové i triacylglycerolové frakci, hladina 4-hydroxy-2-nonenalu (4-HNE), parametry oxidačního stresu a antioxidační kapacity plazmy. Vzorky tkáně byly hodnoceny pomocí elektronové a fluorescenční mikroskopie. Makroskopicky byla rychlost hojení a kontrakce rány hodnocena digitální planimetrií. Výsledky byly statisticky zpracovány neparametrickou statistickou analýzou (C vs. E vs. F, Kruskal-Wallisův test, následovaný vícečetným srovnáním – Mann-Whitneyho post hoc test). Výsledky jsou prezentovány jako medián (dolní a horní kvartil) na hladině významnosti $p < 0,05$.

Výsledky

V základním panelu hematologické analýzy nebyly zjištěny žádné rozdíly mezi skupinami. Dvoutýdenní aplikace tukových emulzí vedla ke změnám hladin MK ve fosfolipidové i triacylglycerolové frakci: celkové množství omega-3 ani omega-6 MK sice nebylo signifikantně změněno, ale ve skupině omega-3 sledovaných MK se blížilo signifikanci (Kruskal-Wallis; $p = 0,061$). Detailní analýza pak odhalila signifikantní změny uvnitř skupin omega-3 (jedna kyselina ze čtyř sledovaných omega-3) a omega-6 (dvě ze šesti sledovaných omega-6) MK v obou frakcích (fosfolipidové i tracylglycerolové) plazmy. V plazmatických hladinách 4-HNE nebyly zjištěny signifikantní rozdíly mezi skupinami. Analýza dalších parametrů oxidačního stresu odhalila signifikantní snížení hydrogen peroxidu a poměru nitrátů/nitritů ve skupině E a F, přičemž nejnižší hodnoty byly naměřeny ve skupině F. Produkce reaktivních forem nitrogenů a nitrátů (reactive nitric species; RNS) se signifikantně zvýšila ve skupině E a F s nejvyšší naměřenou hodnotou opět ve skupině F. Podávání tukových emulzí naopak neovlivnilo produkci reaktivních forem kyslíku (reactive oxygen species; ROS), RNS a celkovou antioxidační kapacitu.

Parametry oxidačního stresu stanovené fluorescenční mikroskopií korelovaly s výsledky z plazmy. Distribuce ROS v experimentálních skupinách E a F byla difuzní, na rozdíl od dobře ohraničené lokalizace u skupiny C. Dobře zabarvená jádra jsou důkazem min. interference a přítomnosti artefaktů. Barvení na RNS ukázalo jejich zvýšené množství ve skupinách E a F s difuzním charakterem distribuce. Tyto výsledky jsou podpořeny vizualizací produktů lipidové peroxidace. V nálezech elektronové mikroskopie dominovaly změny v zastoupení buněčných elementů v experimentálních skupinách E a F ve srovnání se skupinou kontrolní, a to v poměrném nižším zastoupení fibroblastů, vyšší produkci makrofágů a vyšším počtu kolagenních vláken organizovaných do větších shluků. Makroskopická analýza hojení ran digitální planimetrií odhalila větší stupeň kontrakce rány ve skupině C (57,9 %) než v experimentálních skupinách F (46,3 %) a E (32 %).

Diskuze

Hojení kožní rány prochází třemi základními fázemi, které se vzájemně prolínají: 1. zánět; 2. proliferace; 3. maturace. Ovlivnění průběhu hojení rány nenasycenými MK může probíhat na mnoha úrovních a nebylo doposud zcela objasněno. Protektivní účinky omega-3 nenasycených MK na kardiovaskulární systém [7], obezitu, aterosklerózu, diabetes mellitus a jiné civilizační nemoci jsou známy již řadu let a jsou i nadále předmětem mnoha studií. Omega-3 a omega-6 MK mají vzájemně do jisté míry antagonistické působení: omega-3 jsou považovány za protizánětlivé, omega-6 spíše za prozánětlivé. Proto je důležitá jejich vzájemná rovnováha v lidském organizmu, a tedy i jejich optimální poměr v potravě. Toho bývá dosaženo u tzv. středomořského typu stravy (Mediterrian diet), kdy je poměr omega-3 : omega-6 okolo 1 : 1–4, naproti tomu u západního typu diety, „fast food" (Western diet) je vyšší poměr omega-6 a mnohdy dosahuje hodnoty až 1 : 20, což přispívá i k vyšší incidenci civilizačních nemocí v těchto zemích [8]. Proto byly v našem experimentu použity tukové emulze s různým poměrným zastoupením uvedených tříd nenasycených MK. Z našich výsledků plyne, že i krátkodobé podávání nenasycených MK vede ke změnám v celkových plazmatických hladinách omega-3 ve fosfolipidové a triacylglycerolové frakci na hranici signifikance, se signifikantními změnami u některých nenasycených MK v obou frakcích. Tyto výsledky jsou v korelaci s předchozími studiemi [2,9]. Elektronová mikroskopie odhalila intenzivnější produkci kolagenu v experimentálních skupinách, což nás vede k domněnce, že se rány ve skupině E a F v době ukončení experimentu (týden od vytvoření defektu) nacházely již v maturační fázi hojení, zatímco u skupiny C byly ještě v proliferační fázi, event. na

pomezí proliferační a maturační fáze hojení. Tento nález je v souladu s výsledky studie [10] prokazující, že omega-3 nenasycené MK stimulují fibroblasty k vyšší produkci kolagenu. Sledované parametry oxidačního stresu – 4-HNE, jakožto konečný produkt lipidové peroxidace [11], ROS a RNS, potvrdily, že zvířata suplementovaná nenasycenými MK jsou schopna se vyrovnat s oxidačním stresem lépe než zvířata ze skupiny kontrolní.

Nalezení optimálního poměru podávaných nenasycených MK pro hojení ran k ovlivnění zejména zánětlivé fáze hojení rány je problematické. Fáze zánětu pro hojení rány je nepostradatelná z důvodu odstranění cizorodých a avitálních (nekrotických) tkání, nicméně její prodloužení vede k prodloužení hojení a vzniku rány chronické. Přesné objasnění mechanizmu působení nenasycených MK ve fyziologických a patofyziologických procesech na celulární a molekulární úrovni by bylo možné využívat do budoucna k ovlivnění léčby ran dlouhodobě se hojících, tzv. chronických ran (non-healing wounds).

Závěr

Krátkodobé podání MK v experimentu u potkanů před operací a v průběhu hojení vedlo k signifikantním změnám v hladinách některých MK třídy omega-3 a omega-6 ve fosfolipidové a triacylglycerolové frakci, přičemž i suma omega-3 nenasycených MK dosahovala úrovně hraniční signifikance. Výsledky vypovídají, že zvířata v experimentálních skupinách měla vyšší schopnost vyrovnat se s oxidativním stresem v pooperačním období, což koreluje i s výsledky elektronové mikroskopie. Závěrem lze říci, že i krátkodobé podání zvýšeného množství omega-3 nenasycených MK, obzvlášť před plánovaným chirurgickým zákrokem, může pozitivně ovlivnit hojení kožní rány.

Literatura

1. Pokorná A, Benešová K, Mužík J et al. Data sources for monitoring of non healing wounds in a national health information system – epidemiology of non-healing wounds – analysis of the national register of hospitalized patients in 2007–2015. Cesk Slov Neurol N 2017; 80/113 (Suppl 1): S8–S17. doi: 10.14735/amcsnn2017S8.

2. Alexander JW, Supp DM. Role of Arginine and omega-3 fatty acids in wound healing and infection. Adv Wound Care (New Rochelle) 2014; 3(11): 682–690. doi: 10.1089/wound.2013.0469.

3. Armstrong DG, Hanft JR, Driver VR et al. Effect of oral nutritional supplementation on wound healing in diabetic foot ulcers: a prospective randomized controlled trial. Diabet Med 2014; 31(9): 1069–1077. doi: 10.1111/dme.12509.

4. Mohammed BM, Fisher BJ, Kraskauskas D et al. Vitamin C promotes wound healing through novel pleiotropic mechanisms. Int Wound J 2016; 13(4): 572–584. doi: 10.1111/iwj.12484.

5. McDaniel JC, Belury M, Ahijevych K et al. Omega-3 fatty acids effect on wound healing. Wound Repair Regen 2008; 16(3): 337–345. doi: 10.1111/j.1524-475X.2008.00388.x.

6. Futamura A, Higaschiguchi T, Ito A et al. Experimental research on stimulation of wound healing by n-3 fatty acids. Wounds 2013; 25(7): 186–192.

7. Lorente-Cebrián S, Costa AG, Navas-Carretero S et al. Role of omega-3 fatty acids in obesity, metabolic syndrome, and cardiovascular diseases: a review of the evidence. J Physiol Biochem 2013; 69(3): 633–651. doi: 10.1007/s13105-013-0265-4.

8. Somopoulos AP. Importance of omega-6/omega-3 balance in health and disease: evolutionary aspects of diet. World Rev Nutr Diet 2011; 102: 10–21. doi: 10.1159/000327785.

9. Arterburn LM, Hall EB, Oken H. Distribution, interconversion, and dose response of n-3 fatty acids in humans. Am J Clin Nutr 2006; 83 (6 Suppl): 1467S–1476S. doi: 10.1093/ajcn/83.6.1467S.

10. Hankenson KD, Watkins BA, Schoenlein IA et al. Omega-3 fatty acids enhance ligament fibroblast collagen formation in association with changes in interleukin-6 production. Proc Soc Exp Biol Med 2000; 223(1): 88–95.

11. Hlaváčová M, Gurnulec J, Stračina T et al. Different doxorubicin formulations affect plasma 4-hydroxy-2-nonenal and gene expression of aldehyde dehydrogenase 3A1 and thioredoxin reductase 2 in rat. Physiol Res 2015; 64 (Suppl 5): S653–S660.

nutrients

MDPI

Article

Fatty Acid Supplementation Affects Skin Wound Healing in a Rat Model

Alica Hokynková [1], Marie Nováková [2], Petr Babula [2], Miroslava Sedláčková [3], Hana Paulová [4], Miroslava Hlaváčová [4], Daniela Charwátová [2] and Tibor Stračina [2,*]

[1] Department of Burns and Plastic Surgery, Faculty Hospital Brno and Faculty of Medicine, Masaryk University, Jihlavská 20, 625 00 Brno, Czech Republic; alicah@post.cz
[2] Department of Physiology, Faculty of Medicine, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic; majka@med.muni.cz (M.N.); babula@med.muni.cz (P.B.); d.charwatova@gmail.com (D.C.)
[3] Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Kamenice 3, 625 00 Brno, Czech Republic; msedl@med.muni.cz
[4] Department of Biochemistry, Faculty of Medicine, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic; hpaulova@med.muni.cz (H.P.); hlavacova@med.muni.cz (M.H.)
* Correspondence: stracina@med.muni.cz; Tel.: +420-549-496-867

Abstract: Polyunsaturated fatty acids (PUFA) play an important role in reparative processes. The ratio of PUFAs n-3 to n-6 may affect wound healing. The study aimed to evaluate the effect of dietary supplementation with n-3 and n-6 PUFA in two proportions on skin wounds in laboratory rats. Adult male Wistar rats received 20% fat emulsion with a ratio of 1.4:1 (group A) or 4.3:1 (group B) for n-3:n-6 PUFAs at a daily dose of 1 mL/kg. The control group received water under the same conditions. The animals were supplemented a week before and a week after the skin excision performed on the back. The level of wound closure, various parameters of oxidative stress, and plasma fatty acids composition were evaluated. Wound tissue samples were examined by electron microscopy. The administration of fat emulsions led to significant changes in plasma polyunsaturated fatty acid composition. The increased production of reactive nitrogen species, as well as more numerous newly formed blood vessels and a greater amount of highly organized collagen fibrils in both groups A and B may indicate more intensive healing of the skin wound in rats supplemented with polyunsaturated fatty acids in high n-3:n-6 ratio.

Keywords: polyunsaturated fatty acids; wound; healing; oxidative stress; 4-hydroxy-2-nonenal

Citation: Hokynková, A.; Nováková, M.; Babula, P.; Sedláčková, M.; Paulová, H.; Hlaváčová, M.; Charwátová, D.; Stračina, T. Fatty Acid Supplementation Affects Skin Wound Healing in a Rat Model. *Nutrients* 2022, *14*, 2245. https://doi.org/10.3390/nu14112245

Academic Editor: Patrick R.M. Lauwers

Received: 19 April 2022
Accepted: 25 May 2022
Published: 27 May 2022

1. Introduction

Healing wounds of various origins represent a challenge in daily clinical practice and one of the biggest economic burdens for the health system, especially in hospitalized patients [1].

Wound healing represents a complex biological process on a cellular and subcellular level, with numerous intercellular interactions and activation of various chemokines, cytokines, and growth factors. Wound healing always proceeds in the following phases: inflammation, proliferation (granulation), and maturation (remodelling). For each of the healing phases, different cells and their products are typical and/or dominant, and their dominance overlaps [2].

The rate and quality of wound healing depends on numerous factors, including nutrition [3]. The effects of certain nutrients on wound healing have been studied, e.g., arginine [4], glutamine [5], vitamin C [6], and fatty acids (FA) [7]. From all the above mentioned, polyunsaturated fatty acids (PUFA) n-3 modulate the production of pro-inflammatory cytokines (IL-1, IL-6, TNF-alfa) by their end products (such as leukotrienes, prostaglandins, and thromboxane) and thus exert dominant anti-inflammatory effects [8]. In contrast, the

effect of n-6 PUFA is rather pro-inflammatory [9]. The protective effects of n-3 PUFA on the cardiovascular system [10], atherogenesis [11], lipidaemia, immune systems, and certain neurological [12] and psychiatric disorders have been studied repeatedly. The role of n-9 PUFA should not be ignored, as these FA may affect the speed of wound closure [13].

In addition to human studies, animal models are used in wound healing studies. Local and systemic effects of the topical application of essential fatty acid oil on skin wounds were described in the rat model [14,15]. Recently, differences in tissue response during wound healing were reported in a rat model after supplementation with various dietary oils [16,17].

The ratio of PUFA n-3 to n-6 in diet leads to the dominance of a certain type of end-products of their metabolism (based on their mutual metabolic pathways) and as a result to pro-inflammatory or anti-inflammatory effects. The effects of PUFAs administration at different ratios and of various durations were reported recently in animal models [18,19]. The aim of the present study was to evaluate the effect of dietary supplementation with n-3 and n-6 PUFA in two different ratios on skin wound healing in laboratory rats.

2. Materials and Methods

2.1. Experimental Protocol

All experiments were carried out according to the recommendations of the European Community Guide for the Care and Use of Laboratory Animals and according to the experimental protocol (No. MSMT-35672/2016-2) approved by the Committee for Ensuring the Welfare of Laboratory Animals of Masaryk University and licensed by the Ministry of Education, Youth and Sports of the Czech Republic. The animals were housed at the Animal Breeding and Experimental Facility, Faculty of Medicine, Masaryk University in a temperature-, pressure- and humidity-controlled environment, with light cycle 12/12 (light/dark).

A total of 30 Wistar male rats (3 weeks old) were included in the study, with an average body mass of 160.4 ± 12.6 g. Figure 1 shows the scheme of the experimental protocol. The animals were handled for seven days (handling phase) to adapt to application with orogastric tube. Then they were randomly divided into three groups (A, B, and C; n = 10) and housed individually in cages with an enriched environment. All animals had *ad libitum* access to water and a standard diet throughout the experiment. Each rat was daily weighed using KERN 440-43N (KERN & Sohn GmbH, Balingen, Germany). Body mass increments were plotted and statistically evaluated. All manipulations and applications were performed during morning hours.

Seven days before surgery (pre-operative phase), animals in groups A and B received 20% vegetable-derived lipid emulsions A and B (Biomedica Praha, Praha, Czech Republic) by orogastric tube once a day (0.2 g/kg of actual body weight). The content of the main FA in the lipid emulsions A and B is summarized in Table 1. The ratio of n-3:n-6 PUFA was 1.4:1 in the emulsion A and 4.3:1 in the emulsion B, respectively. Animals in the control group (C) received *water for injection* in corresponding volume (1 mL/kg of actual body weight).

Table 1. Content of selected fatty acids in lipid emulsions. The numbers describe the content (in %) of the fatty acid in the experimental lipid emulsions A and B, respectively.

Lipid Emulsion	Emulsion A	Emulsion B
Palmitic acid	14.62	15.08
Stearic acid	3.07	2.73
Oleic acid	31.99	39.37
Linoleic acid (LA)	18.36	6.72
α-Linolenic acid (ALA)	15.32	16.74
Docosahexaenoic acid (DHA)	10.12	12.24

On day 14, the animals were deeply anaesthetized with ketamine (100 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.). The interscapular area was shaved and a round full-thickness skin

excision (2 cm in diameter) was performed. The wound was left without dressing to heal *by second intention*. During the following 7 days (postoperative phase), daily administration of lipid emulsion or water continued under the same conditions.

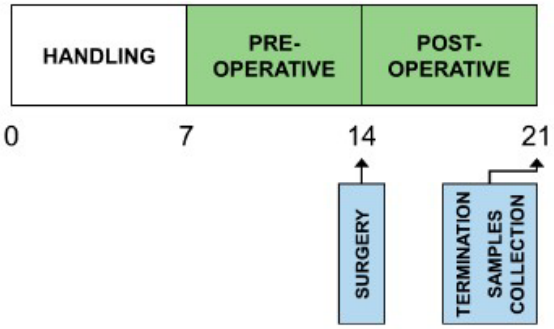


Figure 1. Scheme of the experimental protocol. The boxes represent the phases of the experiment; numbers represent the experimental days. The administration of the lipid emulsions is marked green.

2.2. Analysis of Wound Closure Using Digital Planimetry

The daily area that is yet to heal was measured by a noncontact method using a tablet with specialised planimetric software (Electreasure, HC Electronics, Hradec Králové, Czech Republic). The first measurement was performed immediately after surgery and then the measurements were made daily. The wound snapshot together with the calibration ruler was taken with the built-in camera (Figure S1). Each wound snapshot was calibrated according to the ruler and stored. The wound perimeters were then outlined on the display with a stylus, and the area that had not yet healed (in cm^2) was calculated. The level of wound closure was calculated as follows.

$$WCL = (WA_{D14} - WA_{D21})/WA_{D14} \times 100\,[\%], \quad (1)$$

where WCL—wound closure level; WA_{D14}—wound area on day 14 (immediately after the surgery); WA_{D21}—wound area in the day 21 (day of the termination).

2.3. Sample Collection

On day 21, the animals were deeply anaesthetized, and blood samples were collected by direct intracardial puncture with heparin or EDTA. The animals were sacrificed and the tissue samples from the wound border were promptly excised (Figure 2b).

2.4. Hematological Analyses

Basic hematological parameters—namely red blood cell count, hematocrit, hemoglobin concentration, red blood cell distribution width, platelets count, and white blood cell count were evaluated from EDTA blood samples. The analysis was performed immediately after sample collection using the Mythic 18 blood analyzer (Orphée SA, Plan-les-Ouates, Switzerland). Each sample was measured twice and the average was used for statistical evaluation.

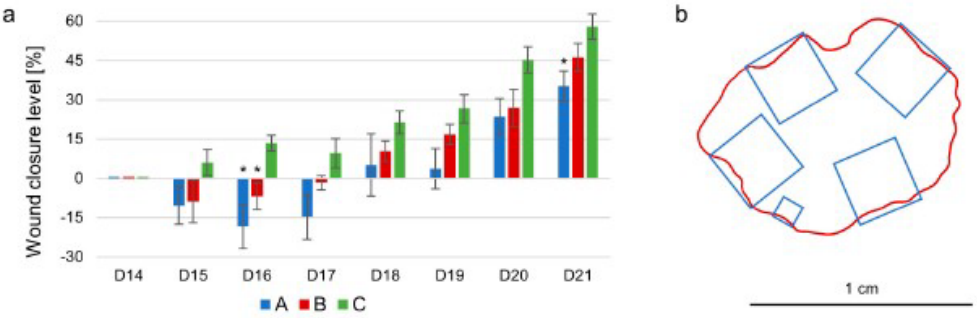


Figure 2. The wound closure level and tissue sampling. (**a**) The graph shows the wound closure level from day 14 (surgery) until day 21 (termination) in groups A (blue), B (red), and C (green), respectively. The columns represent the mean level of wound closure; the bars represent S.E.M.; * indicates statistical significance as compared to control group. (**b**) An example of tissue sample collection from the wound area. From each wound, 5 tissue samples were collected from the border zone; four of them for fluorescence microscopy (3 × 3 × 3 mm), one for electron microscopy (1 × 1 × 1 mm). The bar represents 1 cm.

2.5. Biochemical Analyses

Blood plasma was separated from the heparinised blood sample and stored for further processing at −80 °C. Plasma fatty acid composition was evaluated by gas chromatography as previously described [20,21]. Various plasma oxidative stress parameters were evaluated, including determination of 4-hydroxy-2-nonenal (4-HNE), hydrogen peroxide, nitrite/nitrate ratio, total antioxidant capacity, total reactive nitrogen species, and total reactive oxygen species. The measurement of 4-HNE levels was performed by HPLC as previously described [22] by applying the reaction with 2,4-dinitrophenylhydrazine (DNPH) as derivatization reagent. The fluorometric hydrogen peroxide assay kit (MAK165, Sigma Aldrich, St. Louis, MO, USA) used to determine hydrogen peroxide. To determine the nitrite/nitrate ration, a colorimetric nitrite/nitrate assay kit (23479, Sigma Aldrich, USA) was used. The colorimetric total antioxidant capacity assay kit (MAK187, Sigma Aldrich, USA) was used to evaluate the total antioxidant capacity of blood plasma. All kits were used according to the manufacturer's instructions. The total level of reactive nitrogen species (RNS) was measured using a fluorescence probe 2,3-diaminonaphthalene (DAN, λex = 365/λem = 415 nm, D2757, Sigma Aldrich, USA) according to Rao et al. [23]. The total level of reactive oxygen species (ROS) was measured using a fluorescent probe 2′,7′-dichlorodihydrofluorescein (H_2DCF, λex = 495/λem = 527 nm, D6665, Sigma Aldrich, USA), according to Orozco-Ibarra et al. [24]. The Cytation 3 reader (BioTek Instruments, Winooski, VT, USA) was used for all colorimetric and fluorometric measurements.

2.6. Tissue Sample Analyses

2.6.1. Fluorescence Microscopy

The presence of ROS in wound samples (approximately 3 × 3 × 3 mm) was visualized using fixable CellROX™ Green Reagent (λex = 485/λem = 520 nm, C10444, Thermo Fisher Scientific, Waltham, MA, USA). The RNS were visualized using 2,3-DAN (10 µM in 0.625 M HCl) as a fluorescent probe. Cell nuclei were counterstained with propidium iodide (1 µM solution in 0.9% NaCl, P4170, Sigma Aldrich, USA). Mito Tracker Red FM (750 nM, λex = 581/λem = 644 nm, M22425, Thermo Fisher Scientific, USA) was used to stain mitochondria. The nuclei were counterstained with 4′,6-diamidine-2′-phenylindole dihydrochloride (500 nM, 000000102362760001, Sigma Aldrich, USA). Acridine orange/ethidium bromide fluorescence staining (318337 and E7637, respectively; both chemicals Sigma Aldrich, USA) was used to evaluate cell morphology and apoptosis, respectively. The protocol

according to Jimenez et al. was used [25]. The BODIPY™ 581/591 C11 lipid peroxidation sensor (1 µM, D3861, Thermo Fisher Scientific, USA) was used to determine the rate of lipid peroxidation in wound samples. Depending on the fluorescent probe used, the samples were directly observed or fixed in the fixation (R37602, Image-iT™ Fixation/Permeabilization Kit, Thermo Fisher Scientific, USA). An epifluorescence microscope Nikon Eclipse Ti-S/L100 (Nikon, Tokyo, Japan) and appropriate excitation/emission wavelengths were used for all observations. The NIS-elements software (Nikon, Japan) was used to process images and analyze the resultant pictures.

2.6.2. Electron Microscopy

Small skin blocks (1–2 mm^3) were fixed in 300 mM glutaraldehyde (Sigma Aldrich, USA) dissolved in 100 mM cacodylate buffer for 2 h at room temperature, washed and postfixed with 40 mM osmium tetroxide (Polysciences, Warrington, PA, USA) in the same buffer for 1 h at room temperature. After being rinsed in buffer and dehydration in ethanol, the samples were embedded in araldite resin (Durcupan ACM, Sigma Aldrich, USA). Precise block orientation was made to obtain a perpendicular section of the healing skin defect. Ultrathin sections (60 nm thick) were cut using a Leica EM UC6 ultramicrotome and stained with uranyl acetate and lead citrate. The sections were examined under a FEI Morgagni 268D transmission electron microscope (FEI Company, Hillsboro, OR, USA) at 70 kV.

2.7. Statistical Analyses

Statistical analyses were performed using GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA). Data distribution was tested by the D'Agostino-Pearson normality test. Because of the non-Gaussian data distribution, nonparametric statistical analysis was employed. Between-group (group A vs. group B vs. group C) comparisons were evaluated using the Kruskal–Wallis's test (nonparametric analysis of variance), followed by multiple comparison (Dunn's test). Paired measurements were compared by the Wilcoxon matched pairs test. The results are presented as median (lower quartile–upper quartile) or as mean ± S.E.M., p-values < 0.05 were considered statistically significant.

3. Results

3.1. Wound Closure Level

Figure 2a shows the mean level of wound closure in each group from the day of the surgery to the end of the experiment. In group A, the wound area was significantly enlarged on days 15 and 16 as compared to day 14. From day 17, the wounds started to retract. The same trend was observed in group B. In group C, a continual trend of wound closure was observed. At the end of the experiment, the highest level of wound closure was found in group C (57.94 ± 4.85%). In group B, the wound area decreased by 46.26 ± 5.25%. The lowest level of wound closure was detected in group A (35.35 ± 5.72%), significantly lower compared with group C. No significant differences in wound closure level were detected between groups B and C as well as groups A and B. Representative pictures of wounds are presented in Figure S1.

3.2. Blood and Plasma Parameters

The results of the hematological analyses are summarized in Table S1. No significant differences were found in either of the hematological parameters.

The two-week administration of the lipid emulsion led to alterations in FAs occurrence in both plasma phospholipids and plasma triacylglycerols. A total of 11 PUFAs were analysed, namely 4 acids from the n-3 group (α-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA)) and 7 acids from the n-6 group (linoleic acid (LA), γ-linolenic acid, dihomolinoleic acid, dihomo-γ-linolenic acid, arachidonic acid, adrenic acid, and osbond acid). Full results of the analyses are available in the supplemental file (Tables S2 and S3). The total amounts of PUFAs n-3 and

n-6 were not significantly changed, although total amount of n-3 PUFA in triacylglycerols was close to significance ($p = 0.061$). However, significant changes in two (out of seven) n-6 PUFAs and one (out of four) n-3 PUFAs in phospholipids were observed (Table 2). Similar results were obtained in triacylglycerols (Table 3).

No significant differences in plasmatic levels of 4-HNE were found (10.0 ± 6.9 vs. 6.1 ± 3.4 vs. 5.6 ± 3.7 nmol/L in groups C, A, and B, respectively).

Table 2. Significant changes in PUFAs in plasma phospholipids. The numbers represent the *p* values.

PUFA		Kruskal-Wallis ANOVA	Multiple Comparisons		
			C vs. A	C vs. B	A vs. B
Dihomo-γ-linolenic acid	20:3n-6	0.047 *	0.052	0.260	1.000
Osbond acid	22:5n-6	0.008 *	0.037 *	0.014 *	1.000
Eicosapentaenoic acid (EPA)	20:5n-3	<0.0001 ***	0.008 *	0.0001 ***	0.898

* $p \leq 0.05$, *** $p \leq 0.0001$.

Colorimetric and fluorometric analyses of oxidative stress parameters in blood plasma revealed a significant decrease in the hydrogen peroxide and the nitrates/nitrites ratio in the A and B groups, with the lowest values in group B. Production of RNS and nitrites increased significantly in the A and B groups with the highest values in group B. ROS, total antioxidant capacity, and nitrate production were not significantly affected.

Table 3. Significant changes in PUFAs in plasma triacylglycerols. The numbers represent the *p* values.

PUFA		Kruskal-Wallis ANOVA	Multiple Comparisons		
			C vs. A	C vs. B	A vs. B
Linoleic acid (LA)	18:2n-6	0.0345 *	0.060	1.000	0.074
Osbond acid	22:5n-6	0.0267 *	0.176	0.029 *	1.000
Docosahexaenoic acid (DHA)	22:6n-3	0.048 *	1.000	0.047 *	0.368

* $p \leq 0.05$.

3.3. *Wound Tissue Samples*

The examination of the parameters of oxidative stress by fluorescence microscopy (Figures 3 and 4) corresponded to that obtained from blood plasma. In the control group, the ROS distribution showed accumulation in well-bounded areas as revealed by ROS visualization. In groups A and B, the number of stained nuclei was minimal compared to the control. Well-stained nuclei are evidence of minimal interference, and the presence of the artefacts in the case of the control group and diffuse fluorescence in groups A and B may indicate possible interference due to staining or release of the fluorescent product. The staining of RNS revealed their increased amount in tissues of groups A and B. Compared to the control and similar to ROS, RNS showed diffuse distribution in the A and B groups. These results are supported by visualization of lipid peroxidation products, which were well evident in distinct areas of the tissues of the controls (the ratio between the fluorescence of two wavelengths; a shift to green fluorescence indicates a greater degree of lipid peroxidation). Tissue samples from the control group demonstrated the presence of areas where apoptotic nuclei accumulated. On the other hand, in groups A and B, the apoptotic nuclei were evenly distributed within the tissue, which corresponds to the results obtained using mitochondrial potential staining (not shown).

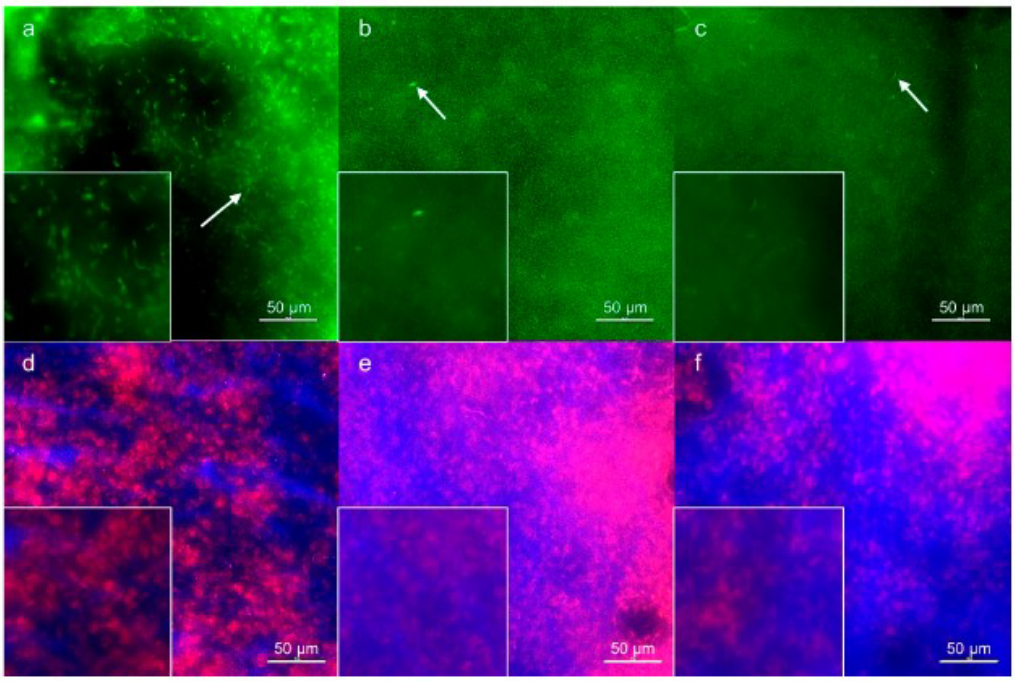


Figure 3. Representative fluorescence microscopy images showing the impact of PUFA administration on ROS/RNS in wound tissue samples. (**a**–**c**): CellROX™ Green Reagent staining; (**a**) control group, (**b**) group A, (**c**) group B. Green fluorescence is proportional to the amount of ROS accumulated in the tissue sample. Note the fluorescence of the nuclei (white arrows) in control group (**a**), group A (**b**), and B (**c**). (**d**,**e**): 2,3-diaminonaphthalene staining; (**d**) control group, (**e**) group A, (**f**) group B. The intensity of blue fluorescence corresponds to the amount of RNS in the tissue sample. The nuclei are counterstained with propidium iodide (red fluorescence). Note that in group B (**f**) the intensity of blue fluorescence overlays fluorescence of the stained nuclei. The insets represent magnified details. The bars represent 50 µm.

Electron microscopic analysis was performed in the region of the skin defect, where epithelization had begun, to a depth of approximately 500 µm. Morphology and relative representation of cellular elements (fibroblasts, macrophages, neutrophils, and lymphocytes), angiogenesis, and the structure of extracellular matrix (ECM) were evaluated.

Fibroblasts with a heavily developed rough endoplasmic reticulum and macrophages were the most numerous cell types found in all samples; neutrophils and lymphocytes were rarely found. The voluminous ECM contained fine collagen fibrils with varying degrees of organization. Compared to the control group, the number of fibroblasts was lower and the number of macrophages was higher in groups A and B. The newly formed blood vessels were more numerous in both groups (A and B), and the ECM contained a greater amount of collagen fibrils that began to organize into larger units (fibres). Thinner fibrils (40 nm) and thicker fibrils (80 nm) were observed (Figure 5). This may indicate a more intensive production of collagen in groups A and B (compared to the control group).

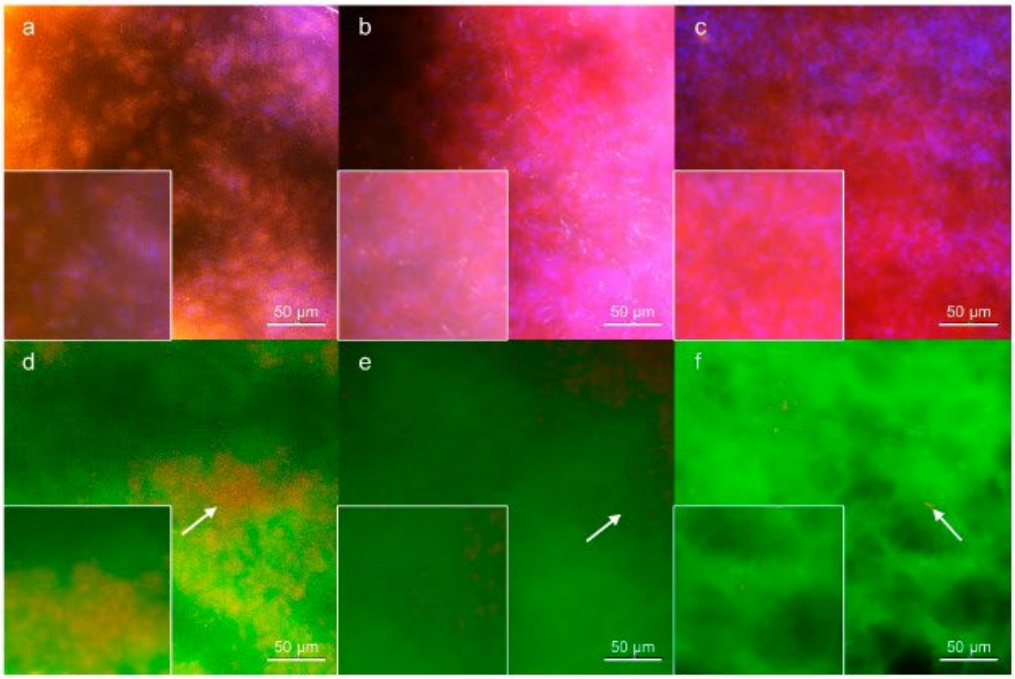


Figure 4. Representative fluorescence microscopy images showing the impact of PUFA administration on lipid peroxidation and apoptosis in wound samples. (**a**–**c**): BODIPY™ 581/591 C11 lipid peroxidation sensor; (**a**) control group, (**b**) group A, (**c**) group B. The rate of lipid peroxidation corresponds to the red/green fluorescence ratio. Nuclei were counterstained with DAPI (blue fluorescence). Staining indicates an increase in the lipid peroxidation in control group (**a**) compared to groups A (**b**) and B (**c**). (**d**,**e**): Acridine orange/ethidium bromide staining; (**d**) control group, (**e**) group A, and (**f**) group B. Staining visualizes apoptotic nuclei (red fluorescence). Note the clustering of apoptotic nuclei in the control group (**d**) and their relatively regular distribution in groups A (**e**) and B (**f**) (white arrows). The insets represent magnified details. The bars represent 50 µm.

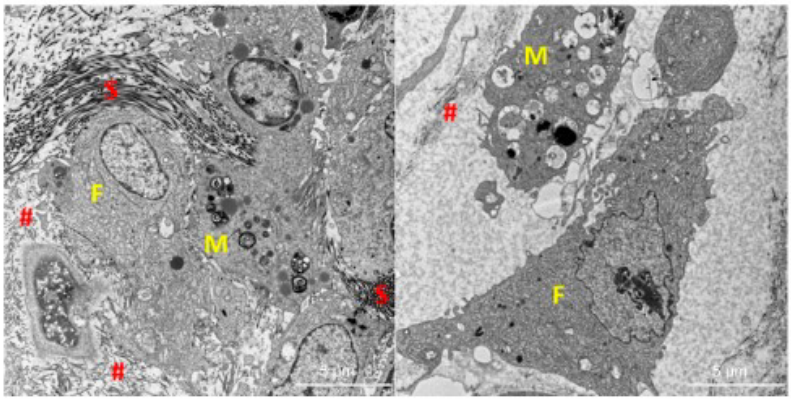


Figure 5. Representative electron microscopy images of healing skin defects in group A (**left**) and control group (**right**). Fibroblasts with heavily developed rough endoplasmic reticulum (F) and macrophages (M) were the most numerous cell types found in all samples. The voluminous extracellular matrix (ECM) contained collagen fibrils with a greater amount and higher degree of organization (fibers) in group A (**left**) and group B than in group C (#—ECM with collagen fibrils; $—collagen fiber). In groups A (**left**) and B, there were thinner fibrils (40 nm) and thicker fibrils (80 nm). This may indicate more intensive collagen production in groups A and B compared to the control group. The bars represent 5 µm.

4. Discussion

Several authors have reported the progression and potentiation of the speed and quality of wound healing with respect to different content of PUFAs, namely n-3 and n-6 PUFAs in a diet [16,26,27]. However, the exact roles of these PUFAs and mainly their mutual relationship in wound healing have not been fully elucidated.

The Western diet, typical for developed countries with a ratio of PUFAs of n-3 and n-6 as high as 1:20, leads to high incidence of cardiovascular diseases, obesity, atherosclerosis, and diabetes mellitus. The incidence of these diseases is lower in populations on a Mediterranean diet (fish products, olive oil, etc.), in which the ratio of PUFAs of n-3 and n-6 varies from 1:1 to 1:4. The protection of the cardiovascular system by n-3 PUFAs has already been known for several years [28].

In the present study, the effects of PUFA supplementation on skin wound healing in rats were studied. The experimental design is unique in several aspects. First, the lipid emulsion with two different ratios between the PUFA n-3 and n-6 was administered; in both emulsions the ratio was quite low, approaching the levels observed in a typical Mediterranean diet. Second, there is only a mild difference between both administered emulsions. Third, short-lasting administration of lipid emulsion was performed only a week before wound formation and a week before the experiment termination. In addition, plasma levels of FAs concentration and the specific lipid peroxidation marker 4-HNE were evaluated. To our knowledge, there is no experimental study taking into account both short duration of changed diet and the low ratio n3:n6 PUFAs at the same time.

In the present study, two-week administration of lipid emulsion with an increased amount of n-3 PUFA leads to increased plasmatic level of n-3 PUFA in triacylglycerols, with borderline significance, which could be explained by the minor differences in the n-3:n-6 ratios in applied emulsion. Significant increases in EPA levels in plasma phospholipids and DHA values in plasma triacylglycerols were observed.

In humans, it has been reported that four-week application of EPA/DHA in the dose of 1.6 and 1.2 g/day, respectively, leads to significant increases in plasma FA levels for both EPA and DHA [7], which was consistent with several previous studies [29]. On the other

hand, a recent study by Mihalj et al. [26] reported a negligible effect of a three-week diet with a higher PUFA content on serum concentrations of n-3 and n-6 PUFA in humans.

The results of the present study correspond to most reports mentioned above. However, our results are barely comparable to the findings from previously reported animal studies. The reasons are different methodological approaches, e.g., long-term PUFA administration, different n-3:n-6 PUFA ratio with high content of n-6 PUFAs, etc. [16,18,19]. In addition, the level of FA was measured mainly in tissue, contrary to our study where plasma concentrations were evaluated.

In addition to plasma concentrations of FAs, reactive oxygen species and products of lipid peroxidation were evaluated. Reactive oxygen species (ROS), including the hydroxyl radical, are known to affect wound healing [30]. ROS was reported to affect the expression of some nuclear-encoded genes crucial for wound healing response and oxidative stress [31]. As expected, areas with ROS accumulation were detected both in the control and experimental groups. A secondary product of lipid peroxidation, 4-HNE, was also analysed as one of the oxidative stress parameters. The aforementioned 4-HNE comes from n-6 PUFAs (e.g., linoleic or arachidonic acid) [32] and is also known as a modulator of cell functions [33]. No significant differences in 4-HNE concentrations were found between the groups. However, the decreasing trend of 4-HNE in groups A and B suggests the positive effect of a higher intake of n-3 PUFAs on lipid peroxidation measured by this parameter. Another study confirmed that the higher intake of n-3 PUFA leads to a decrease in another marker of lipoperoxidation – malondialdehyde in mice tissue [27]. It should be noted that in the case of induced lipoperoxidation (e.g., doxorubicin administration [22]), the concentration of 4-HNE increases and 4-HNE is considered as a good marker reflecting the actual level of lipid peroxidation.

The observed changes in blood plasma were reflected in ultrastructural changes. Electron microscopy revealed a wide range of cell types in the wound healing area: elements that remove decomposed cells or infectious agents (macrophages, neutrophil granulocytes) or elements that participate in healing processes by producing an intercellular matrix (fibroblasts). No significant differences were found among groups A, B and C when representation of particular cell types was compared, most probably due to variability of the samples from the same group. Another possible explanation is the inhomogeneity of the healing area and also the fact that only a small portion of the tissue sample could be examined. In terms of the extracellular matrix, the structure of its fibrous component was studied. No elastic fibres were found in either sample, but all samples contained collagen in the form of microfibrils. In group B, difference in the quantity and quality of collagen was observed: collagen microfibrils were clearly organized into larger aggregates. In addition to very thin microfibrils (which were found in the samples from all groups), microfibrils of a bigger diameter were also observed. The finding probably reflects increased collagen production. This observation is in agreement with a previously published study reporting that n-3 PUFA stimulate fibroblasts to increase collagen production [34]. The significant increase in number of selected n-3 PUFAs in both experimental groups supports this conclusion. The question remains whether increased collagen production is beneficial or whether it might lead in the future to the formation of keloid scars.

The macroscopic picture of wound healing was studied by digital planimetry. Significant wound retraction was observed at the end of the experiment in all groups. However, in animals supplemented with emulsion with a lower content of n-3 PUFA (group A) the retraction was significantly smaller compared to the control group. A similar trend was observed in group B (supplemented by emulsion with a higher content of n-3 PUFAs). At this point, it is necessary to emphasize that the speed of wound closure is not the only or the most important parameter reflecting the healing process. Furthermore, the quality of the formed tissue must be taken into account. As discussed above, the ultramicroscopic picture of newly formed tissue in group B indicates that a higher content of n-3 PUFA stimulated formation of the collagen fibres of higher quantity and quality. The rather surprising widening of the wound area in groups A and B on days 15 and 16 is difficult to

explain. However, it should be noted that this widening was to a smaller extent in group B, which was supplemented with a lipid emulsion with higher content of n-3 PUFA.

As any other model, our experimental setup is burdened by certain limitations. In digital planimetry, the main drawback is the manual evaluation of the wound area. Although the same experimenter always performed the measurement, certain error must be considered since the wound diameter was small (in the range of millimetres) and the wound was not sharp-edged. This error may be minimized by employing automated wound area analysis [35]. In addition, it has to be considered that the results from animal models are not fully transferrable to human medicine. Most of the previously published studies that focused on the effects of PUFAs on wound healing were conducted in humans. However, the rat is a fully accepted model for such a type of study. Furthermore, the results of previously published studies are rather inhomogeneous, which may result from methodological differences, particularly from the different forms and durations of PUFA administration. If the results are compared, origin of lipids should also be considered. In the present study, the vegetable-derived lipid emulsions were administered contrary to most of the previously published studies, where animal-derived lipids were used.

5. Conclusions

It can be concluded that even a short-term administration of lipid emulsions containing higher n-3:n-6 PUFA ratio results in increased plasmatic level of n-3 PUFAs in triacylglycerols of borderline significance, significantly increase in EPA levels in plasma fosfolipids, and significantly increase in DHA plasma levels in triacylglycerols. The decreasing trend of 4-HNE supports this idea. The appearance of numerous newly formed blood vessels and a greater amount of highly organized collagen fibrils, revealed by electron microscopy of the skin defect, support the idea of higher quality wound healing.

More studies are needed to uncover the particular mechanisms behind this beneficial effect of dietary supplementation with higher content of n-3 PUFAs.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/nu14112245/s1, Figure S1: Representative pictures of the wound used for planimetric analysis.; Table S1: Hematological parameters.; Table S2: Polyunsaturated fatty acids (PUFAs) profile in plasma phospholipids.; Table S3: Polyunsaturated fatty acids (PUFAs) profile in plasma triacylglycerols.

Author Contributions: Conceptualization, A.H. and M.N.; methodology, T.S.; validation, A.H., M.N., P.B., M.S. and H.P.; formal analysis, T.S.; investigation, A.H., T.S. and D.C. (animal model, hematological analyzes), H.P. and M.H. (biochemical analyzes), P.B. (fluorescence microscopy), M.S. (electron microscopy); resources, T.S and M.H.; data curation, T.S.; writing—original draft preparation, A.H., M.N., P.B., M.S., H.P., M.H. and T.S.; writing—review and editing, M.N., H.P. and T.S.; visualization, A.H., P.B., M.H. and T.S.; supervision and project administration, M.N.; funding acquisition, M.N and H.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by MASARYK UNIVERSITY, grant numbers MUNI/A/0910/2017, MUNI/A/1255/2018, MUNI/A/1307/2019, and MUNI/A/1246/2020, with the support of the Specific University Research Grant, as provided by the Ministry of Education, Youth and Sports of the Czech Republic in the years 2018, 2019, 2020, and 2021.

Institutional Review Board Statement: The animal study protocol was approved by the Committee for Ensuring the Welfare of Laboratory Animals, Masaryk University and licensed by the Ministry of Education, Youth and Sports of the Czech Republic (Protocol No. MSMT-35672/2016-2, the approval date: 14 November 2016).

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors wish to express their gratitude to Zdeněk Wilhelm who put forward the study and participated in pilot experiments. The authors thank to Jaroslav Nádeníček for technical help. The authors thank also to Milena Kthutová and Pavel Sedláček for language corrections.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Pokorná, A.; Benešová, K.; Mužík, J.; Jarkovský, J. Data sources for monitoring of non-healing wounds in a national health information system-epidemiology of non-healing wounds-Analysis of the national register of hospitalized patients in 2007–2015. *Ceska Slov. Neurol. Neurochir.* **2017**, *80*, S8–S17. [CrossRef]
2. Middleton, J.E. *Wound Healing: Process, Phases, and Promoting (Human Anatomy and Physiology)*; Nova Science Publishers Incorporated: Hauppauge, NY, USA, 2011.
3. Kavalukas, S.L.; Barbul, A. Nutrition and wound healing: An update. *Plast. Reconstr. Surg.* **2011**, *127* (Suppl. 1), 38S–43S. [CrossRef] [PubMed]
4. Alexander, J.W.; Supp, D.M. Role of Arginine and Omega-3 Fatty Acids in Wound Healing and Infection. *Adv. Wound Care* **2014**, *3*, 682–690. [CrossRef] [PubMed]
5. Armstrong, D.G.; Hanft, J.R.; Driver, V.R.; Smith, A.P.; Lazaro-Martinez, J.L.; Reyzelman, A.M.; Furst, G.J.; Vayser, D.J.; Cervantes, H.L.; Snyder, R.J.; et al. Effect of oral nutritional supplementation on wound healing in diabetic foot ulcers: A prospective randomized controlled trial. *Diabet. Med.* **2014**, *31*, 1069–1077. [CrossRef]
6. Mohammed, B.M.; Fisher, B.J.; Kraskauskas, D.; Ward, S.; Wayne, J.S.; Brophy, D.F.; Fowler, A.A.; Yager, D.R.; Natarajan, R. Vitamin C promotes wound healing through novel pleiotropic mechanisms. *Int. Wound J.* **2016**, *13*, 572–584. [CrossRef]
7. McDaniel, J.C.; Belury, M.; Ahijevych, K.; Blakely, W. Omega-3 fatty acids effect on wound healing. *Wound Repair Regen.* **2008**, *16*, 337–345. [CrossRef]
8. Calder, P.C. Omega-3 fatty acids and inflammatory processes: From molecules to man. *Biochem. Soc. Trans.* **2017**, *45*, 1105–1115. [CrossRef]
9. Silva, J.R.; Burger, B.; Kuhl, C.M.C.; Candreva, T.; dos Anjos, M.B.P.; Rodrigues, H.G. Wound Healing and Omega-6 Fatty Acids: From Inflammation to Repair. *Mediat. Inflamm.* **2018**, *2018*, 2503950. [CrossRef]
10. Jain, A.P.; Aggarwal, K.K.; Zhang, P.Y. Omega-3 fatty acids and cardiovascular disease. *Eur. Rev. Med. Pharmacol. Sci.* **2015**, *19*, 441–445.
11. De Caterina, R.; Zampolli, A. Omega-3 fatty acids, atherogenesis, and endothelial activation. *J. Cardiovasc. Med.* **2007**, *8* (Suppl. 1), S11–S14. [CrossRef]
12. Wu, S.; Ding, Y.; Wu, F.; Li, R.; Hou, J.; Mao, P. Omega-3 fatty acids intake and risks of dementia and Alzheimer's disease: A meta-analysis. *Neurosci. Biobehav. Rev.* **2015**, *48*, 1–9. [CrossRef] [PubMed]
13. Cardoso, C.R.; Souza, M.A.; Ferro, E.A.; Favoreto, S.; Pena, J.D. Influence of topical administration of n-3 and n-6 essential and n-9 nonessential fatty acids on the healing of cutaneous wounds. *Wound Repair Regen.* **2004**, *12*, 235–243. [CrossRef] [PubMed]
14. Lania, B.G.; Morari, J.; Almeida, A.R.; Silva, M.N.D.; Vieira-Damiani, G.; Lins, K.A.; César, C.L.; Velloso, L.A.; Maia, N.B.; Cintra, M.L.; et al. Topical essential fatty acid oil on wounds: Local and systemic effects. *PLoS ONE* **2019**, *14*, e0210059. [CrossRef]
15. Zong, J.; Jiang, J.; Shi, P.; Liu, J.; Wang, W.; Li, B.; Zhao, T.; Pan, T.; Zhang, Z.; Bi, L.; et al. Fatty acid extracts facilitate cutaneous wound healing through activating AKT, ERK, and TGF-β/Smad3 signaling and promoting angiogenesis. *Am. J. Transl. Res.* **2020**, *12*, 478–492.
16. Komprda, T.; Sladek, Z.; Sevcikova, Z.; Svehlova, V.; Wijacki, J.; Guran, R.; Do, T.; Lackova, Z.; Polanska, H.; Vrlikova, L.; et al. Comparison of Dietary Oils with Different Polyunsaturated Fatty Acid n-3 and n-6 Content in the Rat Model of Cutaneous Wound Healing. *Int. J. Mol. Sci.* **2020**, *21*, 7911. [CrossRef] [PubMed]
17. Rodrigues, H.G.; Vinolo, M.A.; Magdalon, J.; Vitzel, K.; Nachbar, R.T.; Pessoa, A.F.; dos Santos, M.F.; Hatanaka, E.; Calder, P.C.; Curi, R. Oral administration of oleic or linoleic acid accelerates the inflammatory phase of wound healing. *J. Investig. Dermatol.* **2012**, *132*, 208–215. [CrossRef]
18. Horman, T.; Fernandes, M.F.; Tache, M.C.; Hucik, B.; Mutch, D.M.; Leri, F. Dietary n-6/n-3 Ratio Influences Brain Fatty Acid Composition in Adult Rats. *Nutrients* **2020**, *12*, 1847. [CrossRef]
19. Lepczynski, A.; Ozgo, M.; Michalek, K.; Dratwa-Chalupnik, A.; Grabowska, M.; Herosimczyk, A.; Liput, K.P.; Polawska, E.; Kram, A.; Pierzchala, M. Effects of Three-Month Feeding High Fat Diets with Different Fatty Acid Composition on Myocardial Proteome in Mice. *Nutrients* **2021**, *13*, 330. [CrossRef]
20. Novak, F.; Borovska, J.; Vecka, M.; Rychlikova, J.; Vavrova, L.; Petraskova, H.; Zak, A.; Novakova, O. Plasma Phospholipid Fatty Acid Profile is Altered in Both Septic and Non-Septic Critically Ill: A Correlation with Inflammatory Markers and Albumin. *Lipids* **2017**, *52*, 245–254. [CrossRef]
21. Tvrzická, E.; Vecka, M.; Staňková, B.; Žák, A. Analysis of fatty acids in plasma lipoproteins by gas chromatography–flame ionization detection: Quantitative aspects. *Anal. Chim. Acta* **2002**, *465*, 337–350. [CrossRef]
22. Hlaváčová, M.; Gumulec, J.; Stračina, T.; Fojtů, M.; Raudenská, M.; Masařík, M.; Nováková, M.; Paulová, H. Different doxorubicin formulations affect plasma 4-hydroxy-2-nonenal and gene expression of aldehyde dehydrogenase 3A1 and thioredoxin reductase 2 in rat. *Physiol. Res.* **2015**, *64* (Suppl. 5), S653–S660. [CrossRef] [PubMed]

23. Rao, A.M.; Dogan, A.; Hatcher, J.F.; Dempsey, R.J. Fluorometric assay of nitrite and nitrate in brain tissue after traumatic brain injury and cerebral ischemia. *Brain Res.* **1998**, *793*, 265–270. [CrossRef]
24. Orozco-Ibarra, M.; Medina-Campos, O.N.; Sánchez-González, D.J.; Martínez-Martínez, C.M.; Floriano-Sánchez, E.; Santamaría, A.; Ramírez, V.; Bobadilla, N.A.; Pedraza-Chaverri, J. Evaluation of oxidative stress in D-serine induced nephrotoxicity. *Toxicology* **2007**, *229*, 123–135. [CrossRef] [PubMed]
25. Jimenez, P.C.; Wilke, D.V.; Takeara, R.; Lotufo, T.M.; Pessoa, C.; de Moraes, M.O.; Lopes, N.P.; Costa-Lotufo, L.V. Cytotoxic activity of a dichloromethane extract and fractions obtained from Eudistoma vannamei (Tunicata: Ascidiacea). *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **2008**, *151*, 391–398. [CrossRef]
26. Mihalj, M.; Stupin, A.; Kolobaric, N.; Bujak, I.T.; Matic, A.; Kralik, Z.; Jukic, I.; Stupin, M.; Drenjancevic, I. Leukocyte Activation and Antioxidative Defense Are Interrelated and Moderately Modified by n-3 Polyunsaturated Fatty Acid-Enriched Eggs Consumption-Double-Blind Controlled Randomized Clinical Study. *Nutrients* **2020**, *12*, 3122. [CrossRef]
27. Meng, F.X.; Qiu, J.Y.; Chen, H.J.; Shi, X.J.; Yin, M.F.; Zhu, M.S.; Yang, G. Dietary supplementation with N-3 polyunsaturated fatty acid-enriched fish oil promotes wound healing after ultraviolet B-induced sunburn in mice. *Food Sci. Nutr.* **2021**, *9*, 3693–3700. [CrossRef]
28. Lorente-Cebrián, S.; Costa, A.G.; Navas-Carretero, S.; Zabala, M.; Martínez, J.A.; Moreno-Aliaga, M.J. Role of omega-3 fatty acids in obesity, metabolic syndrome, and cardiovascular diseases: A review of the evidence. *J. Physiol. Biochem.* **2013**, *69*, 633–651. [CrossRef]
29. Arterburn, L.M.; Hall, E.B.; Oken, H. Distribution, interconversion, and dose response of n-3 fatty acids in humans. *Am. J. Clin. Nutr.* **2006**, *83*, 1467S–1476S. [CrossRef]
30. Huo, Y.; Qiu, W.Y.; Pan, Q.; Yao, Y.F.; Xing, K.; Lou, M.F. Reactive oxygen species (ROS) are essential mediators in epidermal growth factor (EGF)-stimulated corneal epithelial cell proliferation, adhesion, migration, and wound healing. *Exp. Eye Res.* **2009**, *89*, 876–886. [CrossRef]
31. Janda, J.; Nfonsam, V.; Calienes, F.; Sligh, J.E.; Jandova, J. Modulation of ROS levels in fibroblasts by altering mitochondria regulates the process of wound healing. *Arch Dermatol. Res.* **2016**, *308*, 239–248. [CrossRef]
32. Ayala, A.; Muñoz, M.F.; Argüelles, S. Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxid. Med. Cell. Longev.* **2014**, *2014*, 360438. [CrossRef] [PubMed]
33. Leonarduzzi, G.; Arkan, M.C.; Başağa, H.; Chiarpotto, E.; Sevanian, A.; Poli, G. Lipid oxidation products in cell signaling. *Free Radic. Biol. Med.* **2000**, *28*, 1370–1378. [CrossRef]
34. Hankenson, K.D.; Watkins, B.A.; Schoenlein, I.A.; Allen, K.G.; Turek, J.J. Omega-3 fatty acids enhance ligament fibroblast collagen formation in association with changes in interleukin-6 production. *Proc. Soc. Exp. Biol. Med.* **2000**, *223*, 88–95. [CrossRef] [PubMed]
35. Magalhes, M.S.F.; Fechine, F.V.; de Macedo, R.N.; Monteiro, D.L.S.; Oliveira, C.C.; Brito, G.A.D.; de Moraes, M.E.A.; de Moraes, M.O. Effect of a combination of medium chain triglycerides, linoleic acid, soy lecithin and vitamins A and E on wound healing in rats. *Acta Cir. Bras.* **2008**, *23*, 262–269. [CrossRef] [PubMed]

3. OXIDATIVE STRESS PERSPECTIVE

Chronic wounds, including PUs, represent a significant clinical challenge due to their prolonged healing process and high recurrence rates. One of the critical factors influencing wound healing is oxidative stress (OS), which arises from an imbalance between reactive oxygen species (ROS) production and antioxidant defences. While controlled ROS levels are essential for initiating key healing processes, such as inflammation, angiogenesis, and tissue remodelling, excessive ROS can lead to prolonged inflammation and cellular damage, contributing to impaired wound healing.

This chapter explores the role of oxidative stress in pressure ulcer management, focusing on its impact on tissue repair and the modulation of ROS and reactive nitrogen species (RNS) during different phases of healing. Particular emphasis is placed on surgical debridement techniques, such as sharp necrectomy and water jet necrectomy, and their influence on oxidative stress parameters and gene expression in chronic wounds. Understanding these biochemical interactions is crucial for optimizing surgical strategies and improving patient outcomes.

Additionally, recent research suggests that targeted therapeutic approaches, including ROS-scavenging nanoparticles and gasotransmitter-based therapies, may offer promising interventions for modulating oxidative stress in both acute and chronic wounds. By integrating biochemical analysis with surgical innovation, this chapter aims to provide new insights into the role of oxidative stress in PUs treatment and contribute to the development of personalized, evidence-based wound management strategies.

3.1 Oxidative stress and wound healing

Hard-to-heal or chronic wounds, such as PUs, require a tailored approach to treatment that varies according to the stage of healing and often takes a long time to heal completely. These wounds often stall in the inflammatory phase due to infection, poor perfusion or systemic problems, such as diabetes, malnutrition, and oxidative stress. Reactive oxygen species (ROS) play a pivotal role in regulating various phases of wound healing. [114] Excessive oxidative stress, characterised by an imbalance between ROS and antioxidant defences [115], can prolong inflammation and impair key processes, such as fibroblast activity and collagen

synthesis. ROS significantly influence the wound healing process by modulating various physiological and pathological mechanisms, including the inflammatory response, cellular proliferation, angiogenesis, granulation tissue development, and extracellular matrix production. [116] Scientific research over the past decade has revealed that OS can play dual role in wound healing, depending on its specific context. In mammals, OS arises either from the accumulation ROS or from a reduction in antioxidant capacity. [117,118] The early stage of wound healing, inflammation, is distinguished by an elevated production of ROS and therefore, [119] prolonged inflammatory response is closely linked to OS, a key factor contributing to delayed wound healing. [120] The elevated production of ROS acts as a defence mechanism against pathogen attacks and ROS accumulation is necessary to prevent infection at the wound site. [121] However, prolonged exposure to high levels of ROS can lead to oxidative stress, resulting in cellular damage. [119] Therapeutical strategies differ according to the stage of healing. In the inflammatory phase, the focus is on controlling infection, reducing oxidative stress and optimising the wound bed through debridement and antimicrobial therapies. A well-regulated ROS response aids in tissue debridement, disinfection, and stimulation of healthy tissue regeneration. Insufficient ROS levels can lead to infection, while excessive ROS can damage otherwise healthy stromal tissue. By understanding and predicting the role of ROS within a wound, we can significantly improve our ability to manage and coordinate the processes involved in wound healing. [122,123] In the proliferative phase, therapies may aim to stimulate granulation tissue formation and angiogenesis using advanced dressings, growth factors, or negative pressure wound therapy. The status of OS and ROS is closely linked to the wound healing process, influencing key stages, such as inflammation, angiogenesis, and granulation tissue formation. [114,124–126] By modulating OS and ROS levels, it may be possible to positively impact all phases of wound healing, from the initial inflammatory response to tissue remodelling and repair. ROS are connected also with gasotransmitters, which also act as a barrier against excessive ROS levels, particularly superoxide radicals. These molecules, defined as endogenous gaseous signalling compounds, freely diffuse across cell membranes to facilitate intercellular communication and regulate numerous physiological and pathological processes. The most extensively studied gasotransmitters - nitric oxide (NO), carbon monoxide (CO), and hydrogen sulfide (H_2S) - are naturally produced within

the body but can also be supplemented exogenously to enhance their therapeutic effects. [127]

Gasotransmitters play critical roles in wound healing by modulating OS, inflammation, and angiogenesis. For instance, NO is essential for vasodilation and oxygen delivery to tissues, while CO exhibits potent anti-inflammatory properties, and H_2S supports angiogenesis and antioxidant defences. Their ability to interact with ROS allows them to protect cells from oxidative damage and promote a balanced inflammatory response, which is vital for effective tissue repair. [119]

Therapeutically, gasotransmitters hold promise for addressing challenges in chronic wound healing. To harness their potential, effective delivery systems must meet key requirements: biocompatibility, controlled release of sufficient gasotransmitter amounts, protection of the wound from pathogens, and maintenance of a moist wound environment. [128] Exogenous administration of gasotransmitters or their donors could provide targeted interventions to regulate oxidative stress and inflammation, promoting faster and more efficient wound healing. As research continues to elucidate the mechanisms of gasotransmitter activity, these molecules represent a promising avenue for innovative therapeutic strategies in wound management and beyond.

Previous research has confirmed the significant role of ROS in various aspects of the wound healing process. To date, several genes have been identified expression of which is directly regulated by ROS. These genes play critical roles in various cellular processes, including inflammation, angiogenesis, apoptosis, and tissue repair. Building on this foundational understanding, numerous therapeutic strategies targeting ROS have been developed. Among these approaches is the use of ROS-scavenging modified nanoparticles, designed to efficiently neutralize ROS and facilitate wound healing. Some studies highlight the advancing application of biomaterials in regulating ROS to support tissue regeneration. [116,129,130]

3.2 Oxidative stress and pressure ulcer surgical management

We sought to explore the role of oxidative stress in chronic wounds by examining various debridement techniques applied to deep category III and IV of PUs. Our objective was to

evaluate whether oxidative stress parameters and related gene expressions in wound tissues differ following sharp necrectomy compared to water jet necrectomy. This ongoing grant project No. NU21-09-00541, “The role of oxidative stress in pressure ulcers treatment in a patient with spinal injury” aims to deepen our understanding of the role of OS in chronic wounds, specifically in category III and IV of PUs. The research focuses on comparing two distinct debridement techniques - sharp necrectomy and water jet necrectomy - and their impact on oxidative stress parameters in tissues. In addition to ROS, the study also examines reactive nitrogen species (RNS), such as nitric oxide synthase (NOS), as key contributors to the wound microenvironment.

As the project is still in the data collection and the analysis phase, the complete results are not yet available. Preliminary results suggest that the mechanical and physical characteristics of these techniques may differentially influence levels of ROS and RNS, potentially affecting the wound’s ability to progress through the healing phases. Moreover, it also indicates certain correlations in the expression of different genes. This investigation aims to provide novel insights into how these biochemical factors can be modulated through surgical techniques to optimize outcomes in patients with deep PUs.

Furthermore, this project lays the groundwork for future research into personalized approaches for managing chronic wounds. By using OS parameters as indicators, clinicians could select the most appropriate therapeutic strategy for each patient. The findings are expected to contribute to the development of clinical guidelines that improve healing efficacy and reduce complications in some challenging cases.

3.3 Summary

This chapter offers a foundational characterization of oxidative stress parameters, focusing on their role in the wound healing process and their potential for therapeutic modulation in both acute and chronic wounds. It explores the dual nature of OS, where controlled levels of ROS and RNS are essential for effective tissue repair, while excessive levels can hinder healing by causing prolonged inflammation and cellular damage.

Additionally, the chapter discusses the methods for measuring these parameters, including their baseline levels and potential dynamic changes during the surgical treatment of PUs. Particular attention is given to the comparison of OS responses to different surgical

techniques, such as sharp necrectomy and water jet necrectomy, which may influence the healing trajectory and outcomes for category III and IV of PUs.

While the potential clinical applications of this knowledge are significant, including personalized treatment approaches and improved surgical planning, it is important to emphasize that this work is part of an ongoing research project. The data and results from these investigations are not yet available, but they are expected to contribute to a deeper understanding of OS dynamics in wound healing and inform future therapeutic strategies and clinical guidelines.

3.4 Articles related to this issue

Hokynková, A., Babula, P., Pokorná, A., Nováková, M., Nártová, L., & Šín, P. Oxidative stress in wound healing - current knowledge. *Česká a slovenská neurologie a neurochirurgie*, Vol. 82/115 (Suppl 1), p. 37-39.

Šín, P., Hokynková, A., Nováková, M., Paulová, H., Babula, P., Pokorná, A., Nártová, L., Coufal, P., & Hendrych, M. (2022). Can different type of the pressure ulcers debridement affect oxidative stress parameters? *Česká a Slovenská Neurologie a Neurochirurgie.* Vol. 85/118 (Suppl 1), p. 34-37.

Samadian, A., Kratochvílová, M., Hokynková, A., Šín., P., Nováková, M., Štěpka, P., Pokorná, A., & Babula, P. (2023). Changes in Gene Expression in Pressure Ulcers Debrided by Different Approaches–a Pilot Study. *Physiological research*, *72*(Suppl 5), S535.

Križanová, O., Penesová, A., Sokol, J., Hokynková, A., Samadian, A., & Babula, P. (2022). Signaling pathways in cutaneous wound healing. *Frontiers in Physiology*, *13*, 1030851.

doi: 10.14735/amcsnn2019537

Oxidative stress in wound healing – current knowledge

Role oxidativního stresu v hojení ran – současné poznatky

Abstract
Wound healing is a complex process based on a subtle coordination of biochemical and physiological interactions. Healing process itself and its quality are affected by numerous factors, both local (type, size, depth, and localization of the wound, bacterial contamination, microcirculation, oxygen supply, etc.) and systemic (age, comorbidities, smoking, nutritional status, etc.). Many studies, using various methodological approaches, focus on wound healing process at various levels. It is well known that reactive oxygen and nitrogen species play an important role in all phases of wound healing. Regardless increasing knowledge about the role of oxidative stress in wound healing process, the conclusions of research in this area are still rather contradictory. Therefore, aim of this paper is to summarize current knowledge about the role of oxidative stress in wound healing process.

Souhrn
Hojení ran je etapovitý proces probíhající na celulární i subcelulární úrovni jako souhra řady biochemických a fyziologických pochodů a interakcí. Rychlost a kvalita hojení jsou ovlivněny mnoha faktory, a to jak lokálními (typ, velikost, hloubka a lokalizace rány, bakteriální biofilm, stav mikrocirkulace, zásobení kyslíkem), tak systémovými (věk, komorbidity, kouření, stav nutrice, aj). Iz tohoto důvodu je proces hojení předmětem zájmu řady studií a je zkoumán různými metodickými přístupy na mnoha úrovních. Je známo, že reaktivní formy kyslíku a dusíku hrají významnou roli ve všech fázích hojení ran. Navzdory rostoucí pozornosti, která je výzkumu role oxidativního stresu v procesu hojení ran věnována, jsou závěry aktuálních studií stále ještě rozporuplné. Cílem příspěvku je tedy poskytnout přehled o současných znalostech o úloze oxidativního stresu v procesu hojení ran.

The authors declare they have no potential conflicts of interest concerning drugs, products, or services used in the study.
Autoři deklarují, že v souvislosti s předmětem studie nemají žádné komerční zájmy.
The Editorial Board declares that the manuscript met the ICMJE "uniform requirements" for biomedical papers.
Redakční rada potvrzuje, že rukopis práce splnil ICMJE kritéria pro publikace zasílané do biomedicínských časopisů.

A. Hokynková[1], P. Babula[2], A. Pokorná[3], M. Nováková[2], L. Nártová[3], P. Šín[1]

[1] Department of Burns and Plastic Surgery, Faculty Hospital Brno, Czech Republic
[2] Department of Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic
[3] Department of Nursing and Midwifery, Faculty of Medicine, Masaryk University, Brno, Czech Republic

✉
Petr Šín, MD, PhD
Department of Burns and Plastic Surgery
University Hospital Brno
Jihlavská 20
625 00 Brno
Czech Republic
e-mail: p.sin@seznam.cz

Accepted for review: 30. 6. 2019
Accepted for print: 11. 7. 2019

Key words
reactive oxygen species – reactive nitrogen species – oxidative stress – wound healing

Klíčová slova
reaktivní formy kyslíku – reaktivní formy dusíku – oxidativní stress – hojení ran

Introduction

Wound healing is a complex process based on a subtle coordination of biochemical and physiological interactions. Healing process in the wound starts by a tissue damage and is finished when a functional scar is formed. Healing process itself and its quality are affected at various levels by numerous factors, both local and systemic. Local factors include type, size, depth, and localization of the wound, then also bacterial contamination, microcirculation, oxygen supply, etc.

Systemic factors are age, comorbidities, nutritional status, and others. Chronic and non-healing wounds (e.g. pressure ulcers, diabetic ulcerations) still represent a major concern not only for patient and his family, but also for public health system due to a steadily growing issue of socioeconomic cost. Specific group of patients at increased risk for development of pressure ulcers and other dermatological complications are those after spinal cord injury [1,2]. Therefore, any therapeutic approach potentiating or accelerating wound healing process at any level is considered beneficial. Several models have been used to evaluate wound healing process from macroscopic down to molecular level, using various experimental approaches from *in silico* (computational model to understand wound healing theoretically), *in vitro* (explaining pathogenesis of wound healing), *ex vivo* (providing 3D model of skin explant), and *in vivo* (using animal or human model) [3]. At present, one of the main topics in the theoretical research in wound healing is the role of oxidative stress in various phases of healing process. It is widely believed that the amount of oxygen/nitrogen radicals might be crucial for further direction of a healing process. However, number of systematic studies presenting detailed insight into reactive oxygen species (ROS) / nitrogen species (RNS) role in particular phases of wound healing is still limited. Aim of this article is to summarize in detail present knowledge about parameters of oxidative stress in particular phases of wound healing in order to provide an integrated, synthesized overview of the current knowledge.

Reactive oxygen and nitrogen species in wound healing – a general view

Wound healing is one of the most complex biological processes. It involves the spatial and temporal synchronization of a variety of cell types with distinct roles in the phases of haemostasis, inflammation, growth, re-epithelialization, and remodelling [4,5]. The first phase "haemostasis" prevents excessive blood loss; it triggers events that lead to local inflammation by neutrophils and then macrophages. The inflammation is followed by the performance of local tissue cells, keratinocytes and fibroblasts. The former cells first migrate into the injured area for the primary coverage and start to proliferate to recover the stratification. The latter transform to the myofibroblasts that are capable of producing extracellular matrix and of tissue contraction. Both cell migration of keratinocytes and fibroblasts-myofibroblasts conversion largely depend on the activity of a potent growth factor, transforming growth factor β (TGFβ), although a set of growth factors are believed to orchestrate the whole process of tissue repair [6]. Changes in the microenvironment, including alterations in mechanical forces, oxygen levels, chemokines, extracellular matrix, and growth factor synthesis directly affect cellular recruitment and activation, leading to impaired states of wound healing. Impaired wound healing, in turn, may lead to post-surgical complications frequently observed in elderly patients, chronic ulcers in diabetic patients, hindered and ineffective pain management, etc. [7]. The mechanism of delayed wound healing has multifactorial causes, including a prolonged inflammatory stage, postponed proliferation and remodelling stages. It has been reported that nuclear factor kappa B (NF-κB) regulates the gene expression of several cytokines, such as interleukin-1beta, interleukin-6, tumor necrosis factor-alpha, and interleukin-10; inducible nitric oxide synthase (iNOS); chemotactic and matrix proteins; immunological responses; and cell proliferation [8]. NF-κB can contribute to inflammation and fibroblast function, which are necessary components of incision and wound healing [9]. It has been shown that inhibition of these signal transduction pathways may provide novel strategies to prevent sepsis but may interfere with healing. The persistence of the inflammatory reaction is associated with oxidative stress, which is one of the most common reasons for the delayed wound healing [10]. The increased production of free radicals and decreased antioxidant activities of enzymes, such as superoxide dismutase, glutathione peroxidase, heme oxygenase-1, and heme oxygenase-2 may aggravate the situation leading to a delay in diabetic wound healing [11]. All these events indicate a pivotal role of ROS in the orchestration of the normal wound healing response. On the other hand, ROS act as secondary messengers to many immunocytes and non-lymphoid cells involved in the repair process and appear to be important in coordinating the recruitment of lymphoid cells to the wound site and effective tissue repair. ROS also possess the ability to regulate the formation of blood vessels (angiogenesis) at the wound site and the optimal perfusion of blood into the wound-healing area [8]. ROS act in the host's defence through phagocytes that induce a ROS burst onto the pathogens present in wounds, leading to their destruction. During this period, excessive ROS leakage into the surrounding environment exhibits further bacteriostatic effects. In light of these important roles of ROS in wound healing and the continued quest for therapeutic strategies to treat wounds, it is necessary to look for ways to manipulate with ROS as a promising avenue for improving wound-healing responses [12]. On the other hand, several applications of ROS in wound healing have been shown. Cold physical plasmas are particularly effective in promoting wound closure, irrespective of its aetiology. These partially ionized gases deliver a therapeutic cocktail of ROS and RNS safely at body temperature and without genotoxic side effects. Specifically, molecular switches governing redox-mediated tissue response, the activation of the nuclear E2-related factor signalling, together with antioxidative and immunomodulatory responses, and the stabilization of the scaffolding function and actin network in dermal fibroblasts are emphasized in the light of wound healing [13]. This example shows the inconsistency of published results and the need for further research in the role of ROS in wound healing.

Reactive oxygen species are closely connected with nitric oxide and other RNS. There is very close interplay between them – they can create common forms of free radicals; in addition, ROS and RNS are able to partake in the modification of thiol groups, suggesting that the final outcome will be dependent on the concentrations and locations of these molecules [14]. Nitric oxide itself is implicated in cellular and molecular events of wound healing, such as vasodilation, angiogenesis, inflammation, tissue fibrosis, or immune responses. Several studies suggested that NO synthesis is essential to the uncomplicated cutaneous wound healing. NO production is mediated by iNOS that is regulated independently of intracellular calcium elevations. Initial injury is followed by infiltration of inflammatory cells, that is, neutrophils and macrophages, fibroblast repopulation and its transformation to myofibroblast, and new vessel formation as well as keratinocyte migration and proliferation. The major source of TGFβ in a tissue under repairing process is macrophage. Recruitment of macrophage to an injured tissue is

stimulated by NO. It is therefore hypothesized that NO might affect the healing process of cutaneous injury [15]. The process of wound healing is completed by action of other molecules. Growth factors such as epidermal growth factor, fibroblast growth factor, TGF-beta1, and vascular endothelial growth factor and several molecules including hypoxia-inducible factor-1alpha are involved in the healing process by stimulating and activating cell proliferation via activation of various reactions, such as angiogenesis, reepithelialisation, differentiation, and production of the extracellular matrix [16]. All above mentioned facts indicate often contradictory information about involvement of ROS and RNS in wound healing.

Conclusion

Reactive forms of oxygen and nitrogen – basic oxidative stress parameters – play an important role in all phases of wound healing. This "overview" of currently available scientific information offers a framework for the exploration of the role of oxidative stress during wound healing process. Despite of growing attention in the field of oxidative stress research, conclusions of contemporary studies are still contradictory, therefore further intense work is needed to fully understand its role in wound healing process. Based on the present knowledge, it can be concluded that balanced ROS response will debride and disinfect a tissue and stimulate healthy tissue turnover; suppressed ROS will result in infection and an elevation in ROS will destroy otherwise healthy stromal tissue. Understanding and anticipating the ROS function within a tissue will greatly enhance our possibilities to orchestrate the processes of wound healing.

References

1. Marbourg JM, Bratasz A, Mo X et al. Spinal cord injury suppresses cutaneous inflammation: implications for peripheral wound healing. J Neurotrauma 2017; 34(6): 1149–1155. doi: 10.1089/neu.2016.4611.
2. Pokorna A, Benesova K, Muzik J et al. The pressure ulcers monitoring in patients with neurological diseases – analyse of the national register of hospitalized patients. Cesk Slov Neurol N 2016; 79/112 (Suppl 1): S8–S14. doi: 10.14735/amcsnn201658.
3. Sami DG, Heiba HH, Abdellatif A. Wound healing models. A systematic review of animal and non-animal models. Wound Med 2019; 24(1): 8–17. doi: 10.1016/j.wndm.2018.12.001.
4. Doshi BM, Perdrizet GA, Hightower LE. Wound healing from a cellular stress response perspective. Cell Stress Chaperones 2008; 13(4): 393–399. doi: 10.1007/s12192-008-0059-8.
5. Rodrigues M, Kosaric N, Bonham CA et al. Wound healing: a cellular perspective. Physiol Rev 2019; 99(1): 665–706. doi: 10.1152/physrev.00067.2017.
6. Lichtman MK, Otero-Vinas M, Falanga V. Transforming growth factor beta (TGF-beta) isoforms in wound healing and fibrosis. Wound Repair Regen 2016; 24(2): 215–222. doi: 10.1111/wrr.12398.
7. Stolzenburg-Veeser L, Golubnitschaja O. Mini-encyclopaedia of the wound healing – Opportunities for integrating multi-omic approaches into medical practice. J Proteom 2018; 188: 71–84. doi: 10.1016/j.jprot.2017.07.017.
8. Sanchez MC, Lancel S, Boulanger E et al. Targeting oxidative stress and mitochondrial dysfunction in the treatment of impaired wound healing: a systematic review. Antioxidants (Basel) 2018; 7(8): 98–112. doi: 10.3390/antiox7080098.
9. O'Sullivan A, O'Malley D, Coffey J et al. Inhibition of nuclear factor-kappa B and p38 Mitogen-activated protein kinase does not always have adverse effects on wound healing. Surg Infect (Larchmt) 2010; 11(1): 7–11. doi: 10.1089/sur.2007.060.
10. Bryan N, Ahswin H, Smart N et al. Reactive oxygen species (ROS) – a family of fate deciding molecules pivotal in constructive inflammation and wound healing. Eur Cell Mater 2012; 24: 249–265.
11. Nouvong A, Ambrus AM, Zhang ER et al. Reactive oxygen species and bacterial biofilms in diabetic wound healing. Phys Genomics 2016; 48(12): 889–896. doi: 10.1152/physiolgenomics.00066.2016.
12. Dunnill C, Patton T, Brennan J et al. Reactive oxygen species (ROS) and wound healing: the functional role of ROS and emerging ROS-modulating technologies for augmentation of the healing process. Int Wound J 2017; 14(1): 89–96. doi: 10.1111/iwj.12557.
13. Schmidt A, Bekeschus S. Redox for repair: cold physical plasmas and Nrf2 signaling promoting wound healing. Antioxidants (Basel) 2018; 7(10): 146–163. doi: 10.3390/antiox7100146.
14. Hancock JT, Whiteman M. Hydrogen sulfide and reactive friends: the interplay with reactive oxygen species and nitric oxide signalling pathways. In: De Kok LJ, Hawkesford MJ, Rennenberg H et al (eds.). Molecular Physiology and Ecophysiology of Sulphur. Förlag: Springer 2015: 153–168.
15. Kitano T, Yamada H, Kida M et al. Impaired healing of a cutaneous wound in an inducible nitric oxide synthase-knockout mouse. Dermatol Res Pract 2017; 2017: 2184040. doi: 10.1155/2017/2184040.
16. Cowburn AS, Alexander LEC, Southwood M et al. Epidermal deletion of HIF-2 alpha stimulates wound closure. J Investig Dermatol 2014; 134(3): 801–808. doi: 10.1038/jid.2013.395.

doi: 10.48095/cccsnn2022S34

Can different type of the pressure ulcers debridement affect oxidative stress parameters?

Ovlivní typ nekrektomie dekubitů parametry oxidativního stresu?

Abstract

Wound debridement is one of the crucial steps in wound bed preparation for surgical closure. Various debridement forms and tools can be employed. *Aim:* The prospective case series study aimed to compare the impact of two different types of debridement on oxidative stress parameters. *Material and methods:* This study included five patients with pressure ulcers of deep category various localisation. The wound was divided into halves. In one, the sharp debridement was performed, in the second Hydrosurgery Versajet® debridement was accomplished. Tissue, blood and urine samples were collected on days 0 and 7 after the surgery. Histopathological evaluation in tissue samples was performed. Oxidative stress parameters in plasma and urine were evaluated. *Results:* Differences in quality and quantity of granulation tissue between two types of debridement were found. An insignificant decrease of oxidative stress markers in blood plasma and urine 7 days after surgery were observed. *Conclusions:* Preliminary and pilot results suggest that the wound healing process is closely associated with markers of oxidative stress that are measurable in blood plasma and urine. These could be indicative of the healing process. A nonsignificant decrease was observed for all oxidative stress parameters on day 7 after the surgery. The pilot study will be followed by a detailed molecular biological analysis of tissue samples.

Souhrn

Debridement je jedním z nejdůležitějších kroků k přípravě rány před chirurgickým úzávěrem, který může být proveden v různých formách a různými nástroji. *Cíl:* Cílem prospektivní případové studie bylo porovnat vliv dvou různých typů debridementu (nekrektomie) na parametry oxidativního stresu. *Soubor a metodika:* Do studie bylo zařazeno celkem pět pacientů s hlubokými dekubity v různých lokalizacích. Dekubitus byl rozdělen na dvě poloviny; jedna polovina rány byla ošetřena ostrou nekrektomií a druhá Versajet® hydrosystémem. Vzorky tkání, krve a moči byly odebrány v nultý a sedmý den po debridementu. Byla provedena histopatologická analýza a vyšetření parametrů oxidativního stresu v plazmě a moči. *Výsledky:* Byly nalezeny rozdíly v kvalitě a kvantitě granulační tkáně mezi dvěma různými typy provedeného debridementu. Zjištěno bylo nesignifikantní snížení markerů oxidativního stresu v plazmě a moči sedmý den po debridementu. *Závěr:* Předběžné a pilotní výsledky naznačují, že proces hojení ran je úzce spojen s markery oxidativního stresu, které jsou měřitelné v krevní plazmě i v moči. Tyto by mohly poukazovat na průběh hojení. U všech parametrů oxidativního stresu bylo pozorováno nesignifikantní snížení sedmý den po zákroku. Na pilotní studii naváže podrobná molekulárně-biologická analýza vzorků tkáně.

The Editorial Board declares that the manuscript met the ICMJE "uniform requirements" for biomedical papers.

Redakční rada potvrzuje, že rukopis práce splnil ICMJE kritéria pro publikace zasílané do biomedicínských časopisů.

P. Šín[1], A. Hokynková[1], M. Nováková[2], H. Paulová[3], P. Babula[2], A. Pokorná[4], L. Nártová[1], P. Coufal[1], M. Hendrych[5]

[1] Department of Burns and Plastic Surgery, Faculty of Medicine, Masaryk University and University Hospital, Brno

[2] Department of Physiology, Faculty of Medicine, Masaryk University, Brno

[3] Department of Biochemistry, Faculty of Medicine, Masaryk University, Brno

[4] Department of Health Sciences, Faculty of Medicine, Masaryk University, Brno

[5] First Department of Pathology, St. Anne's University Hospital Brno and Faculty of Medicine, Masaryk University, Brno

✉

Alica Hokynková, MD, PhD
Department of Burns and Plastic Surgery
Faculty of Medicine
Masaryk University and University Hospital
Jihlavská 20
625 00 Brno
e-mail: alicah@post.cz

Key words

pressure ulcer – pressure injury – debridement – Versajet hydrosurgery system – oxidative stress parameters

Klíčová slova

dekubitus – tlakové poranění – debridement – Versajet hydrosystém – parametry oxidativního stresu

Introduction

Surgical treatment of pressure ulcers (PUs) is primarily intended for PUs of a deep category (III and IV category). Meticulous surgical preparation of wounds in various debridement approaches is necessary for successfully reconstructing Pus [1–3] and represents the most crucial technique in wound management [4,5]. By eliminating bacterial colonisation, debridement decreases an inflammatory response and the risk of sepsis [6]. Moreover, it reduces exudate and odour [7] and improves wound healing [8]. Sharp debridement (using scalpel, scissors or electrocautery) [9] was described in 1950 by Cannon et al [10]. Nowadays, it is mainly used type of debridement in PUs surgical therapy. Other debridement techniques were introduced, such as enzymatic [11], ultrasonic [12], Versajet Hydrosurgery system [13], etc. The decision of which type of debridement should be used depends on the wound size, localisation, character of present avital tissue (thickness of eschar, slough, debris etc.) and on preferences and experience of the surgeon.

Hydrosurgery debridement is based on a high-powered jet of saline, enabling cutting tissue simultaneously with a suction of debrided particles which diminishes an aerosolisation effect in the wound [9]. Numerous studies indicate that reactive oxidative species (ROS) are involved in the wound healing process [14–16]. Aim of the present study was to compare the impact of two different types of debridement on oxidative stress parameters in tissue samples from the same PU. Conventional sharp debridement using scalpel and hydrosurgery Versajet system were compared.

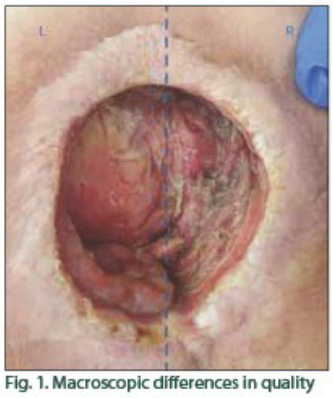


Fig. 1. Macroscopic differences in quality and quantity of granulation tissue in left part of the wound treated by Versajet® hydrosurgery and right part treated by sharp debridement.

Obr. 1. Makroskopické změny v kvalitě a kvantitě granulační tkáně v levé polovině rány (debridement proveden Versajet® hydrosystémem) a v pravé polovině rány (ošetřeno chirurgickou ostrou nekrektomií).

Material and methods

This study included five patients with PUs of deep category (III and IV) and various localisation (sacral, trochanteric and ischial). All these patients were indicated to the surgical therapy. The wound was divided into halves. In one halve, the sharp debridement and the second halve debridement using Hydrosurgery Versajet® were performed. Tissue samples were harvested before debridement from each half in the first phase and the same place one week after debridement, immediately before surgical closure using flap (fasciocutaneous or musculocutaneous) reconstruction. Tissue samples (3) were fixed in formalin and routinely processed into formalin-fixed paraffin-embedded tissue specimens. Histopathological evaluation of tissue samples was performed using hematoxylin-eosin staining and immunohistochemical analysis of alpha-smooth muscle actin (Abcam, Czech Republic). Blood samples were collected on day 0 and day 7 after the necrectomy. After blood processing and deproteinization (10kD Spin Column, Abcam, Czech Republic, ab93349), the thiols in plasma samples were measured fluorimetrically using 7-azido-4-methylcoumarin as a fluorescent probe (λex = 365 nm and λem = 450 nm; Sigma-Aldrich, USA). The method was optimized for plasma samples. The amount of reactive nitrogen species was measured using the enzymatic conversion of nitrate to nitrite by nitrate reductase, followed by the addition of 2,3-diaminonapthalene (DAN, Sigma-Aldrich, USA), and NaOH, which converts nitrite to a fluorescent compound (λex = 365 nm and λem = 450 nm). Total oxidation stress was measured fluorimetrically using 2',7'-dichlorodihydrofluorescein compound (λex = 492 nm and λem = 515 nm; Sigma-Aldrich, USA). The amount of hydrogen peroxide was measured using a fluorimetric hydrogen peroxide assay kit (Sigma-Aldrich, USA, MAK165). Morning urine samples were collected at the same time points as blood samples. Urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) was quantified by liquid chromatography with tandem mass spectrometry (triple quadrupole EVOQ CUBE, Bruker, Germany) after SPE purification.

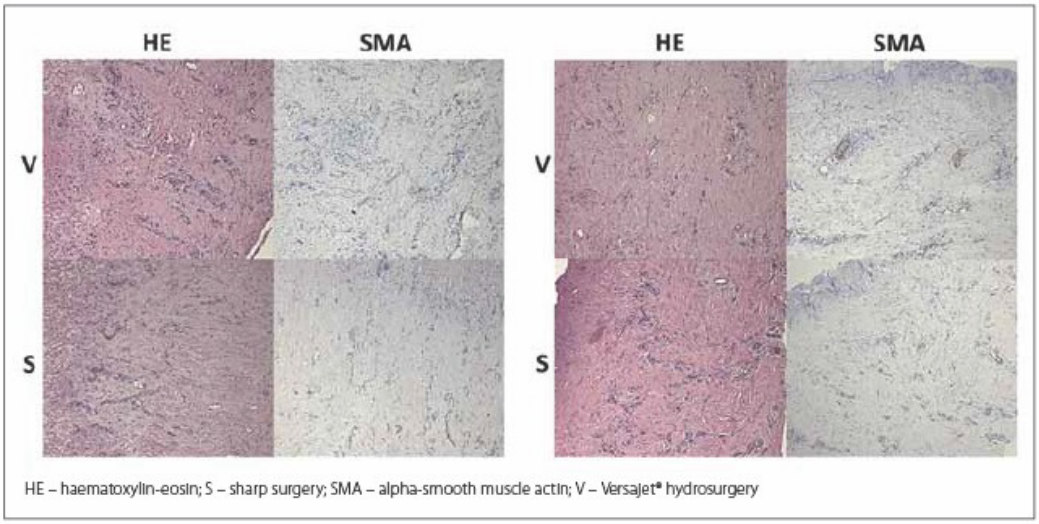
Results

In most cases, a significant difference in the quality of debridement between both types of necrectomy was observed. With hydrosurgery using Versajet®, adequate debridement was obtained in one session only in all cases. In contrast, sharp debridement parts often required further wound bed re-evaluation or debridement during the observed period.

The clinically significant difference in wound bed appearance was also detected after use of Versajet® hydrosurgery, with macroscopically visible signs of improved healing in the form of pink granulation tissue, as shown on the left side in Fig. 1. This difference was even more visible in deep convex surfaces, where effective sharp debridement is technically hard to achieve. It was possible to obtain a macroscopically clean wound bed with overall petechial bleeding even in these complex wounds.

Histopathological analysis of 3 patient's tissue samples revealed granulation tissue without any significant morphological differences in Versajet technique therapy treated samples and sharp surgery. Immunohistochemical expression of alpha-smooth muscle actin displayed increase in smooth muscle cells within the newly formed vessels and stromal myofibroblasts between the first and second tissue samples. No significant differences in different therapy technique were detected (Fig. 2).

Blood analysis was focused on oxidative stress parameters. The results obtained indicate a decrease in the levels of total oxidative stress (68,456.7 ± 15,498.5 A.U. for day 0, 58,459.2 ± 8,889.00 A.U. for day 7, respectively), hydrogen sulphide (106.54 ± 20.71 nmol.l^{-1} for day 0, 101.90 ± 14.06 nmol.l^{-1} for day 7, respectively), hydrogen peroxide (3.40 ± 2.91 µmol.l^{-1} for day 0, 2.73 ± 1.56 µmol.l^{-1} for day 7, respectively), and reactive nitrogen species (12,585.9 ± 7,806.2 A.U. for day 0, 9,993.8 ± 4,400.7 A.U. for day 7, respectively) on day 7 as compared to day 0. However, this decrease was insignificant (Fig. 3). There was an insignificant decrease in the level of 8-hydroxy-2'-deoxyguanosine, a marker of oxidative stress, on day 7 as compared to day 0 (8.63 ± 2.12 ng/mg creatinine for day 0, 8.10 ± 2.41 ng/mg creatinine for day 7, respectively; for details, see Fig. 4).



HE – haematoxylin-eosin; S – sharp surgery; SMA – alpha-smooth muscle actin; V – Versajet® hydrosurgery

Fig. 2. Histopathological evaluation of tissue samples. Mag. 100×.
Obr. 2. Histopatologické vyšetření vzorků tkáně. Zvětšeno 100×.

Discussion

Wound healing is a complex process involving the orchestration of different hormones, growth factors and cytokines [15]. Among the crucial compounds playing a role in wound healing, reactive oxygen species can be found. They are implemented simultaneously in several lines. Due to their toxicity, they are an essential component of protection against pathogenic organisms while at the same time playing a vital signalling role in a wide range of processes [16]. Whereas the importance of reactive oxygen species in wound healing has been intensely studied, data showing their relationship to changes in circulating reactive oxygen species (in blood plasma) concerning wound healing processes have not yet been elucidated. The work of James et al showed a correlation between allantoin and uric acid concentrations in patients suffering from leg ulcers [17]. Significant elevation between al-

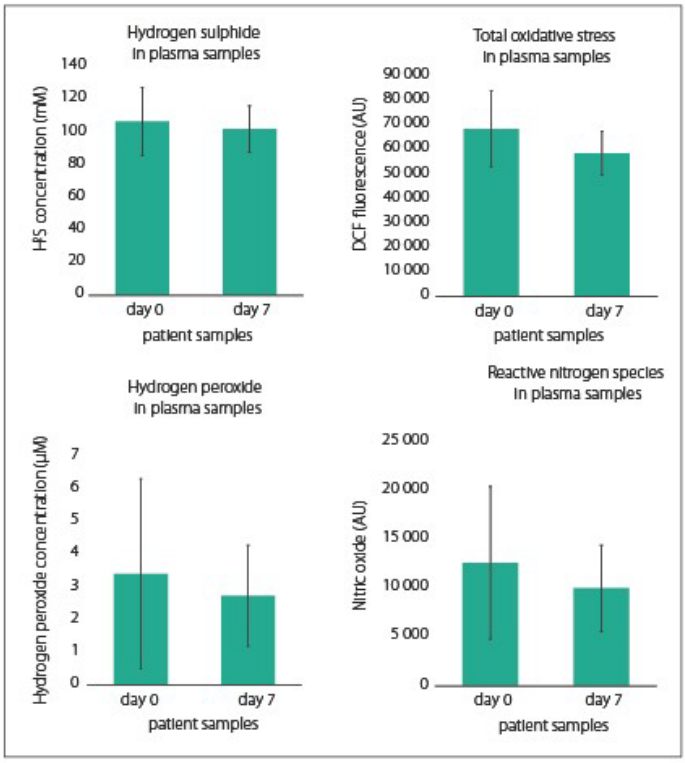


Fig. 3. Concentrations of hydrogen sulfide and hydrogen peroxide and total oxidative stress parameters and reactive nitrogen species in plasma at day 0 and day 7 after surgery (N = 7).
Obr. 3. Koncentrace hydrogen sulfidu a hydrogen peroxidu, parametry oxidativního stresu a reaktivních forem dusíku v plazmě ve dnech 0 a 7 po operaci (n = 7).

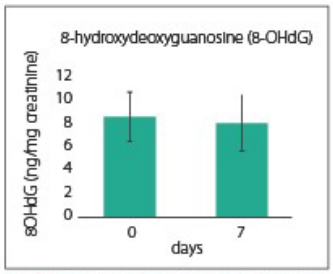


Fig. 4. Changes in concentration of 8-hydroxydeoxyguanosine in urine at day 0 and day 7 after surgery (N = 10).
Obr. 4. Změny koncentrace 8-hydroxy-2´-deoxyguanosin v moči ve dnech 0 and 7 po operaci (n = 10).

lantoin: uric acid percentage ratio was observed in wound fluid from chronic leg ulcer compared to both paired plasma and acute surgical wound fluid. Pressure ulcers development was also studied in an animal model. The elevated level of 8-OHdG was detected in prolonged compressed muscles in mice indicating increased oxidative stress [18]. Moseley et al. discuss the roles of ROS/antioxidants in skin wound healing, their possible involvement in chronic wounds and the potential value of ROS-induced biomarkers in wound healing prognosis [19]. However, the study focuses on the analysis of wound fluids, emphasising total protein carbonyl content, western blot analysis of protein carbonyl content, malondialdehyde content, and total antioxidant capacity in wound fluids. The present study focused on the analysis of blood plasma and urine. The results indicate a decrease of determined markers of oxidative stress in blood plasma and urine on the 7th day after Versajet®/sharp surgery. However, the observed decrease is insignificant. The presented results become from a pilot study; we plan to extend the number of patients included in the study. Then the significance of the results may be expected.

On the other hand, the results indicate the importance of oxidative stress parameters and their changes concerning wound healing. Next, it will be necessary to correlate the obtained data with other outcomes, such as biochemical parameters and blood analysis. In addition, the study will be extended to include gene expression analysis of candidate genes associated with ROS and selected enzyme activities and markers of oxidative stress in tissue samples.

Conclusions

The pilot study results indicate that wound healing is closely connected to amounts of reactive oxygen and nitrogen species and total antioxidant capacity in corresponding tissue.

Ethical aspects

Institutional Ethical Committee approved this study of Faculty Hospital Brno (Reference Number 17-100620/EK, Project Number 68/20, date 14. 6. 2020).

Acknowledgement

This work was supported by the Ministry of Health of the Czech Republic under grant No. NU21-09-00541 "The role of oxidative stress in the healing of pressure ulcers in patients with spinal cord lesions".

Conflict of interest

The authors declare they have no potential conflicts of interest concerning drugs, products, or services used in the study.

References

1. Agren MS, Strömberg HE. Topical treatment of pressure ulcers. A randomized comparative trial of Varidase and zinc oxide. Scand J Plast Reconstr Surg 1985; 19(1): 97–100. doi: 10.3109/02844318509052871.
2. Hokynková A, Šín P, Černoch F et al. Employment of flap surgery in pressure ulcers surgical treatment. Cesk Slov Neurol N 2017; 80/113 (Suppl): S41–S44. doi: 10.14735/amcsnn2017S41.
3. Černoch F, Jelínková Z, Rotschein P. Reconstruction of recurrent ischiadic pressure ulcer using turnover hamstring muscle flap. Cesk Slov Neurol N 2019; 82/115 (Suppl): S15–S18. doi: 10.14735/amcsnn2019S15.
4. Ligresti C, Bo F. Wound bed preparation of difficult wounds: an evolution of the principles of TIME. Int Wound J 2007; 4(1): 21–29. doi: 10.1111/j.1742-481X.2006.00280.x.
5. Pilcher M. Wound cleansing: a key player in the implementation of the TIME paradigm. J Wound Care 2016; 25 (Suppl 5): S7–S9. doi: 10.12968/jowc.2016.25.Sup3.S7.
6. Longe RL. Current concepts in clinical therapeutics: pressure sores. Clin Pharm 1986; 5(8): 669–681.
7. Baranoski S. Pressure ulcers: a renewed awareness. Nursing 2006; 36(8): 36–42. doi: 10.1097/00152193-200608000-00037.
8. Schiffman J, Golinko MS, Yan A et al. Operative debridement of pressure ulcers. World J Surg 2009; 33(7): 1396–1402. doi: 10.1007/s00268-009-0024-4.
9. Shimada K, Ojima Y, Ida Y et al. Efficacy of Versajet hydrosurgery system in chronic wounds: a systematic review. Int Wound J 2021; 18(3): 269–278. doi: 10.1111/iwj.13528.
10. Cannon B, O'leary JJ, O'neil JW et al. An approach to the treatment of pressure sores. Trans Meet Am Surg Assoc Am Surg Assoc 1950; 68: 439–457.
11. Rosenberg L, Krieger Y, Bogdanov-Berezovski A et al. A novel rapid and selective enzymatic debridement agent for burn wound management: a multi-center RCT. Burns 2014; 40(3): 466–474. doi: 10.1016/j.burns.2013.08.013.
12. Messa CA 4th, Chatman BC, Rhemtulla IA et al. Ultrasonic debridement management of lower extremity wounds: retrospective analysis of clinical outcomes and cost. J Wound Care 2019; 28 (Suppl 5): S30–S40. doi: 10.12968/jowc.2019.28.Sup5.S30.
13. Matsumura H, Nozaki M, Watanabe K et al. The estimation of tissue loss during tangential hydrosurgical debridement. Ann Plast Surg 2012; 69(5): 521–525. doi: 10.1097/SAP.0b013e31826d2961.
14. Dunnill C, Patton T, Brennan J et al. Reactive oxygen species (ROS) and wound healing: the functional role of ROS and emerging ROS-modulating technologies for augmentation of the healing process. Int Wound J 2017; 14(1): 89–96. doi: 10.1111/iwj.12557.
15. Schäfer M, Werner S. Oxidative stress in normal and impaired wound repair. Pharmacol Res 2008; 58(2): 165–171. doi: 10.1016/j.phrs.2008.06.004.
16. Hokynková A, Babula P, Pokorná A et al. Oxidative stress in wound healing – current knowledge. Cesk Slov Neurol N 2019; 82/115 (Suppl 1): S37–S39. doi: 10.14735/amcsnn2019S37.
17. James TJ, Hughes MA, Cherry GW et al. Evidence of oxidative stress in chronic venous ulcers. Wound Repair Regen 2003; 11(3): 172–176. doi: 10.1046/j.1524-475x.2003.11304.x.
18. Wong SW, Cheung BC, Pang BT et al. Intermittent vibration protects aged muscle from mechanical and oxidative damage under prolonged compression. J Biomech 2017; 55: 113–120. doi: 10.1016/j.jbiomech.2017.02.023.
19. Moseley R, Hilton JR, Waddington RJ et al. Comparison of oxidative stress biomarker profiles between acute and chronic wound environments. Wound Repair Regen 2004; 12(4): 419–429. doi: 10.1111/j.1067-1927.2004.12406.x.

Physiol. Res. 72 (Suppl. 5): S535-S542, 2023 *https://doi.org/10.33549/physiolres.935222*

Changes in Gene Expression in Pressure Ulcers Debrided by Different Approaches – a Pilot Study

Amir SAMADIAN[1], Monika KRATOCHVÍLOVÁ[1], Alica HOKYNKOVÁ[2], Petr ŠÍN[2], Marie NOVÁKOVÁ[1], Petr ŠTĚPKA[1], Andrea POKORNÁ[3], Petr BABULA[1]

[1]Department of Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic, [2]Department of Burns and Plastic Surgery, University Hospital Brno and Faculty of Medicine, Masaryk University, Brno, Czech Republic, [3]Department of Health Sciences, Faculty of Medicine, Masaryk University, Brno, Czech Republic

Received March 31, 2023
Accepted September 11, 2023

Summary

Pressure ulcers (PUs), also known as pressure injuries, are chronic wounds that represent potential lifelong complications. Pressure ulcers of a deep category (III and IV) are often indicated for surgical treatment – debridement and surgical reconstruction. Sharp surgical debridement is widely used in the debridement of PUs; however, the Versajet® hydrosurgery system is becoming an increasingly popular tool for tangential excision in surgery due to its numerous advantages. This work focused on the expression of selected genes, especially those associated with oxidative stress, in PUs debrided by two approaches – sharp surgical debridement and debridement using Versajet® hydrosurgery system. Expression of following genes was evaluated: *NFE2L2, ACTA2, NFKB1, VEGFA, MKI67, HMOX1, HMOX2, HIF1A,* and *SOD2. ACTB* and *PSMB* were used as housekeeping genes. So far, five patients have been enrolled in the study. Preliminary results suggest no significant difference in gene expression with different pressure ulcer treatment approaches except *NFE2L2*, despite the macroscopic differences. However, the results revealed correlations between the expression of some genes, namely *HIF1A* and *SOD2*, *VEGFA* and *SOD2* and *VEGFA* and *HIF1A*. These results may indicate a connection between hypoxia, oxidative stress, pressure ulcer healing processes and angiogenesis.

Key words

Pressure ulcers • Debridement • Wound healing • Oxidative stress • Gene expression

Corresponding author

A. Hokynková, Department of Burns and Plastic Surgery, University Hospital Brno, Jihlavská 20, 625 00 Brno, Czech Republic, Faculty of Medicine, Masaryk University, Kamenice 5, 625 00, Brno, Czech Republic. E-mail: alicah@post.cz

Introduction

A pressure ulcer (PU) is defined by the European Pressure Ulcer Advisory Panel as an area of localized damage to the skin and underlying tissue caused by pressure, shear, or friction, or a combination of these [1]. Pressure ulcers are caused by a local breakdown of soft tissue as a result of compression between a bony prominence and an external surface [2]. Soft tissue deformation beyond certain tolerance threshold also occurs, leading to direct deformation and damage of the cells due to structural failure of the cytoskeleton and plasma membrane [3,4].

The highest prevalence of PUs is in high-income North America followed by Central America, tropical Latin America and Carribean, and the lowest in the south and central Asia. Compared to high-income North America, central Europe ranks tenth with a prevalence of 7.8 per 100000 population [5]. Between 2010-2019, 264442 patient records with diagnoses L89.0-L89.9 (PUs diagnoses in ICD-International Classification of Diseases and Related Health Problems) were identified (an average of 26444 patients per year) in the Czech Republic. Numbers have increased each year, with a 40 % increase

PHYSIOLOGICAL RESEARCH • ISSN 1802-9973 (online) - an open access article under the CC BY license

Fax +420 241 062 164, e-mail: physres@fgu.cas.cz, www.biomed.cas.cz/physiolres

between 2010 and 2019 [6]. Analyses of national health registries showed that the prevalence of PUs before the onset of the COVID-19 pandemic and during the 2020 pandemic was higher in patients hospitalized with SARS-CoV-2 infection [7]. It is evident that PUs represent a significant socio-economic and health problem. Management of PUs is complex and involves a change in patient care, including the use of various aids to prevent its occurrence, i.e. avoiding pressure, friction or shear (pressure-relieving strategies, repositioning or 'turning' patients), good skin-care regime and managing exacerbating factors, such as urinary or fecal incontinence, nutrition therapy, but also surgical treatment – debridement – followed by pressure ulcer healing management [8]. Multiple techniques, such as mechanical, biological or surgical, are used to debride. Determination of the most appropriate technique mandates the consideration of both host-specific (i.e. comorbidities, compliance, social support, etc.) and wound-related (i.e. infection/contamination, perfusion, viability, etc.) factors, as well as the resources available at the treatment facility. The European Wound Management Association guidelines for debridement provide specific information regarding each technique's indications, contraindications, and potential adverse effects [9,10].

New approaches and procedures are being introduced in the field of surgical debridement. One of these techniques is the hydrosurgery system, which utilizes a high-pressure parallel water jet promoting the Venturi effect. Its use was first described by Klein *et al.* in 2005 as a new tool for tangential dissection [11]. This technique has gradually spread, especially in managing chronic wounds and burns. It is gradually proving to offer a number of advantages, including lower blood loss during the procedure and faster wound healing [12]. Unfortunately, despite the use of Versajet hydrosurgery in clinical practice, our knowledge about its impact on healing is still limited. Therefore this study aimed to compare two approaches to category III and IV PUs treatment – sharp debridement (performed with a scalpel and/or electrocauter) and Versajet® hydrosurgery system. This treatment represented the first surgical step before reconstruction using flap plasty. The obtained samples were analysed for gene expression levels of genes related to oxidative stress and healing processes to gain new information about the differences between the two techniques at the molecular biological level.

Methods

Experimental design

Prospective interventional study in which a total of five patients with PUs larger than 5×5 cm of various localizations were included in the pilot phase. Basic data of patients describes Table 1. The PU bed was divided into two halves, and each half was subsequently debrided with a different approach – sharp or hydrosurgery (Versajet®) debridement. Tissue samples were collected from each half of the PU before and one week after the debridement. Samples were always collected in the same manner with respect to the size and the depth of the PU. Samples collected in this way were processed immediately after collection, i.e. placed in an RNA-later (Roche, Czech Republic).

Tissue samples were mechanically homogenized by microtube pestle. RNA was isolated from homogenized tissue using TriPure Isolation Reagent (Roche, Basel, Switzerland) according to the manufacturers' protocol and then transcribed using using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Waltham, MA, USA) in accordance with the manufacturer's instructions.

Analysis of tissue samples

The quantitative RT-PCR was carried out using TaqMan gene expression assays with the LightCycler®480 II System (Roche, Basel, Switzerland). The amplified cDNA was analyzed by the comparative ddCt method using *PSMB* as a reference. The primer and probe sets for *PSMB2* (Hs01009704_m1, housekeep), *ACTB* (Hs99999903_m1, housekeep), *NFE2L2* (Hs00975961_g1), *ACTA2* (Hs05005341_m1), *NFkB1*(p50/p105) (Hs00765730_m1), *EGF* (Hs01099999_m1), *VEGFA* (Hs00900055_m1), *MKI67* (Hs00606991_m1), *HMOX1* (Hs01110250_m1), *HMOX2* (Hs01558390_m1), *GPX1* (Hs07288100_g1), *HIF1A* (Hs00153153_m1), *SOD2* (Hs00167309_m1), *NOS2* (Hs01075529_m1), and *ANGPT4* (Hs00907074_m1) were selected from the TaqMan Gene Expression Assays (Life Technologies, USA). The selection of genes for expression monitoring was based on available data in the literature with respect to oxidative stress [13,14]. The qRT-PCR was executed under the following amplification conditions: total volume of 20 µl, initial incubation at 50 °C/2 min, denaturation at 95 °C/10 min, then 45 cycles at 95 °C/15 s and 60 °C/1 min.

Table 1. Basic clinical data of patients.

Gender/Age	PU category	Size of PU (length × width × depth)	PU location	Wound bed	Type of flap	Comorbidities	Swab (admission date)
F/55	IV	8×5×3 cm	Sacral	Slough, appropriate secernation, bone not exposed	FC gluteal rotation bilateral flap	Paraplegia, st. p. PU sepsis year ago	*Streptococcus alfa haemolyticus, Staphylococcus aureus, Candida albicans*
M/42	III	9×7×1 cm	Hip-left-sided	Intact fascia, bone not exposed	Tensor fasciae latae muscle flap (MTFL)	Quadriplegia, nefrotic syndrome, amyloidosis, hypothyreosis, mild ventilation disorder, polymorbidities	*Acinetobacter baumanii*
F/58	IV	5×5×2 cm	Sacral	Rolled hard edges, poor granulation, coated pseudocyst	FC gluteal rotation unilateral flap	Quadriparesis, sclerosis multiplex, polymorbidities	*Proteus mirabilis, Acinetobacter baumannii*
M/68	IV	6×6×2 cm	Ischial-right-sided	PU rather sessile, granulation poor, elbow slightly loose, bone not exposed	Dorsal thigh flap+distal portion of gluteus maximus muscle	Quadriplegia, M. Recklinghausen, polymorbidities	*Staphylococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis*
M/63	IV	6×3×2 cm	Ischial-left-sided	Rolled edges, macrosopically clean, bone intact, no signs of inflammation	Dorsal thigh FC flap	Paraplegia, sideropenic anemia	*Beta-haemolytic Streptococcus group G*

Statistical analysis

The statistical analysis was performed using R 4.0.2 language with the following packages: ggplot2, tidyverse, corrplot, rstatix [15-19]. The data from qRT-PCR analysis were evaluated with the "ddCt" method, where the relative expression of each gene was referred to *PSMB* and mean values as control. Considering the distribution of the data, logarithmic values were used for statistical analysis. Multifactorial ANOVA was calculated, but better the Wilcoxon test was used to determine the significant differences between individual treatments, since the data did not meet the assumptions for parametrical testing (Levene's Test for Homogeneity of Variance, Shapiro-Wilk normality test).

Spearman correlations were computed to analyze the relations in the data. For summary, the heatmap with dendrogram was created. For the dendrogram, non-hierarchical cluster analysis was used with calculation of the Euclidean distance both for individual samples and for individual variables. Unless noted otherwise $p \leq 0.05$ was considered significant.

Results

In expression analysis, there were 9 gene expressions in 20 samples (all in duplicates) detected. From each patient (5) in the study were collected 4 different samples (each in duplicate). The sample was

either treated with Versajet®, or collected surgically and also monitored in two individual samplings. The overall effects on expression profiles of treatment or sampling were determined with multifactorial ANOVA, but the data did not meet the requirements for parametric statistical analysis. The mutual differences between individual patients were greater than the effects of the used treatments. For example, the effect of a patient factor on *NFKB1* expression (Welch´s One-way analysis of means, not assuming equal variances, F=4.6394, num. df.=4.000, denom. df.=17.189, p=0.0101), or *VGEFA* expression (F=5.2973, num. df.=4.000, denom. df.=16.599, p=0.006126). Hence individual differences were detected with the Wilcoxon test. This analysis revealed a significant change between the expression of *NFE2L2* after the first (mean=-0.5892) and the second sampling (mean=-0.7358) that were treated with Versajet® (W=77, p=0.04507, n=10) (Fig. 1).

The correlation analysis was performed as well. Spearman correlations were computed for each treatment and sampling, and the 10 most significant ones ($p<0.05$) are presented in Figure 2. The pairs unique for each treatment are worth mentioning. The expression of *NFE2L2* correlates positively with *NFKB1* after the first sampling and surgical treatment (r=0.91, p=0.0002). The only significant negative correlation is found between *ACTA2* and *NFKB1* (r=-0.902, p=0.0004), and between *NFE2L2* and *NFKB1* (r=-0.801, p=0.005, not shown) after the second sampling and surgical treatment. This is in contrast with the results after the first sampling. Some correlations are specific for VersaJet samples, i.e. *VGEFA* and *HMOX2* (r=0.92, p=0.00016, the first sampling; r=0.86, p=0.0013, the second sampling), and *NFKB1* and *HMOX1* (r=0.88, p=0.0008, the second sampling).

All gene expression profile results were summarized in a heatmap with cluster analysis (Fig. 3). The mean expression values after all treatment combinations show several trends. The genes form three separate clusters, the ones that increased their expression after the treatment (*HIF1A*, *SOD2*), the ones that were suppressed (*NFE2L2*, *HMOX2*) and the rest that showed minor changes. Also, the samples created some clusters, where the ones after the first sampling show more similarities, regardless of the treatment.

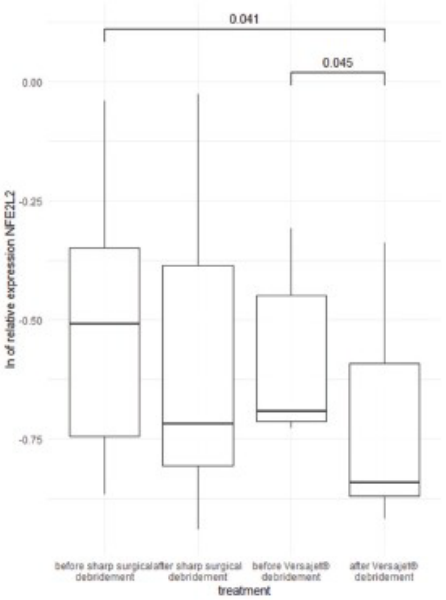


Fig 1. The log of relative expression of NFE2L2. The box and whisker plot presents the median values after all treatments and their significant differences. The hinges correspond to the 1st and 3rd quartiles and the whiskers show 1.5*IQR (interquartile range). The depicted statistical significance is result of Wilcoxon test. The samples treated with Versajet® after the first sampling have higher relative expression of NFE2L2 than the same treated samples after the second sampling (p=0.045). Also, the samples treated surgically have higher relative expression of this gene (p=0.041).

Discussion

Analysis of tissue samples taken from PUs treated with different debridement techniques – sharp surgery (scalpel or electrocauter) versus Versajet® hydrosurgery – yielded almost no differences in the expression of the studied genes. However, the expression of *NFE2L2* was significantly reduced in case of Versajet as compared to sharp surgery (the first sampling: surgery versus Versajet) and in case of the second sampling of Versajet as compared to the first sampling of Versajet. *NFE2L2* is gene coding Nuclear Factor Erythroid 2-Related Factor 2 (NF-E2-Related Factor 2, respectively Nrf-2). It is a transcription factor, which is under normal conditions relatively rapidly degraded in the cells. Still, under oxidative stress conditions, it is transported to the nucleus, where it binds to the DNA promoter region and triggers the expression of genes encoding enzyme–protein–antioxidant mechanisms. This function has been studied in case of the antioxidant action of compounds of natural origin [20,21], but particularly in the context of certain types of cell deaths, especially apoptosis [22],

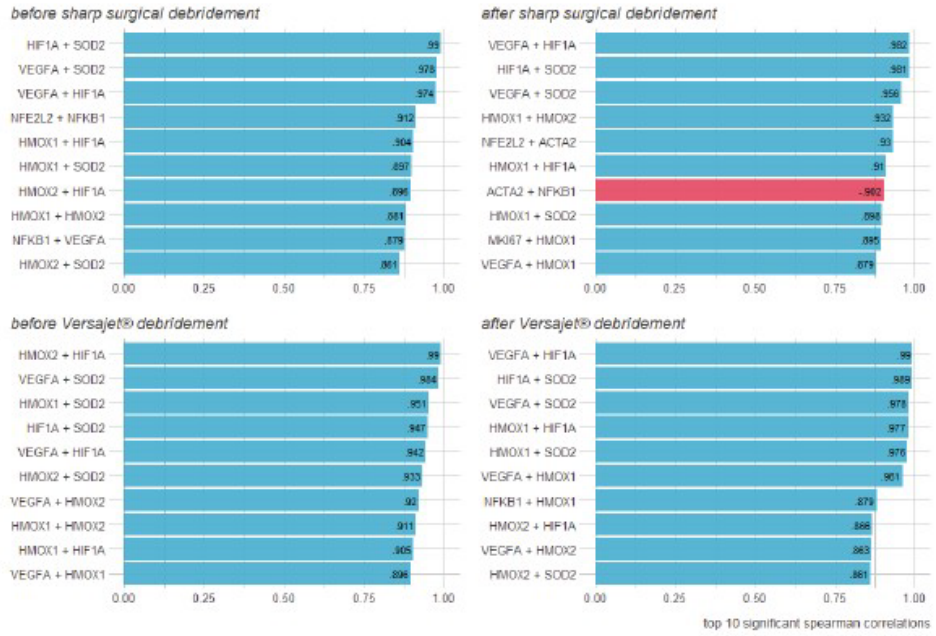


Fig. 2. Ten most important correlations for each treatment and sampling combination. The length of the column expresses the size of the correlation coefficient R (Spearman's correlation), blue shows positive correlation, red is negative. All depicted correlations are significant ($p<0.05$).

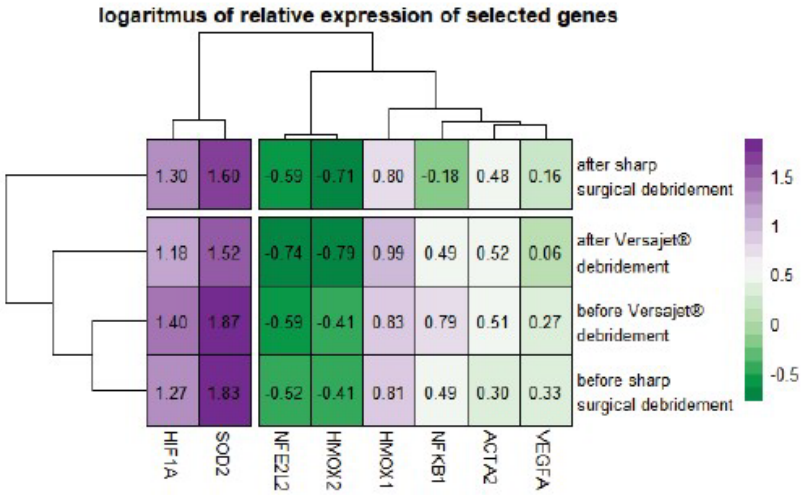


Fig. 3. The picture presents all obtained gene expression results. The mean of relative expression is depicted with color (high expression in purple, low in green). The dendrograms show the differences and similarities between individual genes and treatments. The highest relative expression has SOD2 in all samples, the lowest HMOX2, in particular after the second sampling and Versajet® treatment. Samples from the first samples collection regardless the treatment cluster together. For example, the genes HMOX2 and NFE2L2 show similar trends in expression.

autophagy [23], or pyroptosis [24]. The question arises what is the relationship between the expression of this gene and the pressure ulcer healing process. Reactive oxygen species (ROS) and nitrogen species (RNS) play an essential role in the wound healing process, being involved in different phases of the healing process. Van Huizen *et al.* reports that the first significant accumulation of ROS occurs at the wound site after the first hour, and ROS are required for wound closure [25]. Subsequently, they are implemented in the regulation of actin-mediated epithelial stretching and rearrangement of adjacent cells over the wound surface. Their importance also lies in the regulation of cell signalling during the wound healing process. The importance of ROS in the wound healing process is described and discussed in detail for example, in a review by Krizanova *et al.* [26] or Hokynkova *et al.* [27]. So far, several genes have been identified expression of which is directly controlled by ROS. The same authors [25] describe ROS-dependent jun-1 and HSP70 expression regulation. Jun is a transcription factor that, together with Fos, forms the transcription factor AP-1 (Jun and Fos are its subunits), which in turn modulates the expression of MMP (matrix metallopeptidase)-2 and MMP-9 [28]. Both are significant players in regulating extracellular matrix degradation and deposition essential for wound reepithelialization [29,30]. The importance of HSP70 in the wound healing process has also been described [31]. Nrf-2 is another important protein whose association with the previous ones has been reported, but not sufficiently studied in the wound healing process. Expression and activity of NRF-2 in wounds have been found in keratinocytes as well as in other cells in granulation tissue as a response to ROS [32]. Several studies have shown that elevated levels of Nrf-2 temporarily modulate the expression of vascular genes in wounds, which may accelerate the healing of chronic wounds. The mechanism of action of Nrf-2 is still not precisely known, but Nrf-2 is likely to be essential during the inflammation and proliferation phases of wound repair [33]. From this perspective, the pilot results could indicate a potential benefit of the Versajet technique in Pus healing.

The results of the correlation analysis are also interesting. The association between the expression of genes for neovascularization, antioxidant proteins and HIF1A is shown for each intervention. In case of the Versajet® technique, the specific correlation was found between gene expression of *VGEFA* (vascular endothelial growth factor A) and *HMOX2* (heme oxygenase 2) and *NFKB1* (nuclear factor NF-kappa-B p105 subunit) and *HMOX1* (heme oxygenase 1). Vascular endothelial growth factor A (keratinocyte-derived) binds primarily to endothelial cell receptors to promote angiogenesis which plays a key role in wound healing [34]. Correlation in *VGEFA* and *HMOX2* expression suggests the link between oxidative stress, or hypoxia, and new blood vessel formation [35].

The presented data come from a pilot study, which has so far been conducted on a small sample of patients. Although it suggests some interesting correlations in the expression of various genes, further extension of the study and additional analyses are needed to provide new insights and allow us to determine the potential benefit of Versajet technique in the debridement of PUs category III and IV.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

This work was supported by the Ministry of Health of the Czech Republic under grant no. NU21-09-00541, "The role of oxidative stress in pressure ulcers treatment in a patient with spinal injury".

References

1. Haesler E. European Pressure Ulcer Advisory Panel (EPUAP); National Pressure Injury Advisory Panel (NPIAP); Pan Pacific Pressure Injury Alliance (PPPIA), Prev Treat Press Ulcers Injuries Clin Pract Guidel Int Guidel, 2019.
2. Grey JE, Harding KG, Enoch S. Pressure ulcers. BMJ 2006;332:472-475. https://doi.org/10.1136/bmj.332.7539.472
3. Slomka N, Gefen A. Relationship between strain levels and permeability of the plasma membrane in statically stretched myoblasts. Ann Biomed Eng 2012;40:606-618. https://doi.org/10.1007/s10439-011-0423-1
4. Shoham N, Gefen A. Deformations, mechanical strains and stresses across the different hierarchical scales in weight-bearing soft tissues. J Tissue Viability 2012;21:39-46. https://doi.org/10.1016/j.jtv.2012.03.001
5. Zhang X, Zhu N, Li Z, Xie X, Liu T, Ouyang G. The global burden of decubitus ulcers from 1990 to 2019. Sci Rep 2021;11:21750. https://doi.org/10.1038/s41598-021-01188-4

6. Búřilová P, Pokorná A, Benešová K, Mužík J, Klugar M, Krupová L, Dolanová D, ET AL. A ten-year follow-up of the prevalence of pressure injuries in the Czech Republic: Analysis of the National Registry. Int Wound J 2022;19:1870-1877. https://doi.org/10.1111/iwj.13793
7. Pokorná A, Dolanová D, Benešová K, Búřilová P, Mužík J, Jarkovský J, Krupová L, ET AL. How the COVID-19 pandemic influences the prevalence of pressure injuries in the Czech Republic: A nationwide analysis of a health registry in 2020. J Tissue Viability 2022;31:424-430. https://doi.org/10.1016/j.jtv.2022.06.003
8. Riordan J, Voegeli D. Prevention and treatment of pressure ulcers. Br J Nurs 2009;18:S20,S22,S24-S27. https://doi.org/10.12968/bjon.2009.18.Sup7.45132
9. Anghel EL, DeFazio MV, Barker JC, Janis JE, Attinger CE. Current concepts in debridement: science and strategies. Plast Reconstr Surg 2016;138(3 Suppl):82S-93S. https://doi.org/10.1097/PRS.0000000000002651
10. Strohal R, Dissemond J, Jordan O'Brien J, Piaggesi A, Rimdeika R, Young T, Apelqvist J. EWMA Document: Debridement: An updated overview and clarification of the principle role of debridement J Wound Care 2013;22:5. https://doi.org/10.12968/jowc.2013.22.Sup1.S1
11. Klein MB, Hunter S, Heimbach DM, Engrav LH, Honari S, Gallery E, Kiriluk D-M, Gibran NS. The Versajet water dissector: a new tool for tangential excision. J Burn Care Rehabil 2005;26:483-487. https://doi.org/10.1097/01.bcr.0000185398.13095.c5
12. Shimada K, Ojima Y, Ida Y, Matsumura H. Efficacy of Versajet hydrosurgery system in chronic wounds: A systematic review. Int Wound J 2021;18:269-278. https://doi.org/10.1111/iwj.13528
13. Auf Dem Keller U, Kümin A, Braun S, Werner S. Reactive oxygen species and their detoxification in healing skin wounds. J Investig Dermatol Symp Proc 2006;11:106-111. https://doi.org/10.1038/sj.jidsymp.5650001
14. Růžička J, Dejmek J, Bolek L, Beneš J, Kuncová J. Hyperbaric oxygen influences chronic wound healing - a cellular level review. Physiol Res 2021;70(Suppl 3):S261-S273. https://doi.org/10.33549/physiolres.934822
15. R. D. C. Team, A language and environment for statistical computing. Httpwww R-Proj. Org, 2009.
16. Wickham H, Chang W, Wickham MH. Package "ggplot2", Create Elegant Data Vis. Using Gramm. Graph. Version, vol. 2, no. 1, pp. 1-189, 2016.
17. Wei T, Simko V. R package 'corrplot': Visualization of a Correlation Matrix. 2021. [Online]. Available: https://github.com/taiyun/corrplot
18. Wickham H, Averick M, Brian J. Chang W, D'Agostino McGowan L, Francois R, Grolemund G, ET AL. Welcome to the Tidyverse. J Open Source Softw 2019;4:11686. https://doi.org/10.21105/joss.01686
19. Kassambara A. rstatix: Pipe-Friendly Framework for Basic Statistical Test'. Feb. 01, 2023. Accessed: May 09, 2023. [Online]. Available: https://cran.r-project.org/web/packages/rstatix/index.html
20. Shao D, Gao Z, Zhao Y, Fan M, Zhao X, Wei Q, Pan M, Ma B. Sulforaphane Suppresses H_2O_2-Induced Oxidative Stress and Apoptosis via the Activation of AMPK/NFE2L2 Signaling Pathway in Goat Mammary Epithelial Cells. Int J Mol Sci 2023;24:1070. https://doi.org/10.3390/ijms24021070
21. Li R, Fang H, Shen J, Jin Y, Zhao Y, Wang R, Fu Y, ET AL. Curcumin alleviates LPS-induced oxidative stress, inflammation and apoptosis in bovine mammary epithelial cells via the NFE2L2 signaling pathway. Toxins 2021;13:208. https://doi.org/10.3390/toxins13030208
22. Passero FC Jr, Ravi D, McDonald JT, Beheshti A, David KA, Evens AM. Combinatorial ixazomib and belinostat therapy induces NFE2L2-dependent apoptosis in Hodgkin and T-cell lymphoma, Br J Haematol 2020;188:295-308. https://doi.org/10.1111/bjh.16160
23. Chen X, Yu L, Zhou J, Ren J, Wang S, Tan Y, Ding Y. Inhibition of p62 and/or NFE2L2 induced autophagy impaires esophageal squamous cell cancer metastasis by reversing EMT. Gene 2023;858:147194. https://doi.org/10.1016/j.gene.2023.147194
24. Bai Z, Liu W, He D, Wang Y, Yi W, Luo C, Shen J, Hu Z. Protective effects of autophagy and NFE2L2 on reactive oxygen species-induced pyroptosis of human nucleus pulposus cells. Aging (Albany NY) 2020;12:7534-7548. https://doi.org/10.18632/aging.103109
25. Van Huizen AV, Kinsey LJ, Greene JM, Beane WS. Reactive Oxygen Species Signaling: A Therapeutic Target For Wound Healing And Regeneration. In: *Wound Repair and Regeneration*. Wiley, NJ USA, 2021, pp A14-A14.
26. Krizanova O, Penesova A, Sokol J, Hokynkova A, Samadian A, Babula P. Signaling pathways in cutaneous wound healing. Front Physiol 2022;13:1030851. https://doi.org/10.3389/fphys.2022.1030851

27. Hokynková A, Babula P, Pokorná A, Nováková M, Nártová L, Šín P. Oxidative stress in wound healing - current knowledge. Czech Slovak Neurol Neurosurg 2019;82(Suppl 1):S37-S39. https://doi.org/10.14735/amcsnn2019S37
28. Tombulturk FK, Soydas T, Sarac EY, Tuncdemir M, Coskunpinar E, Polat E, Sirekbasan S, Kanigur-Sultuybek G. Regulation of MMP 2 and MMP 9 expressions modulated by AP-1 (c-jun) in wound healing: improving role of Lucilia sericata in diabetic rats. Acta Diabetol 2019;56:177-186. https://doi.org/10.1007/s00592-018-1237-5
29. Yang L, Zheng Z, Zhou Q, Bai X, Fan L, Yang C, Su L, Hu D. miR-155 promotes cutaneous wound healing through enhanced keratinocytes migration by MMP-2. J Mol Histol 2017;48:147-155. https://doi.org/10.1007/s10735-017-9713-8
30. Caley MP, Martins VLC, O'Toole EA. Metalloproteinases and wound healing. Adv Wound Care (New Rochelle) 2015;4:225-234. https://doi.org/10.1089/wound.2014.0581
31. Rouhollahi E, Moghadamtousi SZ, Hajiaghaalipour F, Zahedifard M, Tayeby F, Awang K, Abdulla MA, Mohamed Z. Curcuma purpurascens BI. rhizome accelerates rat excisional wound healing: involvement of Hsp70/Bax proteins, antioxidant defense, and angiogenesis activity. Drug Des Devel Ther 2015;9:5805-5813. https://doi.org/10.2147/DDDT.S88196
32. Braun S, Hanselmann C, Gassmann MG, auf dem Keller U, Born-Berclaz C, Chan K, Kann YW, Werner S. Nrf2 transcription factor, a novel target of keratinocyte growth factor action which regulates gene expression and inflammation in the healing skin wound. Mol Cell Biol 2002;22:5492-5505. https://doi.org/10.1128/MCB.22.15.5492-5505.2002
33. Liu Y, Yang X, Liu Y, Jiang T, Ren S, Chen J, Xiong H, Yuan M, ET AL. NRF2 signalling pathway: new insights and progress in the field of wound healing. J Cell Mol Med 2021;25:5857-5868. https://doi.org/10.1111/jcmm.16597
34. Ong HT, Dilley RJ. Novel non-angiogenic role for mesenchymal stem cell-derived vascular endothelial growth factor on keratinocytes during wound healing. Cytokine Growth Factor Rev 2018;44:69-79. https://doi.org/10.1016/j.cytogfr.2018.11.002
35. Muñoz-Sánchez J, Chánez-Cárdenas ME. A review on hemeoxygenase-2: focus on cellular protection and oxygen response. Oxid Med Cell Longev 2014;2014:604981. https://doi.org/10.1155/2014/604981

frontiers | Frontiers in Physiology

TYPE Review
PUBLISHED 25 November 2022
DOI 10.3389/fphys.2022.1030851

OPEN ACCESS

EDITED BY
Razvigor Darienski,
Trakia University, Bulgaria

REVIEWED BY
Thomas S. Lisse,
University of Miami, United States
Kanhaiya Singh,
Indiana University–Purdue University Indianapolis, United States

*CORRESPONDENCE
Petr Babula,
babula@med.muni.cz

SPECIALTY SECTION
This article was submitted to Skin Physiology, a section of the journal Frontiers in Physiology

RECEIVED 29 August 2022
ACCEPTED 07 November 2022
PUBLISHED 25 November 2022

CITATION
Krizanova O, Penesova A, Sokol J, Hokynkova A, Samadian A and Babula P (2022). Signaling pathways in cutaneous wound healing.
Front. Physiol. 13:1030851.
doi: 10.3389/fphys.2022.1030851

Signaling pathways in cutaneous wound healing

Olga Krizanova[1,2,3], Adela Penesova[1], Jozef Sokol[2], Alica Hokynkova[4], Amir Samadian[3] and Petr Babula[3]*

[1]Institute of Clinical and Translational Research, Biomedical Research Center SAS, Bratislava, Slovakia, [2]Department of Chemistry, Faculty of Natural Sciences, University of St. Cyril and Methodius, Trnava, Slovakia, [3]Department of Physiology, Faculty of Medicine, Masaryk University, Brno, Czechia, [4]Department of Burns and Plastic Surgery, Faculty of Medicine, Masaryk University and University Hospital, Brno, Czechia

Wound healing is a very complex process, where variety of different pathways is activated, depending on the phase of healing. Improper or interrupted healing might result in development of chronic wounds. Therefore, novel approaches based on detailed knowledge of signalling pathways that are activated during acute or chronic cutaneous wound healing enables quicker and more effective healing. This review outlined new possibilities of cutaneous wound healing by modulation of some signalling molecules, e.g., gasotransmitters, or calcium. Special focus is given to gasotransmitters, since these bioactive signalling molecules that can freely diffuse into the cell and exert antioxidative effects. Calcium is an important booster of immune system and it can significantly contribute to healing process. Special interest is given to chronic wounds caused by diabetes mellitus and overcoming problems with the inflammation.

KEYWORDS

wound healing, gasotransmitters, calcium, hydrogen peroxide, diabetes mellitus

Introduction

Skin as the biggest organ in humans provides several important functions for the organism—it acts as a barrier maintaining skin integrity and homeostasis against harmful pathogens and physical stressors. Acute (mechanical injury, surgery, burn, etc.) or chronic (diabetic ulcers, etc.) cutaneous damage can have serious consequences to the whole body. Therefore, wound healing as a multistep process is an important move in the maintenance

Abbreviations: AE2, bicarbonate transporter type 2; CBS, cystathionine β-synthase; CSE, cystathionine γ-lyase; CRBP-1, cellular retinoid-binding protein 1; DFU, diabetic foot ulcers; ECM, extracellular matrix; EGF, epidermal growth factor; eNOS, endothelial NO synthase; FGF, fibroblast growth factor; HIF, hypoxia-inducible factor; HO-1, heme oxygenase-1; IGF, insulin-like growth factor; iNOS, inducible NO synthase; IP3Rs, inositol 1,4,5-trisphosphate receptors; MSCs, mesenchymal stromal cells; MST, 3-mercaptopyruvate-sulfurtransferase; nNOS, neuronal NO synthase; Nrf2, nuclear factor (erythroid-derived 2)-like 2; PAD, peripheral arterial disease; PDGF, platelet-derived growth factor; PPAR, peroxisome proliferator-activated receptors; RARs, retinoic acid receptors; ROS, reactive oxygen species; RXRs, retinoid X receptors; RyRs, ryanodine receptors; TGF, transforming growth factor; TNF, tumor necrosis factor; TRP, transient receptor potential channel; VEGF-A, vascular endothelial growth factor.

of human health and well-being. Hemostasis, inflammation, proliferation, and remodelling belong to the main steps in wound healing (Borena et al., 2015).

Skin consists of two layers—thin epithelial membrane (epidermis) and a thicker layer (dermis), composed of connective tissue. These layers differ in the composition and also in the function. Various types of cells can be recognized in both layers. In dermis, six cell types that differently contribute to wound healing were identified. Also, myofibroblasts and macrophages may change the skin wound healing fates by modulating critical signalling pathways (Chen et al., 2022). Single cell analysis revealed heterogeneity in large wounds (Guerrero-Juarez et al., 2019). In murine skin wounds the dynamic nature of fibroblast identities was shown during healing with formation subclusters of the wound fibroblasts into distinct cell populations. Also, the wound induced plasticity of myeloid lineage cells was demonstrated on this model (Guerrero-Juarez et al., 2019). Major variations in epithelial, fibroblast, and immune cell populations were observed in young and aged skin during wound healing (Vu et al., 2022). It is well known that wound healing declines with age, which contributes to a variety of health complications, and to decreased lifespan. Aged skin wounds exhibited more inflammatory profile than young equivalents, probably due to dysregulated growth factor, chemokine, and cytokine pathways during wound healing in aged skin (Vu et al., 2022). Moreover, aged basal epidermal keratinocytes isolated from the wound edge appeared to be more recalcitrant to activation, as judged by their markedly reduced transcriptional activity of genes involved in important processes of wound-repair (Keyes et al., 2016).

Wound healing is a complex process that involves the interaction between different cell types, growth hormones, cytokines, antioxidants and a stable supply of metal ions (e.g., calcium, zinc, and magnesium) (Dehkordi et al., 2019). After the skin is damaged, several cell systems and signalling pathways are activated in the wound to defend the body. Therefore, and also due to complexity of the skin, diverse approaches are needed to improve cutaneous wound healing (Zeng et al., 2018). Due to differences in signalling, healing strategy of acute and chronic wounds diverge. While acute wound heals in 3–4 weeks depending on the size, localization, origin, patient's co-morbidities, age, etc., chronic wound basically stops in the certain phase of healing, generally in the inflammatory phase. Also, chronic wounds are characterized by persistent infections, formation of drug-resistant microbial biofilms and the inability of dermal and/or epidermal cells to respond to reparative stimuli (Table 1, Demidova-Rice et al., 2012). Besides inflammatory phase, basic differences between acute and chronic wounds occur also in proliferative phase (Martin and Nunan, 2015). In the acute wounds platelets release platelet-derived growth factor and transforming growth factors A1 and 2, which attract inflammatory cells that release reactive oxygen species (ROS) and effectively clear the wound from bacteria (Demidova-Rice et al., 2012). Afterwards, growth factors are produced to induce and maintain cellular proliferation while initiating cellular migration. Finally, granulation tissue is formed to support epithelialization (Demidova-Rice et al., 2012). In chronic wounds, lower density of growth factor receptors occur that decrease the mitogenic potential of dermis and epidermis. Keratinocytes derived from chronic ulcers have increased expression of several cell cycle–associated genes, such as cyclin-dependent protein kinase 2 and cyclin B1, which point to the hyperproliferative status. However, these chronic wound–derived keratinocytes with increased proliferative marker Ki67 exhibit impaired migratory potential (Demidova-Rice et al., 2012; Martin and Nunan, 2015). Therefore, chronic wounds caused by progression of some diseases (e.g., diabetes) require repetitive or periodical medical intervention to prevent complications.

Inflammation is the basic response to cutaneous wounds that helps to protect the tissue from further damage and set up conditions that promote repair. Inflammation as the early step of the wound healing is characterized by the overproduction of ROS. Although the precise role of ROS in the process of wound healing is still not fully clear, increasing evidence suggests that ROS might be crucial for wound repair, not only as germicides but also for cellular signalling (Roy et al., 2006) in different phases of wound healing (for review see André-Lévigne et al., 2017). To eliminate excessive ROS production antioxidants and/or gasotransmitters are among the interest in many fields of medicine. Gasotransmitters are signalling molecules that easily penetrate through the plasma membrane and they have well defined and specific functions at physiologically relevant concentrations (Shefa et al., 2017). Exogenous application of gasotransmitters to wounds can significantly improve their treatment. Calcium ions play an unmistakable role in wound healing. It was proved that dietary

TABLE 1 Major differences between acute and chronic wounds.

Acute wounds	Chronic wounds
Time of healing is 3–4 weeks	Time of healing is long, or wounds are nontreatable
Activation of resident immune cells	Persistent inflammation and formation of drug-resistant microbial biofilms
Release of cytokines	Alterations in inflammatory cytokines
Stimulation of fibroblasts	Fibroblast senescence
Deposition of extracellular matrix	Decreased extracellular matrix
Neovascularization, angiogenesis	Impaired angiogenesis

10.3389/fphys.2022.1030851

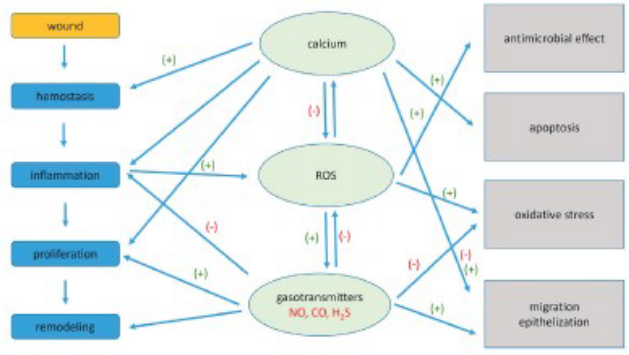


FIGURE 1
Schematic interaction of signalling molecules (calcium ions, ROS and gasotransmitters) in the individual phases of wound healing and their effects. Calcium ions affect hemostasis, inflammation and also proliferative phase of wound healing, which have an impact on the process of apoptosis, epithelization and migration. Gasotransmitters affect inflammation, proliferation and remodelling and significantly affect the amount of reactive oxygen species (ROS) and thus oxidative stress and also epithelization and migration. (+) represents positive effect, (–) represents negative effect.

calcium deficiency caused delayed wound healing and higher prevalence of chronic wound formation (Lansdown, 2002). Recently, photothermal injectable hydrogel composed of Ca^{2+} and alginate solution with α-lipoic acid modified palladium nanoparticles was developed, and possess anti-oxidative and anti-inflammatory properties (Luo et al., 2022). Role of the calcium ions in healing process is well documented not only during inflammation, but also in the proliferation phase (Subramaniam et al., 2021). Thus, new approaches based on calcium therapy (and combined calcium and vitamin D therapy) can result in more effective wound healing. Also, ROS can affect calcium signalling through targeting its influx through calcium channels (Gorlach et al., 2015). Mutual communication of calcium signalling, ROS and gasotransmitters is shown in Figure 1.

This review is focused on possibilities to utilize gasotransmitters and calcium ions and/or their combination in wound healing under normal and special conditions (diabetes). We focus on wound healing in diabetes as a modern civilization burden, which significantly contributes to chronic wound healing problems.

Wound healing, reactive oxygen species, and gasotransmitters

Increased reactive species (ROS) production serves as a defence to fight against pathogen attacks. Thus, ROS accumulation is required to prevent infection in the area of the wound (Muzumdar et al., 2019). However, long-term exposure to high concentrations of ROS generally causes oxidative stress, which damages cells (Figure 1). ROS contributes to the increasing group of gaseous mediators in the control of wound healing. Inhibiting excessive ROS production is an important feature in wound healing. From this point, antioxidants might play an important role in this process. Nrf2-activating compounds were studied to prevent and treat chronic inflammatory and degenerative disorders. It has been shown that Nrf2-inducing bioactive compounds that improve the wound healing process may be a promising therapeutic approach for treating chronic wounds (Suntar et al., 2021). Li and co-workers have shown that a hydrogen-rich medium relieved oxidative stress *via* activation of the Nrf-2/heme oxygenase-1 (HO-1) pathway (Li et al., 2022). Also, gasotransmitters serve as a barrier to increased ROS, particularly to superoxide radicals. To promote gas-healing therapy, the following requirements should be fulfilled: 1) biocompatibility, 2) ability to provide adequate and controlled amounts of gasotransmitter, 3) protection of the wound against pathogens, and 4) retaining a favourable moist wound environment (Schneider et al., 2009). Three gasotransmitters, nitric oxide (NO), carbon monoxide (CO), and hydrogen sulfide (H_2S) are important players in wound healing. These gasotransmitters are endogenously produced, but they could be donated also exogenously.

Nitric oxide, the best-described gasotransmitter, is deeply involved in the modulation of a variety of cellular functions, especially in the heart and nervous system (Förstermann and Sessa, 2012). It can be produced endogenously by three types of NO synthases (NOS)—neuronal (nNOS), endothelial (eNOS), inducible (iNOS), or added to the cells exogenously. NO was also

described to affect cutaneous functions, like proliferation, differentiation, or keratinocyte migration (Krischel et al., 1998; Zhan et al., 2015). NO seems to have a biphasic effect on wound healing. Low levels of NO can increase the permeability of the endothelium and facilitate the migration of inflammatory cells to the affected site, thus positively affecting cytokine expression. On the other side, high levels of NO can inhibit cutaneous inflammation, probably by inhibiting migration and adhesion of inflammatory cells (Man et al., 2022a). In wounds, NO is generated mainly by iNOS. The iNOS plays an essential role in non-specific defence against microorganisms (Man et al., 2022b). It was shown that iNOS-deficient mice showed a severely delayed epithelial wound closure (Yamasaki et al., 1998). Also, NO can sensitize and enhance the antibacterial effectiveness of many therapeutic approaches, such as antibiotics. To provide an adequate and accurate amount of NO to the wound, several new approaches were tested and developed. Polymer matrices are of special interest for NO molecular systems functionalization due to their high versatility and similarities with living tissues (for review see work of Pinto et al., 2022). Recently, NO-releasing oxidized bacterial cellulose/chitosan crosslinked hydrogel was shown to eliminate polymicrobial wound infection, where linear polyethyleneimine diazeniumdiolate was used as the NO donor (Hasan et al., 2022). This newly developed NO-releasing hydrogel represents a promising approach for the treatment of various skin infections. Another approach utilizes a type of gold nanostar/hollow polydopamine Janus nanostructure with precise near-infrared - controlled NO release property, which effectively eliminated methicillin-resistant *Staphylococcus aureus* from infected wounds and promoted wound healing through a synergistic photothermal and NO therapeutic effect (Liang et al., 2022).

Carbon monoxide (CO) is endogenously produced by heme oxygenases (HO) and its beneficial effect is also dependent on its concentration. Three isoforms of this enzyme were described up to now, inducible type HO-1, and constitutively expressed types HO-2 and HO-3. HO-1 protects against oxidative stress and is regulated by the redox-sensitive transcription factor, the nuclear factor (erythroid-derived 2)-like 2 (Nrf2). Abundantly produced CO in activated macrophages can enhance proliferation, differentiation, and polarization towards anti-inflammatory effects on cells (Kang et al., 2021). Vectorization of CO releasing molecules by gold nanoparticles was shown to improve the anti-inflammatory effect of CO (Fernandes et al., 2020). Recently, a new strategy of the activation of CO-release from 3-hydroxyflavone moieties through a photooxygenation mechanism was described, thus enabling CO to release under red light irradiation, exerting a selective antimicrobial effect on *S. aureus* bacteria (Cheng et al., 2021). Combined and simultaneous release of NO and CO from a single donor molecule (obtained by covalent grafting of NO-releasing N-nitrosamine onto the CO-releasing 3-hydroxyflavone derivatives under visible light irradiation) exerted a synergistic antibacterial effect against *S. aureus* (Gao et al., 2022). Nevertheless, application of an exogenous CO might be a problem, since it is difficult to quantify precise amount of administered CO and its administration might increase plasma carboxyhemoglobin to toxic levels (Takagi et al., 2022).

The third gasotransmitter—hydrogen sulfide (H_2S)—was described to affect a variety of body functions, including cardiovascular, neurological, reproductive, and endocrine systems. Also, it was shown to affect the cancer proliferation, but also apoptosis, since its effect is bell-shaped (Cao et al., 2019; Kajsik et al., 2022). H_2S is also involved in wound healing, mainly because of the anti-inflammatory properties and attenuation of oxidative-stress-related tissue injury (Figure 1). Mechanism of the beneficial effect on exogenous supplementation can cover also vascular endothelial growth factor upregulation, which might promote blood vessel formation, increase blood perfusion around the wound, and finally accelerates wound healing (Xu et al., 2019). Kutz and co-workers have found that H_2S mediates cutaneous vasodilation and has a functional interaction with both NO and cyclooxygenase signalling pathways (Kutz et al., 2015). H_2S is produced endogenously by three enzymes—cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptopyruvate-sulfurtransferase (MST). CSE appeared to be the most relevant H_2S-producing enzyme in wound tissue (Goren et al., 2019). Wu and co-workers developed a novel PCL fibrous matrix coated with pH-controllable H_2S releasing donor JK1 (Wu et al., 2016). This matrix promoted wound healing efficiency through H_2S's unique cytoprotective characteristics *in vivo*. Other carriers for JK1 encapsulation—sodium alginate or a hyaluronic acid-based hydrogel were also tested (Chattopadhyay et al., 2016; Zhao et al., 2020). Up to now, the controversial effect of the H_2S in inflammation caused by burns was described. The effect of H_2S might depend on the extent of burn degree, the course of the burn, or dosage of H_2S, and the treatment time with H_2S donors. Due to the biphasic effects of H_2S on burn wounds, H_2S supplementation in the late, but not the early stage of a burn may be helpful to accelerate healing (Xu et al., 2021).

Hydrogen peroxide (H_2O_2) is an endogenous reactive oxygen species that contributes to oxidative stress directly as a molecular oxidant and indirectly through free radical generation. It has antimicrobial properties and can act as a debriding agent through its effervescence, making low-concentration H_2O_2 useful for wound care. H_2O_2 has also been shown to promote venous insufficiency ulcer healing (Murphy and Friedman, 2019). H_2O_2 is very important signalling molecule. In the zebrafish animal model, where the wound was induced mechanically, H_2O_2 production was detected in the wound margins, with its concentration increasing over time along with leukocyte recruitment with a peak at 20 min. Its formation is mediated predominantly by NADPH oxidase, which converts oxygen to the superoxide anion radical, which is further converted by superoxide dismutase to hydrogen peroxide. NADPH oxidase

is activated/stimulated not only by mechanical injury but also by pathogenic microorganisms or pro-inflammatory cytokines (Zhu et al., 2017). In the phase of haemostasis, hydrogen peroxide stimulates the exposure of tissue factor to the surface of the relevant cells involved in haemostasis, initiating a cascade of actions leading to the generation of thrombin, the central molecule of haemostasis. It also affects platelet adhesion and aggregation (Sen and Roy, 2008). In the inflammatory phase, hydrogen peroxide affects the efficiency of macrophages at the level of protease secretion, stimulates the release of pro-inflammatory cytokines and also stimulates the recruitment of additional macrophages. It is involved in neutrophil extracellular trap formation. Hydrogen peroxide as a non-radical form of ROS is able to participate in the formation of microbially or oxidatively more efficient compounds such as hypothiocyanite (Zhu et al., 2017). In some works, H_2O_2 has been shown to have the ability to induce TNF-β production and stimulate fibroblast proliferation, leading to increased fibrotization. Here we are already at the level of cell proliferation and remodelling phases. Excessive stimulation of TGF-β leads to accelerated wound healing, but it is accompanied by increased fibrosis and scar formation. Hydrogen peroxide stimulates the production of certain growth factors, such as VEGF, which is released by macrophages and stimulates angiogenesis, and this effect is concentration dependent. In *in vivo* models, ability to affect keratinocyte viability and migration has been demonstrated (Urban et al., 2019). The effect of hydrogen peroxide on the secretion of other physiologically active molecules involved in wound healing remains a question. Here, it would certainly be worth mentioning cyclooxygenase-2 (COX-2), which is crucial for the formation of prostacyclins and prostaglandins. These actions influence a variety of processes including blood flow, vascular tone or angiogenesis. Work by Eligini et al. (2009) showed the ability of hydrogen peroxide to stimulate COX-2, but in endothelial cells. Thus, this area remains virtually unanswered and further studies are necessary.

Use of all mentioned compounds is extremely dependent on the type of dressing. Dressing generally depends on the type of wound, its stage, but also on the type of compound it has to carry. Dressings can be classified from different points, e.g., thein function in the wound healing, type of material, physical form, etc. [for review see (Boateng et al., 2008)]. Modern systems capable of controlled oxygen release are based on oxygen releasing polymeric microspheres [by incorporating hydrogen peroxide into poly (lactic-co-glycolic acid)] and hydrogel scaffolds (Choi et al., 2018), cyanoacrylate-encapsulated calcium peroxide (Zhang et al., 2020), OxOBand composed of antioxidant polyurethane (PUAO), as highly porous cryogels with sustained oxygen releasing properties (Shiekh et al., 2020), oxygenated-bacterial-cellulose nanofibers (Sarkandi et al., 2022), or injectable hydrogel based on hyaluronic acid-graft-dopamine and polydopamine coated Ti3C2 MXene nanosheets (Li et al., 2022). ROS-responsive oxygen and NO releasing systems based on encapsulated biosafe NO donor L-arginine and hydrogen peroxide were developed too (Yu et al., 2022). Other therapeutic approaches include topical application of growth factors and cytokines and some other agents such as hyaluronic acid or erythropoietin. These are also being tested in topical forms, but in controlled-release systems (Legrand and Martino, 2022). Examples include various polymers, particularly modified celluloses, which have the ability to form hydrogels and release growth factors in a controlled manner. Their major advantages include in particular their biocompatibility. Most recently, Hao et al. (2022) have prepared multifunctional benzaldehydeterminated 4-arm PEG (4-arm-PEG-CHO)/carboxymethyl chitosan (CMCS)/basic fibroblast growth factor (bFGF) hydrogels, that have shown the ability to increase Ki67, increase generation of epithelialization and collagen, induces the formation of hair follicles, and enhanced neovascularization by upregulating the production of CD31 and CD34 (Hao et al., 2022). A similar approach was taken by Cheng et al. (2020), who, however, used metal-free CO-releasing polymers based on photoresponsive 3-hydroxyflavone derivatives (Cheng et al., 2020).

Involvement of calcium channels in wound healing

A variety of ions is indisputably involved in different stages of wound healing. Calcium ions are involved in both, normal skin function and also in wound healing. Calcium ions are prerequisite for keratinocyte differentiation and corneocyte formation. To cope with the different calcium needs of keratinocytes (low calcium concentrations for proliferation, high calcium for differentiation) epidermis built up calcium gradient (Rinnerthaler and Richter, 2018). Calcium can enter the cytoplasm of cells either from outside, through special types of calcium channels, or by release from the intracellular stores, mainly from the endoplasmic reticulum (for review see Babula and Krizanova, 2022). The function of individual calcium transport systems in wound healing is unwinded from their role in healthy skin. For example, ryanodine receptors (RyRs) that are localized in the membranes of the endoplasmic reticulum are expressed in keratinocytes and can affect their differentiation and barrier homeostasis (Denda et al., 2012). After the skin wound creation, the initiation of keratinocyte migration is among the first reparation mechanisms (O'Toole, 2001). In this process, an increase in the intracellular calcium concentration was determined, which probably results in the upregulation of bicarbonate transporter type 2 (AE2). An increase in AE2 expression is probably involved in cell migration and results in wound closure (Hwang et al., 2020). Inhibition of RyRs by specific antagonists (e.g., dantrolene) can accelerate wound closure *in vivo* through the process of epithelialization (Degovics et al., 2019). RyRs are probably activated by exposure

to ROS (Csordas and Hajnoczky, 2009). Thus, the limitation of calcium release by inhibition of RyRs resulted in a decrease in ROS formation (Degovics et al., 2019). Based on these results authors have concluded that dantrolene might be another tool for the acceleration of wound healing. The role of other store-operated channels—inositol 1,4,5-trisphosphate receptors (IP_3Rs)—in wound healing is still elusive. In general, IP_3Rs type 1 and 2 were shown to have proapoptotic effects in cancer cells, while type 3 IP_3R has anti-apoptotic effect (Rezuchova et al., 2019). Their importance in wound healing has not yet been fully elucidated. It was already shown that IP_3Rs activated by phospholipase C are active in human keratinocytes (Tu et al., 2005).

Transient receptor potential (TRP) channels are a diverse group of channels with different function in various tissues. In non-excitable cells, TRP channels regulate intracellular calcium concentrations, which are related to keratinocytes proliferation and differentiation to influence the skin barrier (Moran, 2018). The family of TRP channels comprises a large number of channels that can be divided into 6 subtypes—TRPA (ankyrin), TRPC (canonical), TRPM (melastatin), TRPML (mucolipin), TRPP (polycystin), and TRPV (vanilloid) (Montell et al., 2002). Different TRP channels participate in different skin homeostasis and barrier functions. A variety of TRPC channels was shown to be expressed in keratinocytes and probably playing role in keratinocyte differentiation (Caterina and Pang, 2016). TRPV channels are sensitive to various tissue-damaging signals and their activation is generally perceived as pain (for review see Wang, 2021a). Non-selective ion channel—transient receptor potential vanilloid 1 (TRPV1) is a potential drug target for improving the outcome of inflammatory/fibrogenic wound healing, especially cornea (Nidegawa et al., 2014).

During aging, changes in pH and calcium transport are detectable in skin. The pH of the epidermis goes up and the calcium gradient goes down (Rinnerthaler and Richter., 2018). Decrease in calcium levels is due to a failure to transport calcium into the stratum granulosum. As a consequence, skin pH is increased and aged skin is more vulnerable to bacterial infection.

Wound healing in diabetes

Diabetic foot ulcers (DFU) are the most common chronic wounds characterized by poor healing. Patients with diabetes mellitus have a 15%–25% lifetime risk of developing DFU, of which 40%–80% become so severely infected that they suffer from bone infection, leading to osteomyelitis. Wound healing disorders in patients with diabetes also occur due to a higher incidence of infectious complications, vascular changes at the level of microangiopathy and macroangiopathy, and in some cases repeated pressure on the wound increasing local ischemia. The issue of wound healing is a complex matter, so attention should be paid to the control of several parameters. The most important factor involves poor glycemic control (Dissanayake et al., 2020). Chronic decompensation of diabetes helps to develop ischemic lower limb disease, neuropathy, and other abnormalities that are modified not only at the systemic but also at the local level. Prolonged poor controlled diabetes leads to dysfunction of immune cells involved in repair processes and to the formation of late glycation products, which affect wound healing directly by reacting with some components of the healing process, or indirectly through diabetic neuropathy or angiopathy individual stages of the wound healing process (Fournet et al., 2018).

The early stages of wound healing are characterized by hypoxia, which induces the activation of hypoxia-inducible factor (HIF) -1α and stimulates the stimulation of vascular endothelial growth factor (VEGF-A). HIF is very important to promote the migration and proliferation of each cell type as well as the release of growth factors (Hong et al., 2014). HIF-1 is involved in many wound healing processes; such as cell migration, cell metabolism under hypoxic conditions, cell differentiation, cell growth factor release, cell survival, and synthesis of signal molecules throughout the healing process. Both overexpression of HIF-1, as well as HIF-1 deficiency, are associated with reduced adaptive responses to hypoxia during diabetic wound healing (Li et al., 2021). Overexpression of HIF-1 leads to an increased production of profibrotic factors associated with the overproduction of collagenous matrix (Kimura et al., 2008). On the other hand, HIF-1 deficiency and subsequent impaired response to hypoxic stimuli contribute to the formation of non-healing ulcers. Targeted wound healing therapy using regulators of HIF-1 production has many important aspects that can lead to tissue repair (Li et al., 2021). However, more preclinical and clinical studies are needed to validate the feasibility of treating diabetic wounds by manipulating HIF-1α activity.

In the damaged tissue, monocytes are activated, which become macrophages and they mediate phagocytosis as well as the production of growth factors such as platelet-derived growth factor (PDGF), tumor necrosis factor (TNF), and transforming growth factor (TGF-β). Growth factors influencing wound healing include PDGF, which increases macrophage migration and collagen synthesis, promotes granulation tissue formation and accelerates epithelialization, fibroblast growth factor (FGF) supporting angiogenesis and fibroblast proliferation, insulin-like growth factor (IGF-1) increasing fibroblast proliferation, collagen synthesis and epithelialization (Patel et al., 2019; Garoufalia et al., 2021). Decreased IGF-1 expression in diabetic individuals has been reported, which in turn has slowed the healing process (Garoufalia et al., 2021; Liu et al., 2021). Many (23 types) of FGF have been identified and divided into seven subfamilies. Recently, with the increasing research on the function of FGF, more and more studies are focused on FGF therapeutic approach.

TABLE 2 Involvement of gasotransmitters and calcium transport systems in wound healing.

Wound healing	Acute wounds	Chronic wounds
Inflammation	CO-antiinflammatory effect	H_2S—attenuates inflammation
	NO- antimictobial effect	NO—suppresses inflammation, ROS scavenging
	H_2S- antimicrobial effect	
	Ca^{2+} through TRPV—improves inflammatory wound healing	
Proliferation differentiation	CO-increases proliferation, differentiation	Ca^{2+} blocking by azelnipidine promotes fibroblast proliferation
	Ca^{2+} through RyR—promotes differentiation in keratinocytes	
	Ca^{2+} through AE2—promotes keratinocytemigration	
	Ca^{2+} through TRP—affects proliferation, differentiation	
Remodeling angiogenesis	H_2S—increases blood perfusion around wounds	H_2S—promotes angiogenesis
		NO through iNOS—enhancing angiogenesis
		Ca^{2+} blocking by azelnipidine promotes angiogenesis

AE2, bicarbonate transporter type 2; CO, carbon monooxide; H_2S, hydrogen sulfide; iNOS, inducible NO synthase; NO, nitric oxide; ROS, reactive oxygen species; RYR, ryanodine receptors; TRP, transient receptor potential channel; TRPV, vanilloid transient receptor potential channel.

It has been applying FGF-1, FGF-2, FGF-4, FGF-7, FGF-21, and FGF-23 topically to DFU with good therapeutic effects (Liu et al., 2021).

The other three factors—TGF-beta, TNF-alpha, and IL-1 (interleukin-1) promote angiogenesis and collagen synthesis and therefore regulate epidermal stem cells in wound epithelialization (Xiao et al., 2020). The last factors influencing wound healing are GSF (granulocyte stimulating factor) and VEGF (vascular endothelial growth factor). The latter was significantly reduced during wound healing (by 50%) in an experiment performed on diabetic rats compared to healthy controls (Kurkipuro et al., 2022).

The anti-inflammatory effect of epidermal growth factor (EGF) has been intensively studied for more than 20 years. Many studies showed that the intralesional administration of EGF has emerged as an effective treatment for DFU (Mendoza-Mari et al., 2022). EGF injected into the ulcer matrix enhanced cell proliferation and migration, leading to peri- and intra-lesion infiltration. Therefore, it accelerates the healing of deep and complex ulcers, both ischemic and neuropathic, and reduces diabetes-related amputations (Berlanga et al., 2013). EGF helps diabetic wound healing, reaching responsive cells while avoiding the deleterious effect of proteases and the biofilm on the wound's surface.

Gasotransmitter's use was tested also in treatment of chronic diabetic wounds. Nitric oxide and NO-releasing compounds can significantly contribute to diabetic wound healing. Since in diabetes endogenous production of NO is affected, need for the topical supply of NO from exogenous sources is desirable (Takagi et al., 2022). Azelnipidine, a new dihydropyridine blocker of L-type calcium channels, increased wound fluid NO level, enhanced fibroblast proliferation and promotes angiogenesis, which participates to the acceleration of wound healing in type 1 diabetic rats (Bagheri et al., 2011). The NO-donors attached to patches or matrices to treat diabetic wounds are under development. Recently, preparation of anti-bacterial and nano-enzyme-containing hydrogel with inflammation-suppressing, ROS-scavenging, oxygen and nitric oxide-generating properties was published (Tu et al., 2022).

Insufficient intracellular H_2S production in diabetes impairs angiogenic property and ischemic tissue injury, probably *via* interrupting the balance between pro- and anti-angiogenic factors (Cheng and Kishore, 2020). Exogenous donation of H_2S by NaHS improved diabetic wound healing in ob/ob mice via promoting angiogenesis and attenuating inflammation (Zhao et al., 2017).

A meta-analysis was performed by Lin et al. (2022) focused on the association between vitamin D levels, respectively vitamin D hypovitaminosis, and wound healing in diabetic patients. Most significant association has been found between low vitamin D levels and foot ulcer wounds. Patients with foot ulcer wounds had significantly lower levels of vitamin D. Also, higher prevalence of vitamin D deficiency as well as higher prevalence of severe vitamin D deficiency was associated with higher incidence of foot ulcer wounds compared with non-diabetic non-ulcerated diabetic subjects. Some studies suggest that vitamin D supplementation in diabetic patients may have a positive effect on foot ulcer wound healing (Yammine et al., 2020; Halschou-Jensen et al., 2021; Kurian et al., 2021). Severe vitamin D deficiency is associated with elevated inflammatory cytokine concentrations in diabetic patients, particularly in those with foot infection (Tiwari et al., 2014). Negative correlation was observed between vitamin D and circulating concentrations of IL-1 beta and IL-6, but not for TNF-alpha and IFN-gamma. The question remains as to the mechanism of action of vitamin D in wound healing. Topically applied vitamin D in diabetic patients promotes corneal wound healing and nerve regeneration, reduces neutrophil infiltration and stimulates the transition of macrophages from M1 to M2, which is accompanied by suppression of excessive activation of the

NLRP3 inflammasome (Wang et al., 2021b). Vitamin D downregulates the expression of MMP-1 and MMP-10 in keratinocytes from diabetic food ulcer cultivated *in vitro*. In contrast, increased expression of these genes was found in diabetic patients with diabetic food ulcer (Lopez-Lopez et al., 2014). MMP-1 breaks down the interstitial collagens I, II, and III, MMP-10 is intensively studied in connection with processes of metastasizing. The stem cells' secreted bioactive molecules (the secretome) mediate paracrine and autocrine functions. Mesenchymal stromal cells (MSCs) are multipotent cells that reside in tissues and can give rise to bone, cartilage, adipocytes, or vascular smooth muscle cells (Laloze et al., 2021).

Meta-analyses of Jaluvka et al. (2020) reveals that cell therapy in peripheral arterial disease (PAD) treatment can prevent or delay foot amputation (Jaluvka et al., 2020). The wound healing process with stem cell therapy can be at least twice as shorter when compared with the standard conservative therapy. It can lead to improvement of perfusion and tissue oxygenation parameters in the wound, even more to pain regression. The available evidence-based medicine data showed that cells-based therapy is safe, associated with minimum complications or adverse events, and effective (Jaluvka et al., 2020). MSCs have been identified in tissues other than the bone marrow, including the umbilical cord, placenta, dental pulp, and adipose tissue (AD-MSCs). From the stem cell types, AD-MSCs have been intensively studied in terms of improving chronic wound healing (Ajit and Ambika Gopalankutty, 2021). The influence of ASC-secretome on cell types associated with the wound healing process can provide a basis for further and more targeted investigations that are useful for addressing the ways of accelerating chronic non-healing wound closure (Lombardi et al., 2019). A recent study showed that MSCs under TNF-α stimulation (MSC-CM-T) can release numerous trophic and survival molecules that have a promising prospect in wound healing acceleration in an animal model of wound healing. The topical gel of MSC-CM-T is more effective in accelerating wound closure healing through increasing platelet-derived growth factor (PDGF) levels and wound closure percentages and fibroblast density appearances in the skin defect animal models (Laloze et al., 2021; Putra et al., 2022).

Conclusion

Wound healing is an extremely complex and complicated process that involves the integration of a variety of mechanisms at different time intervals along a timeline. Hemostasis, inflammation, proliferation, and remodelling are four stages of wound healing. Effect of gasotransmitters and modulation of calcium levels by calcium transport systems on individual phases of wound healing is summarized in Table 2. Development of new drugs targeting individual stages can provide a tool that can more effectively treat different types of wounds (e.g., diabetic wounds). Thus, new treatments based on precise knowledge of pathways activated in every stage can facilitate the process of wound healing. Calcium ions are known to play the crucial role in cell signalling. Several experimental studies have shown that calcium-releasing materials can significantly stimulate wound healing. They stimulate angiogenesis, collagen and extracellular matrix protein synthesis and overall tissue granulation. Polymeric composite dressings containing calcium-releasing nanoparticles are investigated as novel calcium-releasing systems that significantly accelerated wound healing in a diabetic (db/db) mouse model. Among new approaches, use of gasotransmitters provides an excellent tool for treatment, since they easily penetrate into the cells and they might stimulate proliferation (especially NO), they enhance vascularization and decrease period of treatment, especially in diabetic wounds.

In summary, skin wounds often represent a burden to the patient, generally limiting his comfort. Therefore, effective healing, possibly without scars, represents a goal in dermatology. Development of new strategies of wound healing is based on current knowledge of modulated signaling pathways in the wound. Recently, modern treatments based on impregnation of hydrogels and nanoparticles with gasotransmitters, blockers of calcium transport, vitamins, etc. form a powerful tool for effective wound healing.

Author contributions

OK—gasotransmitters, hydrogen peroxide and calcium, revision of text. AP—diabetes mellitus, revision of text. JS—gasotransmitters, revision of text. AH—clinical implications of wound healing. AS—revision of text, language corrections. PB—supervising, discussion, revision of text.

Funding

The work of OK was supported by grants APVV-20-0176, VEGA 2/0040/22 and partially by VEGA 2/0047/22. The work of AP was supported by grants APVV-17-0099 and VEGA 2/0129/20. The work of PB was supported by the Ministry of Health of the Czech Republic under Grant No. NU21-09-00541 "The role of oxidative stress in the healing of pressure ulcers in patients with spinal cord lesions".

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Ajit, A., and Ambika Gopalankutty, I. (2021). Adipose-derived stem cell secretome as a cell-free product for cutaneous wound healing. *3 Biotech.* 11, 413. doi:10.1007/s13205-021-02958-7

André-Lévigne, D., Modarressi, A., Pepper, M. S., and Pittet-Cuénod, B. (2017). Reactive oxygen species and NOX enzymes are emerging as key players in cutaneous wound repair. *Int. J. Mol. Sci.* 18, 2149. doi:10.3390/ijms18102149

Babula, P., and Krizanova, O. (2022). Involvement of calcium signaling in different types of cell death in cancer. *Neoplasma* 69, 264–273. doi:10.4149/neo_2022_220127N121

Bagheri, M., Jahromi, B. M., Mirkhani, H., Solhjou, Z., Noorafshan, A., Zamani, A., et al. (2011). Azelnidipine, a new calcium channel blocker, promotes skin wound healing in diabetic rats. *J. Surg. Res.* 169, e101–e107. doi:10.1016/j.jss.2011.02.039

Berlanga, J., Fernandez, J. L., Lopez, E., Lopez, P. A., Del Rio, A., Valenzuela, C., et al. (2013). Heberprot-P: A novel product for treating advanced diabetic foot ulcer. *MEDICC Rev.* 15, 11–15. doi:10.1590/s1555-79602013000100004

Boateng, J. S., Matthews, K. H., Stevens, H. N. E., and Eccleston, G. M. (2008). Wound healing dressings and drug delivery systems: A review. *J. Pharm. Sci.* 97, 2892–2923. doi:10.1002/jps.21210

Borena, B. M., Martens, A., Broeckx, S. Y., Meyer, E., Chiers, K., Duchateau, L., et al. (2015). Regenerative skin wound healing in mammals: State-of-the-Art on growth factor and stem cell based treatments. *Cell. Physiol. biochem.* 36, 1–23. doi:10.1159/000374049

Cao, X., Ding, L., Xie, Z. Z., Yang, Y., Whiteman, M., Moore, P. K., et al. (2019). A review of hydrogen sulfide synthesis, metabolism, and measurement: Is modulation of hydrogen sulfide a novel therapeutic for cancer? *Antioxid. Redox Signal.* 31, 1–38. doi:10.1089/ars.2017.7058

Caterina, M. J., and Pang, Z. (2016). TRP channels in skin biology and pathophysiology. *Pharmaceuticals* 9, E77. doi:10.3390/ph9040077

Chattopadhyay, M., Kodela, R., Duvalsaint, P. L., and Kashfi, K. (2016). Gastrointestinal safety, chemotherapeutic potential, and classic pharmacological profile of NOSH-naproxen (AVT-219) a dual NO- and H2S-releasing hybrid. *Pharmacol. Res. Perspect.* 4, e00224. doi:10.1002/prp2.224

Chen, C. J., Kajita, H., Takaya, K., Aramaki-Hattori, N., Sakai, S., Asou, T., et al. (2022). Single-cell RNA-seq analysis reveals cellular functional heterogeneity in dermis between fibrotic and regenerative wound healing fates. *Front. Immunol.* 13, 875407. doi:10.3389/fimmu.2022.875407

Cheng, J., Gan, G. H., Shen, Z. Q., Gao, L., Zhang, G. Y., and Hu, J. M. (2021). Red light-triggered intracellular carbon monoxide release enables selective eradication of MRSA infection. *Angew. Chem. Int. Ed. Engl.* 60, 13513–13520. doi:10.1002/anie.202104024

Cheng, J., Zheng, B., Cheng, S., Zhang, G. Y., and Hu, J. M. (2020). Metal-free carbon monoxidereleasing micelles undergo tandem photochemical reactions for cutaneous wound healing. *Chem. Sci.* 11, 4499–4507. doi:10.1039/d0sc00135j

Cheng, Z. J., and Kishore, R. (2020). Potential role of hydrogen sulfide in diabetes-impaired angiogenesis and ischemic tissue repair. *Redox Biol.* 37, 101704. doi:10.1016/j.redox.2020.101704

Choi, J., Hong, G., Kwon, T., and Lim, J. O. (2018). Fabrication of oxygen releasing scaffold by embedding H2O2-PLGA microspheres into alginate-based hydrogel sponge and its application for wound healing. *Appl. Sci. (Basel).* 8, 1492. doi:10.3390/app8091492

Csordas, G., and Hajnoczky, G. (2009). SR/ER-mitochondrial local communication: Calcium and ROS. *Biochim. Biophys. Acta* 1787, 1352–1362. doi:10.1016/j.bbabio.2009.06.004

Degovics, D., Hartmann, P., Nemeth, I. B., Arva-Nagy, N., Kaszonyi, E., Szel, E., et al. (2019). A novel target for the promotion of dermal wound healing: Ryanodine receptors. *Toxicol. Appl. Pharmacol.* 366, 17–24. doi:10.1016/j.taap.2019.01.021

Dehkordi, A. N., Babaheydari, F. M., Chehelgerdi, M., and Dehkordi, S. R. (2019). Skin tissue engineering: Wound healing based on stem-cell-based therapeutic strategies. *Stem Cell Res. Ther.* 10, 111. doi:10.1186/s13287-019-1212-2

Demidova-Rice, T. N., Hamblin, M. R., and Herman, I. M. (2012). Acute and impaired wound healing: Pathophysiology and current methods for drug delivery, part 1: Normal and chronic wounds: Biology, causes, and approaches to care. *Adv. Skin. Wound Care* 25, 304–314. doi:10.1097/01.ASW.0000416006.55218.d0

Denda, S., Kumamoto, J., Takei, K., Tsutsumi, M., Aoki, H., and Denda, M. (2012). Ryanodine receptors are expressed in epidermal keratinocytes and associated with keratinocyte differentiation and epidermal permeability barrier homeostasis. *J. Invest. Dermatol.* 132, 69–75. doi:10.1038/jid.2011.256

Dissanayake, A., Vandal, A. C., Boyle, V., Park, D., Milne, B., Grech, R., et al. (2020). Does intensive glycaemic control promote healing in diabetic foot ulcers? - a feasibility study. *Bmj Open* 10, e029009. doi:10.1136/bmjopen-2019-029009

Eligini, S., Arenaz, L., Barbieri, S. S., Faleri, M. L., Crisci, M., Tremoli, E., et al. (2009). Cyclooxygenase-2 mediates hydrogen peroxide-induced wound repair in human endothelial cells. *Free Radic. Biol. Med.* 46, 1428–1436. doi:10.1016/j.freeradbiomed.2009.02.026

Fernandes, A. R., Mendonca-Martins, I., Santos, M. P. A., Raposo, L. R., Mendes, R., Marques, J., et al. (2020). Improving the anti-inflammatory response via gold nanoparticle vectorization of CO-releasing molecules. *ACS Biomater. Sci. Eng.* 6, 1090–1101. doi:10.1021/acsbiomaterials.9b01936

Förstermann, U., and Sessa, W. C. (2012). Nitric oxide synthases: regulation and function. *Eur. Heart J.* 33, 829–837. 837a.–837d. doi:10.1093/eurheartj/ehr304

Fournet, M., Bonte, F., and Desmouliere, A. (2018). Glycation damage: A possible hub for major pathophysiological disorders and aging. *Aging Dis.* 9, 880–900. doi:10.14336/AD.2017.1121

Gao, L., Cheng, J., Shen, Z. Q., Zhang, G. Y., Liu, S. Y., and Hu, J. M. (2022). Orchestrating nitric oxide and carbon monoxide signaling molecules for synergistic treatment of MRSA infections. *Angew. Chem. Int. Ed. Engl.* 61, e202112782. doi:10.1002/anie.202112782

Garoufalia, Z., Papadopetraki, A., Karatza, E., Vardakostas, D., Philippou, A., Kouraklis, G., et al. (2021). Insulin-like growth factor-I and wound healing, a potential answer to non-healing wounds: A systematic review of the literature and future perspectives. *Biomed. Rep.* 15, 66. doi:10.3892/br.2021.1442

Goren, I., Kohler, Y., Aglan, A., Pfeilschifter, J., Beck, K. F., and Frank, S. (2019). Increase of cystathionine-gamma-lyase (CSE) during late wound repair: Hydrogen sulfide triggers cytokeratin 10 expression in keratinocytes. *Nitric Oxide* 87, 31–42. doi:10.1016/j.niox.2019.03.004

Görlach, A., Bertram, K., Hudecova, S., and Krizanova, O. (2015). Calcium and ROS: A mutual interplay. *Redox Biol.* 26, 260–271. doi:10.1016/j.redox.2015.08.010

Guerrero-Juarez, C. F., Dedhia, P. H., Jin, S., Ruiz-Vega, R., Ma, D., Liu, Y., et al. (2019). Single-cell analysis reveals fibroblast heterogeneity and myeloid-derived adipocyte progenitors in murine skin wounds. *Nat. Commun.* 10, 650. doi:10.1038/s41467-018-08247-x

Halschou-Jensen, P. M., Sauer, J., and Bouchelouche, P., Fabrin, J., Brorson, S., and Ohrt-Nissen, S. (2021). Improved healing of diabetic foot ulcers after high-dose vitamin D: A randomized double-blinded clinical trial. *Int. J. Low. Extrem. Wounds.* doi:10.1177/15347346211020268

Hao, Y. P., Zhao, W. W., Zhang, H., Zheng, W. P., and Zhou, Q. H. (2022). Carboxymethyl chitosanbased hydrogels containing fibroblast growth factors for triggering diabetic wound healing. *Carbohydr. Polym.* 287, 119336. doi:10.1016/j.carbpol.2022.119336

Hasan, N., Lee, J., Ahn, H. J., Hwang, W. R., Bahar, M. A., Habibie, H., et al. (2022). Nitric oxide-releasing bacterial cellulose/chitosan crosslinked hydrogels for the treatment of polymicrobial wound infections. *Pharmaceutics* 14, 22. doi:10.3390/pharmaceutics14010022

Hong, W. X., Hu, M. S., Esquivel, M., Liang, G. Y., Rennert, R. C., Mcardle, A., et al. (2014). The role of hypoxia-inducible factor in wound healing. *Adv. Wound Care* 3, 390–399. doi:10.1089/wound.2013.0520

Hwang, S., Shin, D. M., and Hong, J. H. (2020). Intracellular Ca2+-mediated AE2 is involved in the vectorial movement of HaCaT keratinocyte. *Int. J. Mol. Sci.* 21, E8429. doi:10.3390/ijms21228429

Jaluvka, F., Ihnat, P., Madaric, J., Vrtkova, A., Janosek, J., and Procházka, V. (2020). Current status of cell-based therapy in patients with critical limb ischemia. *Int. J. Mol. Sci.* 21, E8999. doi:10.3390/ijms21238999

Kajsik, M., Chovancova, B., Liskova, V., Babula, P., and Krizanova, O. (2022). Slow sulfide donor GYY4137 potentiates effect of paclitaxel on colorectal carcinoma cells. *Eur. J. Pharmacol.* 922, 174875. doi:10.1016/j.ejphar.2022.174875

Kang, I. S., Kim, R. L., and Kim, C. (2021). Carbon monoxide regulates macrophage differentiation and polarization toward the M2 phenotype through upregulation of heme oxygenase 1. *Cells* 10, 3444. doi:10.3390/cells10123444

Keyes, B. E., Liu, S., Asare, A., Naik, S., Levorse, J., Polak, L., et al. (2016). Impaired epidermal to dendritic T cell signaling slows wound repair in aged skin. *Cell* 67, 1323–1338. doi:10.1016/j.cell.2016.10.052

Kimura, K., Iwano, M., Higgins, D. F., Yamaguchi, Y., Nakatani, K., Harada, K., et al. (2008). Stable expression of HIF-1alpha in tubular epithelial cells promotes interstitial fibrosis. *Am. J. Physiol. Ren. Physiol.* 295, F1023–F1029. doi:10.1152/ajprenal.90209.2008

Krischel, V., Bruch-Gerharz, D., Suschek, C., Kröncke, K. D., Ruzicka, T., and Kolb-Bachofen, V. (1998). Biphasic effect of exogenous nitric oxide on proliferation and differentiation in skin derived keratinocytes but not fibroblasts. *J. Invest. Dermatol.* 111, 286–291. doi:10.1046/j.1523-1747.1998.00268.x

Kurian, S. J., Miraj, S. S., Benson, R., Munisamy, M., Saravu, K., Rodrigues, G. S., et al. (2021). Vitamin D supplementation in diabetic foot ulcers: A current perspective. *Curr. Diabetes Rev.* 17, 512–521. doi:10.2174/1573399816999201012195735

Kurkipuro, J., Mierau, L., Wirth, T., Samaranayake, H., Smith, W., Karkkainen, H.-R., et al. (2022). Four in one-Combination therapy using live Lactococcus lactis expressing three therapeutic proteins for the treatment of chronic non-healing wounds. *Plos One* 17, e0264775. doi:10.1371/journal.pone.0264775

Kutz, J. L., Greaney, J. L., Santhanam, L., and Alexander, L. M. (2015). Evidence for a functional vasodilatatory role for hydrogen sulphide in the human cutaneous microvasculature. *J. Physiol.* 593, 2121–2129. doi:10.1113/JP270054

Lalone, J., Fievet, L., and Desmoulière, A. (2021). Adipose-derived mesenchymal stromal cells in regenerative medicine: State of play, current clinical trials, and future prospects. *Adv. Wound Care* 10, 24–48. doi:10.1089/wound.2020.1175

Lansdown, A. B. G. (2002). Calcium: A potential central regulator in wound healing in the skin. *Wound Repair Regen.* 10, 271–285. doi:10.1046/j.1524-475x.2002.10502.x

Legrand, J. M. D., and Martino, M. M. (2022). Growth factor and cytokine delivery systems for wound healing. *Cold Spring Harb. Perspect. Biol.* 14, a041234. doi:10.1101/cshperspect.a041234

Li, G., Ko, C.-N., Li, D., Yang, C., Wang, W., Yang, G.-J., et al. (2021). A small molecule HIF-1 alpha stabilizer that accelerates diabetic wound healing. *Nat. Commun.* 12, 3363. doi:10.1038/s41467-021-23448-7

Li, Y., Fu, R. Z., Duan, Z. G., Zhu, C. H., and Fan, D. D. (2022). Artificial nonenzymatic antioxidant MXene nanosheet-anchored injectable hydrogel as a mild photothermal-controlled oxygen release platform for diabetic wound healing. *Acs Nano* 16, 7486–7502. doi:10.1021/acsnano.1c10575

Li, Y. J., Shen, C. C., Zhou, X., Zhang, J. H., Lai, X. Y., and Zhang, Y. M. (2022). Local treatment of hydrogen-rich saline promotes wound healing *in vivo* by inhibiting oxidative stress via nrf-2/HO-1 pathway. *Oxid. Med. Cell. Longev.* 2022, 2949824. doi:10.1155/2022/2949824

Liang, Z. Y., Liu, W. K., Wang, Z. Q., Zheng, P. L., Liu, W., Zhao, J. F., et al. (2022). Near-infrared laser-controlled nitric oxide-releasing gold nanostar/hollow polydopamine Janus nanoparticles for synergistic elimination of methicillin-resistant *Staphylococcus aureus* and wound healing. *Acta Biomater.* 143, 428–444. doi:10.1016/j.actbio.2022.02.029

Lin, J., Mo, X. X., Yang, Y. J., Tang, C., and Chen, J. (2022). Association between vitamin D deficiency and diabetic foot ulcer wound in diabetic subjects: A meta-analysis. *Int. Wound J.* doi:10.1111/iwj.13836

Liu, Y., Lin, Y., Deng, J., Li, W., and Nie, X. (2021). Fibroblast growth factor in diabetic foot ulcer: Progress and therapeutic prospects. *Front. Endocrinol.* 12, 744868. doi:10.3389/fendo.2021.744868

Lombardi, F., Palumbo, P., Augello, F. R., Cifone, M. G., Cinque, B., and Giuliani, M. (2019). Secretome of adipose tissue-derived stem cells (ASCs) as a novel trend in chronic non-healing wounds: An overview of experimental *in vitro* and *in vivo* studies and methodological variables. *Int. J. Mol. Sci.* 20, E3721. doi:10.3390/ijms20153721

Lopez-Lopez, N., Gonzalez-Curiel, I., Trevino-Santa Cruz, M. B., Rivas-Santiago, B., Trujillo-Paez, V., Enciso-Moreno, J. A., et al. (2014). Expression and vitamin D-mediated regulation of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) in healthy skin and in diabetic foot ulcers. *Arch. Dermatol. Res.* 306, 809–821. doi:10.1007/s00403-014-1494-2

Luo, Y., Zhou, X., Liu, C., Lu, R., Jia, M., Li, P., et al. (2022). Scavenging ROS and inflammation produced during treatment to enhance the wound repair efficacy of photothermal injectable hydrogel. *Biomater. Adv.* 141, 213096. doi:10.1016/j.bioadv.2022.213096

Man, M. Q., Wakefield, J. S., Mauro, T. M., and Elias, P. M. (2022a). Regulatory role of nitric oxide in cutaneous inflammation. *Inflammation* 45, 949–964. doi:10.1007/s10753-021-01615-8

Man, M. Q., Wakefield, J. S., Mauro, T. M., and Elias, P. M. (2022b). Role of nitric oxide in regulating epidermal permeability barrier function. *Exp. Dermatol.* 31, 290–298. doi:10.1111/exd.14470

Martin, P., and Nunan, R. (2015). Cellular and molecular mechanisms of repair in acute and chronic wound healing. *Br. J. Dermatol.* 173, 370–378. doi:10.1111/bjd.13954

Mendoza-Mari, Y., Garcia-Ojalvo, A., Fernandez-Mayola, M., Rodriguez-Rodriguez, N., Martinez-Jimenez, I., and Berlanga-Acosta, J. (2022). Epidermal growth factor effect on lipopolysaccharide-induced inflammation in fibroblasts derived from diabetic foot ulcer. *Scars Burn. Heal.* 8, 20595131211067380. doi:10.1177/20595131211067380

Montell, C., Birnbaumer, L., Flockerzi, V., Bindels, R. J., Brudorf, E. A., Caterina, M. J., et al. (2002). A unified nomenclature for the superfamily of TRP cation channels. *Mol. Cell* 9, 229–231. doi:10.1016/s1097-2765(02)00448-3

Moran, M. M. (2018). TRP channels as potential drug targets. *Annu. Rev. Pharmacol. Toxicol.* 58, 309–330. doi:10.1146/annurev-pharmtox-010617-052832

Murphy, E. C., and Friedman, A. J. (2019). Hydrogen peroxide and cutaneous biology: Translational applications, benefits, and risks. *J. Am. Acad. Dermatol.* 81, 1379–1386. doi:10.1016/j.jaad.2019.05.030

Muzumdar, S., Hiebert, H., Haertel, E., Greenwald, M. B., Bloch, W., Werner, S., et al. (2019). Nrf2-Mediated expansion of pilosebaceous cells accelerates cutaneous wound healing. *Am. J. Pathol.* 189, 568–579. doi:10.1016/j.ajpath.2018.11.017

Nidegawa, Y., Sumioka, T., Okada, Y., Miyajima, M., Reinach, P. S., Saika, S., et al. (2014). Impairment of corneal epithelial wound healing in a TRPV1-deficient mouse. *Invest. Ophthalmol. Vis. Sci.* 55, 3295–3302. doi:10.1167/iovs.13-13077

O'toole, E. A. (2001). Extracellular matrix and keratinocyte migration. *Clin. Exp. Dermatol.* 26, 525–530. doi:10.1046/j.1365-2230.2001.00891.x

Patel, S., Srivastava, S., Singh, M. R., and Singh, D. (2019). Mechanistic insight into diabetic wounds: Pathogenesis, molecular targets and treatment strategies to pace wound healing. *Biomed. Pharmacother.* 112, 108615. doi:10.1016/j.biopha.2019.108615

Pinto, R. V., Carvalho, S., Antunes, F., Pires, J., and Pinto, M. L. (2022). Emerging nitric oxide and hydrogen sulfide releasing carriers for skin wound healing therapy. *Chemmedchem* 17, e202100429. doi:10.1002/cmdc.202100429

Putra, A., Ibrahim, S., Muhar, A. M., Kuntardjo, N., Dirja, B. T., Pasongka, Z., et al. (2022). Topical gel of mesenchymal stem cells-conditioned medium under TNF-alpha precondition accelerates wound closure healing in full-thickness skin defect animal model. *J. Med. Life* 15, 214–221. doi:10.25122/jml-2019-0103

Rezuchova, I., Hudecova, S., Soltysova, A., Matuskova, M., Durinikova, E., Chovancova, B., et al. (2019). Type 3 inositol 1, 4, 5-trisphosphate receptor has antiapoptotic and proliferative role in cancer cells. *Cell Death Dis.* 10, 186. doi:10.1038/s41419-019-1433-4

Rinnerthaler, M., and Richter, K. (2018). The influence of calcium on the skin pH and epidermal barrier during aging. *Curr. Probl. Dermatol.* 54, 79–86. doi:10.1159/000489521

Roy, S., Khanna, S., Nallu, K., Hunt, T. K., and Sen, C. K. (2006). Dermal wound healing is subject to redox control. *Mol. Ther.* 13, 211–220. doi:10.1016/j.ymthe.2005.07.684

Sarkandi, A. F., Montazer, M., and Rad, M. M. (2022). Oxygenated-bacterial-cellulose nanofibers with hydrogel, antimicrobial, and controlled oxygen release properties for rapid wound healing. *J. Appl. Polym. Sci.* 139, 51974. doi:10.1002/app.51974

Schneider, A., Wang, X. Y., Kaplan, D. L., Garlick, J. A., and Egles, C. (2009). Biofunctionalized electrospun silk mats as a topical bioactive dressing for accelerated wound healing. *Acta Biomater.* 5, 2570–2578. doi:10.1016/j.actbio.2008.12.013

Sen, C. K., and Roy, S. (2008). Redox signals in wound healing. *Biochim. Biophys. Acta* 1780, 1348–1361. doi:10.1016/j.bbagen.2008.01.006

Shefa, U., Yeo, S. G., Kim, M. S., Song, I. O., Jung, J., Jeong, N. Y., et al. (2017). Role of gasotransmitters in oxidative stresses, neuroinflammation, and neuronal repair. *Biomed. Res. Int.* 2017, 1689341. doi:10.1155/2017/1689341

Shiekh, P. A., Singh, A., and Kumar, A. (2020). Exosome laden oxygen releasing antioxidant and antibacterial cryogel wound dressing OxOBand alleviate diabetic and infectious wound healing. *Biomaterials* 249, 120020. doi:10.1016/j.biomaterials.2020.120020

Subramaniam, T., Fauzi, M. B., Lokanathan, Y., and Law, J. X. (2021). The role of calcium in wound healing. *Int. J. Mol. Sci.* 22, 6486. doi:10.3390/ijms22126486

Suntar, I., Cetinkaya, S., Panieri, E., Saha, S., Buttari, B., Profumo, E., et al. (2021). An overview of Nrf2 signaling pathway and its role in inflammation. *Molecules* 26, E5474. doi:10.3390/molecules25225474

Takagi, T., Okayama, T., Asai, J., Mizushima, K., Hirai, Y., Uchiyama, K., et al. (2022). Topical application of sustained released-carbon monoxide promotes cutaneous wound healing in diabetic mice. *Biochem. Pharmacol.* 199, 115016. doi:10.1016/j.bcp.2022.115016

Tiwari, S., Pratyush, D. D., Gupta, S. K., and Singh, S. K. (2014). Vitamin D deficiency is associated with inflammatory cytokine concentrations in patients with diabetic foot infection. *Br. J. Nutr.* 112, 1938–1943. doi:10.1017/S0007114514003018

Tu, C. L., Chang, W. H., and Bikle, D. D. (2005). Phospholipase cgammal is required for activation of store-operated channels in human keratinocytes. *J. Invest. Dermatol.* 124, 187–197. doi:10.1111/j.0022-202X.2004.23544.x

Tu, C. X., Lu, H. D., Zhou, T., Zhang, W. Y., Deng, L. W., Cao, W. B., et al. (2022). Promoting the healing of infected diabetic wound by an anti-bacterial and nano-enzyme-containing hydrogel with inflammation-suppressing, ROS-scavenging, oxygen and nitric oxide-generating properties. *Biomaterials* 286, 121597. doi:10.1016/j.biomaterials.2022.121597

Urban, M. V., Rath, T., and Radtke, C. (2019). Hydrogen peroxide (H2O2): A review of its use in surgery. *Wien. Med. Wochenschr.* 169, 222–225. doi:10.1007/s10354-017-0610-2

Vu, R., Jin, S., Sun, P., Haensel, D., Nguyen, Q. H., Dragan, M., et al. (2022). Defining epidermal basal cell States during skin homeostasis and wound healing using single-cell transcriptomics. *Cell Rep.* 40, 3932–3947. doi:10.1016/j.celrep.2020.02.091

Wang, G. (2021a). Lipid-dependent sequential allosteric activation of heat-sensing TRPV1 channels by anchor-stereoselective "hot" vanilloid compounds and analogs. *Biochem. Biophys. Rep.* 28, 101109. doi:10.1016/j.bbrep.2021.101109

Wang, Y. D., Wan, L. Q., Zhang, Z. Z., Li, J., Qu, M. L., and Zhou, Q. J. (2021b). Topical calcitriol application promotes diabetic corneal wound healing and reinnervation through inhibiting NLRP3 inflammasome activation. *Exp. Eye Res.* 209, 108668. doi:10.1016/j.exer.2021.108668

Wu, J., Li, Y., He, C. C., Kang, J. M., Ye, J. J., Xiao, Z. C., et al. (2016). Novel H2S releasing nanofibrous coating for *in vivo* dermal wound regeneration. *ACS Appl. Mat. Interfaces* 8, 27474–27481. doi:10.1021/acsami.6b06466

Xiao, T., Yan, Z., Xiao, S., and Xia, Y. (2020). Proinflammatory cytokines regulate epidermal stem cells in wound epithelialization. *Stem Cell Res. Ther.* 11, 232. doi:10.1186/s13287-020-01755-y

Xu, M. T., Hua, Y. Y., Qi, Y., Meng, G. L., and Yang, S. J. (2019). Exogenous hydrogen sulphide supplement accelerates skin wound healing via oxidative stress inhibition and vascular endothelial growth factor enhancement. *Exp. Dermatol.* 28, 776–785. doi:10.1111/exd.13930

Xu, M. T., Zhang, L. L., Song, S., Pan, L. L., Arslan, I. M., Chen, Y., et al. (2021). Hydrogen sulfide: Recent progress and perspectives for the treatment of dermatological diseases. *J. Adv. Res.* 27, 11–17. doi:10.1016/j.jare.2020.02.003

Yamasaki, K., Edington, H. D. J., Mcclosky, C., Tzeng, E., Lizonova, A., Kovesdi, I., et al. (1998). Reversal of impaired wound repair in iNOS-deficient mice by topical adenoviral-mediated iNOS gene transfer. *J. Clin. Invest.* 101, 967–971. doi:10.1172/JCI2067

Yammine, K., Hayek, F., and Assi, C. (2020). Is there an association between vitamin D and diabetic foot disease? A meta-analysis. *Wound Repair Regen.* 28, 90–96. doi:10.1111/wrr.12762

Yu, J., Zhang, R. L., Chen, B. H., Liu, X. L., Jia, Q., Wang, X. F., et al. (2022). Injectable reactive oxygen species-responsive hydrogel dressing with sustained nitric oxide release for bacterial ablation and wound healing. *Adv. Funct. Mat.* 32, 2202857. doi:10.1002/adfm.202202857

Zeng, R. J., Lin, C. Q., Lin, Z. H., Chen, H., Lu, W. Y., Lin, C. M., et al. (2018). Approaches to cutaneous wound healing: Basics and future directions. *Cell Tissue Res.* 374, 217–232. doi:10.1007/s00441-018-2830-1

Zhan, R. X., Yang, S. W., He, W. F., Wang, F., Tan, J. L., Zhou, J. Y., et al. (2015). Nitric oxide enhances keratinocyte cell migration by regulating rho GTPase via cGMP-PKG signalling. *Plos One* 10, e0121551. doi:10.1371/journal.pone.0121551

Zhang, T., Han, Z. Y., Zhang, W., Wang, J., and Xu, L. (2020). Cyanoacrylate-encapsulated calcium peroxide achieved oxygen-sustained release and promoted wound healing. *Int. J. Polym. Mater. Polym. Biomaterials* 69, 703–708. doi:10.1080/00914037.2019.1600518

Zhao, H. C., Lu, S. X., Chai, J. H., Zhang, Y. C., Ma, X. L., Chen, J. C., et al. (2017). Hydrogen sulfide improves diabetic wound healing in ob/ob mice via attenuating inflammation. *J. Diabetes Complicat.* 31, 1363–1369. doi:10.1016/j.jdiacomp.2017.06.011

Zhao, X., Liu, L., An, T. Z., Xian, M., Luckanagul, J. A., Su, Z. H., et al. (2020). A hydrogen sulfide-releasing alginate dressing for effective wound healing. *Acta Biomater.* 104, 85–94. doi:10.1016/j.actbio.2019.12.032

Zhu, G., Wang, Q., Lu, S., and Niu, Y. (2017). Hydrogen peroxide: A potential wound therapeutic target. *Med. Princ. Pract.* 26, 301–308. doi:10.1159/000475501

4. ARTIFICIAL INTELLIGENCE PERSPECTIVE

Artificial Intelligence (AI) has become an integral part of modern medicine, offering significant advancements in diagnostics, treatment planning, and patient care. As AI technologies continue to evolve, their application in healthcare is expanding, providing solutions for complex clinical challenges. One of the most promising areas of AI in medicine is Machine Learning (ML), which enables computers to analyse vast datasets, identify patterns, and make predictive decisions. In reconstructive and wound care surgery, AI-driven approaches hold great potential for improving risk assessment, treatment personalization, and clinical outcomes.

This chapter provides an overview of artificial intelligence, its role in healthcare, and its specific application in pressure ulcer management. It begins with the definition and evolution of AI, exploring how the field has developed and how different AI models work. The chapter then shifts to AI applications in medicine, highlighting its role in diagnostics, predictive modelling, and personalized treatment strategies. Special attention is given to ML-based pressure ulcer prediction, leveraging large-scale clinical databases like MIMIC to enhance early detection and prevention strategies.

The chapter further explores how AI-powered tools can support clinicians in the management of PUs, from early-stage prediction to postoperative monitoring following surgical interventions, such as flap plasties. Future perspectives include the integration of AI-driven decision support systems into electronic health records, bedside monitoring, and the development of standardized clinical databases to refine predictive models. By harnessing the power of AI, clinicians can move toward a more proactive, data-driven approach to wound care, ultimately reducing complications and improving patient outcomes.

4.1 Definition of artificial intelligence

Despite the increased interest from academia, industry, and public institutions in Artificial Intelligence (AI), no precise definition of AI exists. Certain approaches characterize AI in relation to humans or intelligence in general. [131] Many definitions of AI refer to machines that mimic human behaviour or are capable of tasks requiring intelligence. [132,133] Numerous

attempts have been made to objectively quantify human intelligence due to the difficulty of defining and measuring it. [134] In reality, however, intelligence is a subjective and abstract concept that creates a false impression of achievable precision. [135] The founder of this discipline is considered to be John McCarthy, who in 1955 described the AI process *"as the ability to make a machine behave in a way that could be called intelligent if a human behaved that way"*. [136] According to Nilsson, AI is an activity dedicated to the creation of intelligent machines, and intelligence is the property that enables an entity to function appropriately and correctly and to anticipate in its environment. [137] The AI Study Panel concurs with Nilsson that intelligence exists on a multidimensional spectrum. According to this viewpoint, the distinction between the arithmetic ability of a calculator and the intellect of the human brain is not limited to type, but also includes range, speed, intelligence level, and the ability to compute. [138] The European Parliament defines AI as *"the ability of machines to mimic human abilities such as reasoning, learning, planning, or creativity. Artificial intelligence enables technical systems to respond to inputs from their environment, solve problems and achieve specific goals. The embedded computer receives data – which has already been prepared or is collected by its own sensors and cameras – and then evaluates and reacts to it. AI systems are able to work autonomously and also change and adapt their actions based on an evaluation of the effects of previous actions."* [139]

4.2 Artificial intelligence in healthcare and medicine

AI is concerned with the development of algorithms, models and actuators that simulate human thought and behaviour. Its use in medicine and healthcare systems has enormous potential to improve diagnosis, treatment, and patient care, or to save medical and non-medical staff time by efficiently managing administration, so long as the legal and ethical aspects of the technology are upheld. AI is slowly transforming medical practice. With recent advances in digital data collection and big data in general, deep learning, and computational infrastructure, AI applications are becoming increasingly accessible, expanding into areas that were once the exclusive domain of humans. [140] Given the broad scope of AI in medicine, the following text will focus specifically on ML, as our investigation centres on its application in predicting the risk of pressure ulcers in ICU settings, see *Figure 8*.

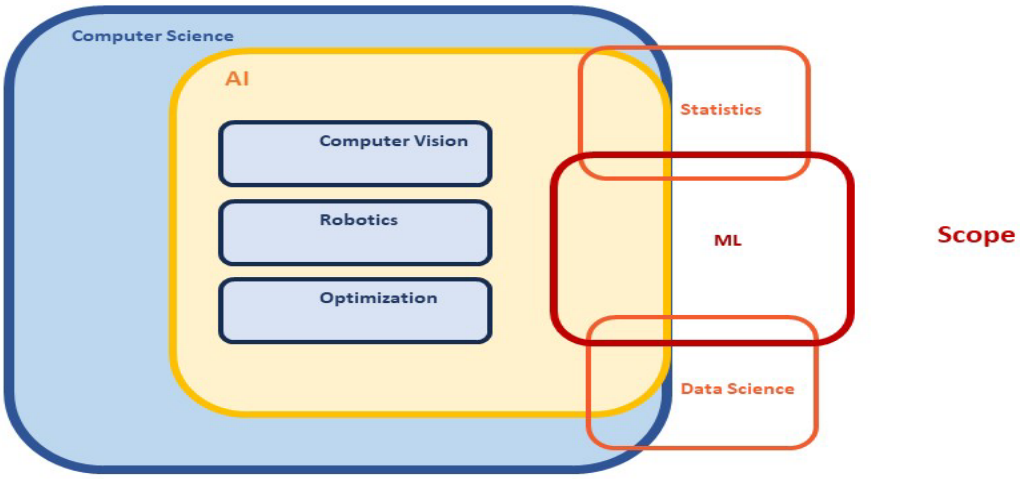


Figure 8: Relationship between computer science, AI and ML and further scope definition

A typical process of ML process consists of several layers [141]: first, relevant data sources need to be identified, then the data need to be pre-processed and prepared for training and testing ML models, which can then perform the required tasks, such as anomaly detection, classification or prediction, depending on the quality and quantity of the available training data, see *Figure 9*.

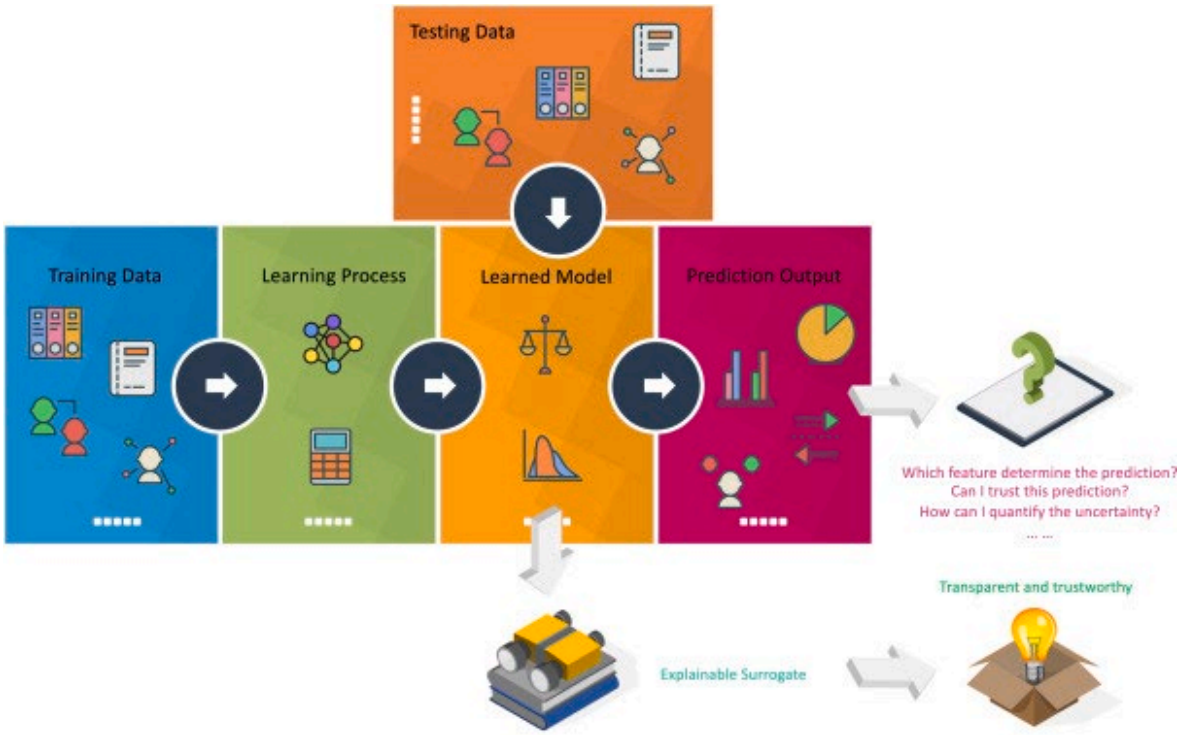


Figure 9: Schematic of typical ML framework. Adopted from [141]

There are many AI techniques and methods in what follows, we focus on the subfield of ***Machine Learning (ML)***, that explores the ability of computers to learn from data. [142,143] It

emerges at the intersection of statistics, data and computer science, utilizing a number of efficient computational algorithms such as optimization or regression. This fusion of mathematics and computer science is a result of the unique computational challenges posed by constructing statistical models from potentially enormous data sets containing billions or trillions of data points. [144] ML can be divided into supervised learning, semi-supervised, unsupervised learning, and reinforced learning. Supervised learning is based on annotated and labelled training data and is the most commonly used method utilizing existing real-world data. [144] In medicine, this may entail training a model to establish a correlation between a person's characteristics (e.g., height, weight, smoking) and a specific outcome (e.g., incidence of diabetes within five years). After effective training, the algorithm will be able to predict outcomes when applied to new data. [145] In clinical practice, it is used for automated ECG interpretation, prediction of cardiovascular events [146] or COVID-19 screening based on CT lung images. [147] In contrast, unsupervised ML does not require pre-processed training data and is therefore mostly exploratory, such as identifying undefined patterns or clusters in the dataset. [145] ***Artificial Neural Networks (ANN)*** are ML models inspired by the biological nervous system, consisting of numerous neurons that process and transmit information and are interconnected. Common applications of neural networks include image recognition, natural language processing, and prediction. [148] ***Deep convolutional neural networks*** have recently achieved remarkable success in a variety of medical image analysis tasks, including image denoising (noise removal from images) [149] and classification. These data are essential for disease diagnosis and management of therapy. [150] Another popular family of methods is inspired by evolutionary mechanisms, such as ***Genetic Algorithms (GA).*** They use selection, crossover, and mutation to determine the optimal solution to a given problem. [151] Frequently, GA are employed for optimization, planning, and design. Evolutionary "generation" is the sequence of operations in space and/or time. A fitness function simulates competition between individuals by selecting the healthiest members of an entire generation. [152]

A cross-section of other popular ML methods includes Linear Regression [153], Logistic Regression [154], Decision tree [155], Support Vector Machine (SVM) algorithm [156], Naive Bayes algorithm [157], K-Nearest Neighbors algorithm (KNN) [158], K-means [159], Random Forest algorithm [160], Dimensionality Reduction algorithms [161], and Gradient Boosting algorithm. [162] ML specialists are responsible for most appropriate model selection. From a medical

point of view, the list of ML methods and tools will be less fascinating than their medical applications. On a semantic graph (constructed from Web of Science search results and plotted using VOS Viewer software), Jiang et al. illustrated the degree of influence and interconnectedness of the most relevant AI-related keywords, as shown in *Figure 10*. According to the colour of the connections, the "application" of AI has received considerable attention in the literature. This term is closely associated with "systems" sciences, whereas "neural networks", "classification," and "prediction" are the primary algorithmic focuses. [163] All of these characteristics are utilized in various branches of medicine and the healthcare system to enhance disease diagnosis, treatment, prevention, prognosis, etc. In order to implement these theories in clinical practice, it is necessary to adhere to ethical, legal, and security considerations pertaining not only to the preservation of personal data, but also to the precision and dependability of the employed technologies.

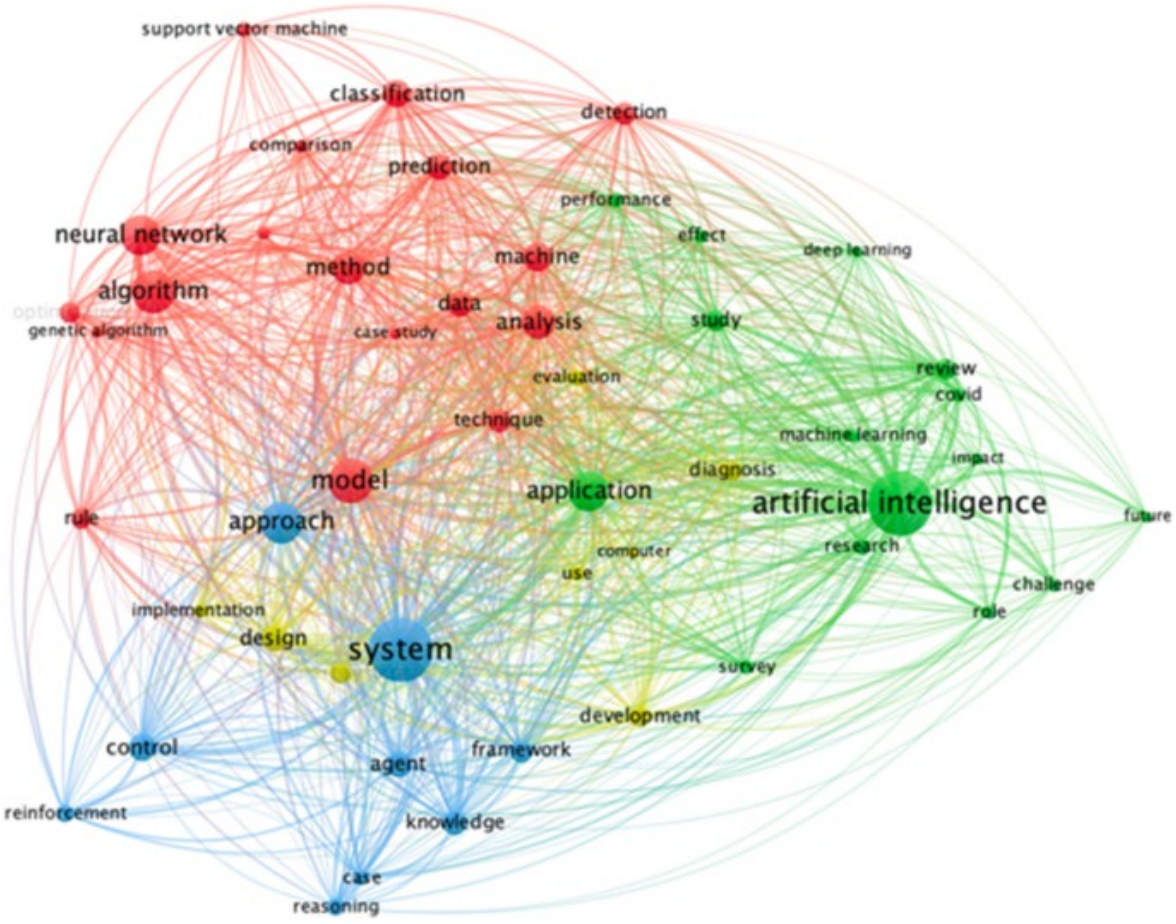


Figure 60: Semantic graph according to Jiang et al. Adopted from [163]

Image data analysis

Deep learning, in which neural networks learn patterns directly from raw data, has been very successful in classifying images [164] from different modalities, such as CT, X-ray, and MRI, in the past few years. Some fields are already using them. In the field of radiology, there has been a change in how accurate mammography interpretation [165] and lung cancer screening [166] are. This has a positive effect not only on diagnosis but also on risk prediction and, eventually, on treatment. [167] In the area of pathology, ML is used to diagnose cancer, mostly because it can be used with whole slides. [168] Neural networks can also be taught to recognize primary tumours and can better predict the survival of different types of cancer [169], thus improving the pathological interpretation of cancer findings. In gastroenterology, AI techniques are used to detect colorectal cancer and predict automatically the malignancy of tumours of this site. [170] In addition to image classification, deep learning models can also learn from text, numbers, and combinations of these data types. Recent research has utilized a variety of rich data sources, such as molecular information (improving protein structure prediction [171], non-invasive cancer detection, prognosis, and tumour origin identification based on detection of circulating cell-free DNA [172], natural language, medical signals like electroencephalogram (EEG) data, and multimodal data). [164] *Figure 11* depicts the prospective applications in medical clinical practice.

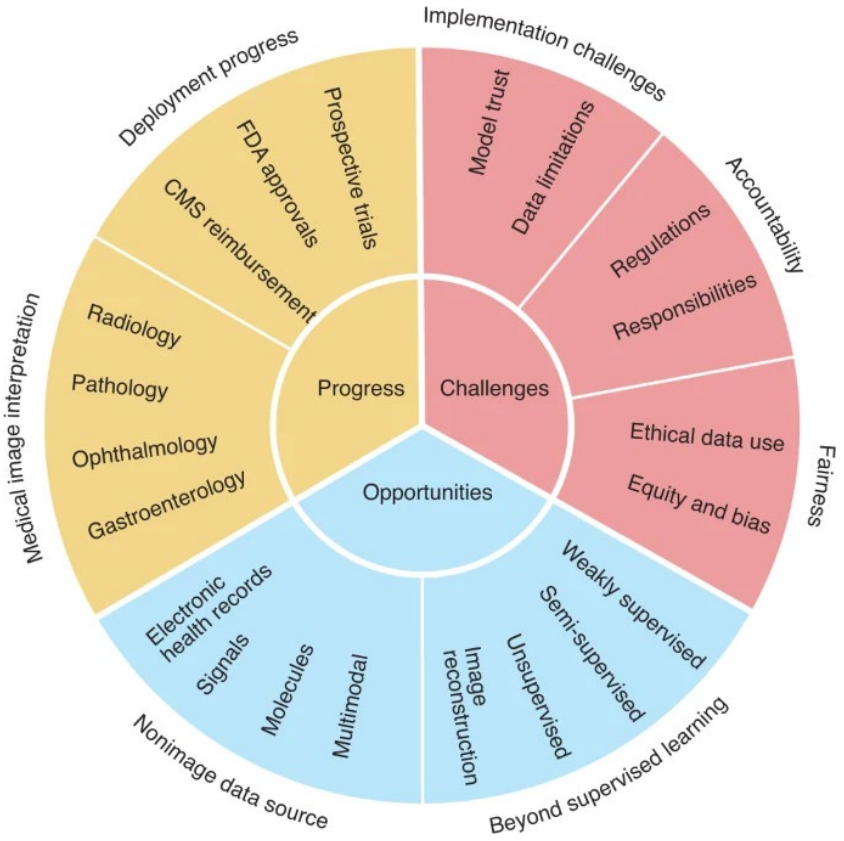


Figure 7: ML application in medical clinical practice. Adopted from [164]

Genomics and personalisation of treatment

Genomics uses ML to analyse disease-specific genomic data in order to tailor treatment protocols individually to the needs of a given patient. It determines the most appropriate type or combination of drugs, drug interactions, dosage, or response to treatment. Side effects are reduced while the desired performance is maximised, which translates directly into improved length and quality of life for individuals. [173]

Robotic Surgical Procedures

Robotic surgery uses AI on multiple levels. Various surgical procedures can be trained on simulators. In a 2020 meta-analysis comparing virtual reality training to traditional apprenticeship training, the authors found that virtual reality training increases trainee efficiency, improves tissue handling, and reduces surgical errors in comparison to traditional apprenticeship-style surgical training. [174] AI methods can also help in data analysis and preparation for 3D printing vascular phantoms of aortic aneurysms, cutting guides in mandibular reconstruction using free vascularized fibula, skeletal prostheses, etc.

[175] ML in surgery gives potential in pre-operative planning of surgery based on existing medical records and imaging. By incorporating artificial intelligence, surgical robotics would be able to detect and comprehend complex environments, make decisions in real time, and execute surgical interventions with greater precision, safety, automation, and efficiency. For instance, current robots can perform basic surgical tasks such as suturing and knot tying automatically. [176,177] Future predictions also include the possibility of bioprinted organs and tissues for transplant surgery. [178,179] An example of the potential use of AI in the preoperative, perioperative and postoperative phases of neurosurgical treatment of brain tumours [180] is shown in *Figure 12*.

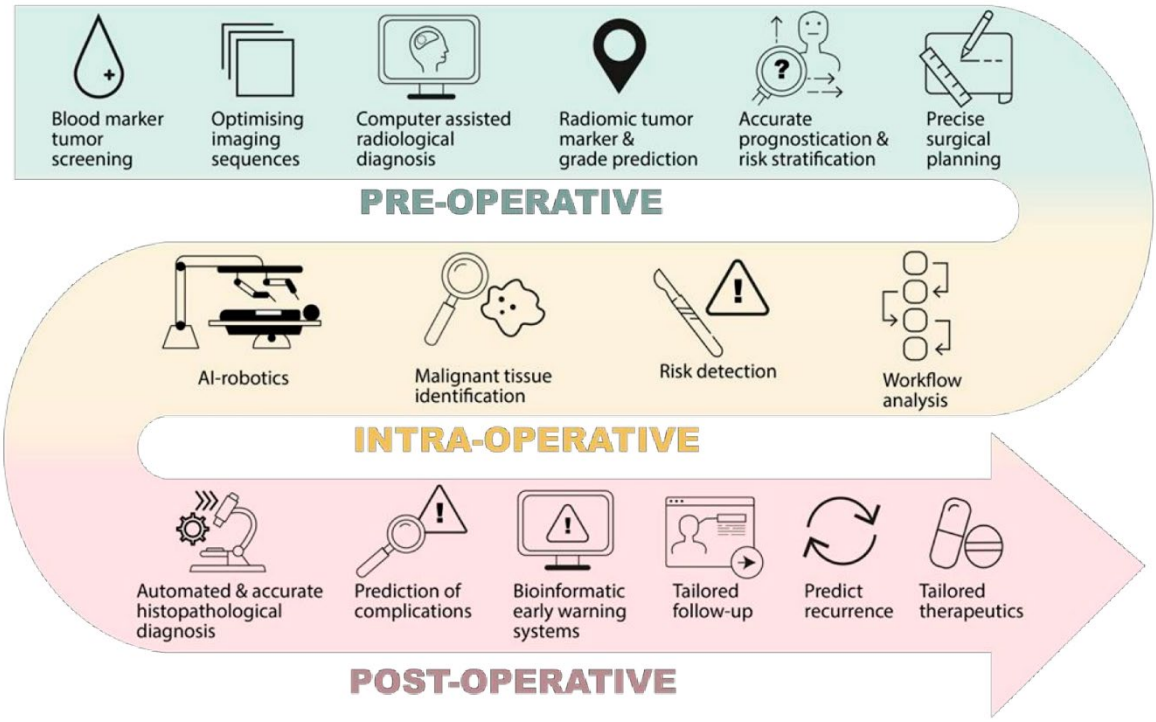


Figure 82: Potential clinical impacts of AI in the neurosurgical management of brain tumours, in the pre-operative, intra-operative, and post-operative phase. Adopted from [180]

Drug development

ML is capable of analysing vast amounts of information about drug compounds and their interactions with biological targets. Using ML, it is possible to identify potential drug candidates, predict their efficacy and interactions with other drugs, and individualize therapies. These technologies not only improve the efficiency and accuracy of the drug discovery and development process, but in some cases they also reduce or eliminate the need for clinical trials by conducting simulations instead, thereby reducing costs and ethical concerns. [181]

Patient monitoring

ML can analyse data from medical devices that measure vital patient parameters such as heart rate, blood pressure, and sleep, thus enabling remote patient monitoring, abnormality detection, and personalised care. Telemedicine and remote patient monitoring have broadened the scope of conventional clinical practice, enabling physicians to monitor patients with chronic or acute illness in remote locations, the elderly in home care, and even hospitalized patients. [182] The use of AI in various health apps on mobile phones or smartwatches is positively altering people's attitudes toward their own health. [183]

Digital medical records

AI tools and technologies can utilize the vast quantity of electronic health record data from digital records for analysis, pattern recognition, outcome prediction, and clinical decision support. Roosan et al. describe a model for using AI in the form of a blockchain to securely share health data with community pharmacies, which has the potential to improve patient outcomes, optimize medication safety, and strengthen pharmacists' roles in patient care. [184] Based on image data, clinical data, e.g., in the form of laboratory results, AI can be used to predict various types of pathologies and diseases, improving the diagnosis and individual treatment of diseases.

Mental health

AI also demonstrates considerable promise in the field of mental health. By analysing speech types, social media, and other types of data, it can assist in the identification of symptoms of various pathological mental conditions (such as depression, anxiety, etc.). By monitoring symptoms, these capabilities can expedite the diagnosis of mental illness, predict the course of the illness, positively influence clinical intervention, and facilitate psychoeducation. The use of AI in mental health is unquestionably crucial, particularly during quarantine measures in COVID-19 pandemics or other biological disasters. There are already numerous AI applications that monitor individuals' mental health. Phama et al. discuss emerging AI-based interventions in the form of chatbots and therapy bots, including conversational apps that teach users coping mechanisms for emotions and provide support for people with communication difficulties, computer-generated facial images that form

the basis of avatar therapy, and intelligent animal-like bots with new advancements in digital psychiatry. [185]

Prevention, prediction, prognosis, and survival

In healthcare, AI plays a significant role in the prevention, prediction, prognosis, and survival of various diseases. Risk factors for specific diseases or pathologies are determined using clinical and genetic data and factors. Based on this analysis, a personalized treatment plan can be developed, and thus the risk of complications can be eliminated.

Decision support systems in healthcare

AI is capable of creating decision support systems based on the analysis of clinical data and medical records using its tools. This aids physicians in determining treatment plans and procedures through the decision-making process. It is primarily employed in critical care, including mortality prediction in acute trauma [186], bleeding after trauma (risk assessment, amount of transfusion administration, detection of bleeding, risk of coagulopathy, etc.) [187], fracture detection and classification in orthopaedics, etc. [188]

Ethical aspects of AI in healthcare

Identifiable, foreseen, and contemplated are the implications of AI on fundamental ethical principles, just as every technological advancement generates ethical discussions. Included among these are data collection, anonymity, privacy, consent, data ownership, security, bias, transparency, accountability, autonomy, and beneficence. Another debated aspect of AI is its application in writing scientific publications. Tools like ChatGPT can serve as valuable aids for researchers and scientists by assisting with material organization, drafting, and proofreading. However, ethical concerns remain, particularly regarding potential inaccuracies, risks of plagiarism, and the necessity of oversight by human experts. [189]

4.3 Clinical medical databases

The contribution of artificial intelligence is also significant in clinical medical databases. Clinical medical databases are extensive registries of structured and organized medical data that play a crucial role in healthcare and medical research. These databases are intended to store, manage, and retrieve voluminous quantities of clinical data, such as patient records, laboratory test results, medical images, and treatment plans. They provide

healthcare professionals, researchers, and policymakers with valuable resources for accessing, analysing, and interpreting medical data for a variety of purposes.

Modern hospitals are well-equipped with monitoring and data collection devices, which provide a relatively low-cost method for accumulating and storing data in internal and intra-hospital information systems. The large amounts of data collected by medical databases necessitate the use of specialized tools for data storage and retrieval, data analysis, and effective data utilization. Particularly, the increase in data volume makes it extremely difficult to acquire relevant information for decision support. Traditional manual data analysis has become ineffective, necessitating the use of effective computer analysis techniques. To meet this demand, medical informatics can utilize technologies developed in the new interdisciplinary field of knowledge discovery in databases, which includes statistical, pattern recognition, machine learning, and visualization tools that facilitate data analysis and the discovery of patterns encoded in data. [190] There are several clinical medical databases that are widely used in healthcare and medical research. Exact research or clinical topic, as well as the volume of information needed, will determine which database is used. Several well-known clinical medical databases that preserve and provide patient information, clinical assessments, laboratory results, and medical imaging available are listed below.

Medical Information Mart for Intensive Care - MIMIC

The MIMIC database is a data repository that contains comprehensive clinical de-identified information collected as part of routine clinical care for>60,000 ICU admissions at the Beth Israel Deaconess Medical Centre in Boston, Massachusetts. [191,192] There are clinical data, physiological histories, test results, prescription information, and survival rates included. The MIMIC-III dataset is readily accessible to researchers worldwide and has been utilized extensively in the creation of predictive models, epidemiological studies, and educational courses. MIMIC is frequently used for research and the construction of prediction models in critical care. MIMIC-IV was created by updating MIMIC-III, incorporating current data and improving many aspects of MIMIC-III. [193,194]

MIMIC-IV takes a modular approach to data organization, emphasizing the origin of data and facilitating the individual and combined use of diverse data sources. MIMIC-IV is

intended to build on the success of MIMIC-III and support a broad set of health care applications.

Observational Medical Outcomes Partnership-OMOP

OMOP is an open-source database, consolidated and standardized electronic health record (EHR) data from diverse healthcare systems. It contains de-identified data about individuals, illnesses, ailments, therapies, medications, tests, etc. OMOP makes it easier to conduct comparative effectiveness studies and extensive observational research. All researchers' data must adhere to the same format according to the OMOP common data model, which makes it possible to use analytic tools like Structured Query Language (SQL) among locations. [195,196]

UK Biobank

Over 500,000 people in the UK participated in this databank. Biobank is a huge prospective cohort study that collects considerable biological samples and health data. It includes details on the history of medicine, physical features, imaging data, genetics, etc. Research on a range of illnesses and health consequences is made feasible by the database. [197]

Optum Clinformatics Data Mart

A commercially available database called Optum Clinformatics Data Mart contains de-identified health care data for lots of US citizens. It contains pharmacological data from test results, prescriptions, pharmacies, and clinician notes. The database is commonly used for research on health consequences, analysis of real-world data, and study of healthcare utilization. [198]

Surveillance, Epidemiology, End-Results Monitoring Program – SEER

The National Cancer Institute (NCI)'s SEER Program collects available cancer data from a variety of sources. It includes information on survival rates, cancer characteristics, and demography. SEER data are used to support analysis of health policy, epidemiological investigations, and cancer research.[199]

BioLINCC

BioLINCC is the information coordination centre for biological specimen and data archives. BioLINCC, a resource of the National Heart, Lung, and Blood Institute (NHLBI), facilitates

access to clinical and biological data from numerous cardiovascular and pulmonary research projects. It includes clinical data, diagnostic results, genetic information, and samples. BioLINCC supports collaboration and secondary research in pulmonary and cardiovascular investigations. [200]

The Cancer Imaging Archive - TCIA

TCIA is a database that concentrates on medical imaging data for cancer research and is accessible to the public. It contains a vast assortment of imaging studies, such as CT, MRI, and PET, as well as relevant clinical data. TCIA grants researchers access to and analysis of imaging data for a vast array of cancer varieties. [201]

Clinical medical databases, in conclusion, are useful instruments that support data-driven decision making, research, and healthcare improvement. These databases hold great promise for expanding medical knowledge, enhancing patient care and public health outcomes, promoting innovation, and influencing the course of medical research. Researchers, medical professionals, and policymakers can use these systems to find and analyse large amounts of patient data, clinical examinations, laboratory tests, and medical images. They make it possible to do a wide range of studies, such as study on clinical results, epidemiological studies, and the creation of predictive models.

4.4 Artificial intelligence and pressure ulcers

The integration of AI into plastic surgery has opened new avenues for improving patient care, particularly in the prevention and treatment of PUs. Our work, as detailed in the article *Machine Learning-Based Pressure Ulcer Prediction in Modular Critical Care Data* (*Diagnostics*, 12(4), 850), focuses on utilizing ML to predict the risk of PUs in critical care settings. This approach uses large-scale patient data to identify risk factors and support targeted prevention strategies.

Pressure ulcers are a significant challenge in critically ill patients, where immobility and comorbidities increase the risk of tissue damage. Traditional risk assessment tools often rely on subjective evaluations, leading to inconsistencies and delayed interventions. Our study applied ML algorithms to the MIMIC IV database, which contains de-identified health records of ICU patients. By analysing these records, ML models identified key risk factors,

such as nutritional parameters, vital signs, and comorbidities, and provided predictive insights to assist in early prevention measures. The predictive power of ML in this context allows for personalized patient care, optimizing interventions, such as the use of specialized mattresses, frequent repositioning, and nutritional support. By applying these AI-driven insights, clinicians can reduce the incidence and severity of pressure ulcers, ultimately improving patient outcomes. The MIMIC IV database was instrumental in our research, offering a robust source of de-identified patient data. Accessing this database requires specific steps to ensure compliance with ethical and regulatory standards. Researchers must complete 11 modules, each culminating in a test, and obtain special certificates, as outlined in *Appendices 2-4.* Building on our findings, AI-driven tools could be further expanded to support decision-making in PUs treatment, including wound classification, monitoring of healing progress, and optimization of surgical interventions such as flap plasties. Integration of AI into electronic health records and bedside devices in ICUs could enhance real-time risk prediction and intervention strategies. By utilizing ML in this innovative manner, we aim to revolutionize the management of PUs in plastic surgery, bridging the gap between advanced technology and personalized patient care.

4.5 Summary

For decades, risk factors and classification systems for pressure ulcers have been well-documented. Modern advancements emphasize the integration of contemporary technologies, particularly AI, across medical disciplines. AI, with its ML capabilities, holds immense potential for predicting pressure ulcer risk by analysing predictive factors, such as laboratory values and a patient's physical and mental condition. By stratifying these risk factors, AI can assess a patient's likelihood of developing PUs upon hospital admission, enabling the immediate implementation of preventive measures, such as specialized mattresses, positioning schedules, and other interventions.

In reconstructive surgery for deep PUs, while procedures like flap plasties have proven effective, recurrence rates remain comparatively high. This has necessitated deeper analysis of risk factors influencing both the development of PUs and the outcomes of surgical interventions. By leveraging AI, specific risk factors for acute, subacute, and long-term complications can now be identified and addressed, allowing for more precise predictions of recurrence rates following reconstructive procedures.

ML offers several advantages in this field, including improved diagnostic accuracy, enhanced surgical planning, better monitoring of healing progress, and personalized treatment strategies. These technologies can optimize the management of PUs by reducing recovery times, minimizing complications, and tailoring interventions to each patient's unique needs.

Although some AI-driven tools are still in the research or clinical trial stages, their continued development and integration into clinical practice promise to revolutionize the treatment and prevention of PUs. By harnessing the potential of ML, we can improve patient outcomes, enhance the effectiveness of surgical interventions, and make advanced care more accessible to a broader population.

4.6 Article related to this issue

Šín, P., Hokynková, A., Nováková M., Pokorná A., Krč, R., & Podroužek, J. (2022). Machine learning-based pressure ulcer prediction in modular critical care data. *Diagnostics*, *12*(4), 850.

diagnostics

MDPI

Article

Machine Learning-Based Pressure Ulcer Prediction in Modular Critical Care Data

Petr Šín [1], Alica Hokynková [1,*], Nováková Marie [2], Pokorná Andrea [3], Rostislav Krč [4] and Jan Podroužek [4]

[1] Department of Burns and Plastic Surgery, Faculty Hospital Brno and Faculty of Medicine, Masaryk University, Jihlavská 20, 625 00 Brno, Czech Republic; p.sin@seznam.cz
[2] Department of Physiology, Faculty of Medicine, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic; majka@med.muni.cz
[3] Department of Health Sciences, Faculty of Medicine, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic; apokorna@med.muni.cz
[4] Institute of Computer Aided Engineering and Computer Science, Faculty of Civil Engineering, Brno University of Technology, Veveří 331/95, 602 00 Brno, Czech Republic; rostislav.krc@gmail.com (R.K.); podrouzek.j@fce.vutbr.cz (J.P.)
* Correspondence: alicah@post.cz; Tel.: +420-532-233-004

Abstract: Increasingly available open medical and health datasets encourage data-driven research with a promise of improving patient care through knowledge discovery and algorithm development. Among efficient approaches to such high-dimensional problems are a number of machine learning methods, which are applied in this paper to pressure ulcer prediction in modular critical care data. An inherent property of many health-related datasets is a high number of irregularly sampled time-variant and scarcely populated features, often exceeding the number of observations. Although machine learning methods are known to work well under such circumstances, many choices regarding model and data processing exist. In particular, this paper address both theoretical and practical aspects related to the application of six classification models to pressure ulcers, while utilizing one of the largest available Medical Information Mart for Intensive Care (MIMIC-IV) databases. Random forest, with an accuracy of 96%, is the best-performing approach among the considered machine learning algorithms.

Keywords: pressure ulcer; pressure injury; machine learning; MIMIC database; MIMIC-IV; open data; artificial neural network; random forest

Citation: Šín, P.; Hokynková, A.; Marie, N.; Andrea, P.; Krč, R.; Podroužek, J. Machine Learning-Based Pressure Ulcer Prediction in Modular Critical Care Data. *Diagnostics* 2022, 12, 850. https://doi.org/10.3390/diagnostics12040850

Academic Editors: Keun Ho Ryu and Nipon Theera-Umpon

Received: 3 March 2022
Accepted: 28 March 2022
Published: 30 March 2022

1. Introduction

Pressure ulcers (PUs), also called pressure injuries (PIs), are classified into the category of non-healing or complicated healing wounds in most cases [1,2]. PUs burden not only the patients (necessity of wound care, pain, limited social interactions and a consequently worsening psychological status, etc.) but also represent a significant financial load on the health care services/systems (hospital, home care, caregivers, etc.). Non-healing wounds often reflect comorbidity or multimorbidity and represent the so-called silent epidemic affecting a large proportion of the world's population [3].

The incidence of pressure injuries worldwide and the prevalence of pressure injuries in healthcare settings ranges from 0% to 72.5% [4–7]. It is estimated that around 10% of hospital patients and 5% of community care patients suffer from PUs and that 72% of all PUs occur in persons older than 65 years [8,9]. Differences in prevalence and incidence statistics are influenced by data collection and analysis methodology [10,11]. In the Czech Republic, there are two main sources for PUs monitoring. In the national adverse event reporting, the PUs are reported from all inpatient healthcare providers nationwide. The Adverse Event Reporting System (AERS) in the Czech Republic monitors the adverse events' (AEs) occurrence in clinical practice and the subsequent data transmission to

the central system [12]. The methodological documents are regularly updated, and the national AERS online portal was created [12] as a professional communication platform for healthcare providers (HCP). The data are reported by nurses or quality managers. AERS is a convenient tool for cultivating the quality of care at a national level, with data mandatorily provided by all inpatient facilities. The most commonly reported AEs for each reporting period were pressure ulcers—PUs (n = 48,704/2018; n = 48,779/2019; n = 47,755/2020). The data reported based on the ICD-10 codes are collected in the National Registry of Reimbursed Health Services (NRRHS). This registry is part of the National Health Information System (NHIS) and serves as a database where patients are reported to health care providers. The database contains data from health insurance companies, including complete data on reported diagnoses, procedures, and treatments. Patients with PUs are all diagnosed with L89* in the primary or secondary position on any medical document in a given year. Between 2010 and 2019, an average of 26,444 PUs per year were identified. In 2019, a diagnosis of L89 was reported in 30,590 patients, or 287 cases per 100,000 population. Most patients were reported to have category II (26.1%) or category III (23.9%) of all PUs [13]. Data analysis showed an increasing trend in PUs reporting, which may be related to improved ability to identify PUs and certainly to reimbursement of care for the higher PUs category.

Pressure ulcers are defined, according to the latest edition (2019) of the International Guideline for "Prevention and Treatment of Pressure Ulcers/Injuries", as a localized damage to the skin and/or underlying tissue, as a result of pressure along or in combination with shear forces [2,14]. Prolonged pressure or shear results from contact between a bony prominence and the base or layer (bed, wheelchair, etc.). It predominantly concerns sacral, hip-trochanteric, and ischial areas. PUs can occur in various clinical forms, from non-blanchable skin erythema, superficial defects with affected subcutaneous tissue, and fistulas, to deep extensive defects with damaged muscles or bones. Clinical appearance of PUs is the basis for its classification—reversible PUs (category I and II), irreversible PUs (category III and IV), unstageable PUs, deep-tissue PUs, and specific PUs (medical device-related PUs and mucosal membrane PUs). [15] The pressure ulcer's category determines its specific treatment—namely, conservative or surgical.

Prevention, early diagnosis, and adequate treatment play the most important role in skin and wound care in patients who are predisposed to PUs or who already suffer from them. Prevention goes hand-in-hand with an assessment of controlling risk factors of PUs; therefore, many assessment scale systems and tools were established to evaluate them. The Braden, Norton, or Waterloo scale systems are mostly used in clinical practice and are focused on moisture, incontinence, nutrition, mobility of the patient, etc. [16,17]. Superficial PUs (category I and II) are often omitted in lists of primary (main/principal) and secondary diagnoses during hospitalization. On the other hand, deep PUs (category III or IV) represent a serious complication that may increase the mortality of the patients, especially in intensive care units (ICUs) [18,19].

Therefore, establishing the predicting factors of PUs can help to eliminate the risk of the formation of hospital-acquired PUs (HAPUs). The formation and progression of PUs is affected by numerous factors; in other words, the causation is multifactorial. The determination of predictive factors, especially in case of hospital-acquired PUs and in critically ill patients in the ICU, can play an important role in their prevention. Predisposing factors for PUs formation are both intrinsic (comorbidities, poor nutritional status, limited mobility, etc.) [20,21] and extrinsic (excessive moisture, pressure from bed mattresses, shear forces from muscle spasms) [22]. In this paper, the following predictive factors of hospital-acquired PUs were included: basal constant demographic factors, such as age, gender and ethnicity.

Predictive factors related to gender are rather inconsistent. Kottner et al. presents that hospital-acquired PUs are a little more frequently found in men than in women. However, since this difference was slight, they concluded that gender should not be taken into consideration as an independent risk factor for PU development [23]. On the contrary,

age is considered a basal risk factor of PUs formation [24]. It has been reported that up to 70% of PUs are found in patients aged 65 and older [25]. As far as ethnicity is concerned, Redelings et al. found that mortality related to PUs was higher among Black patients, as compared to Caucasians [26].

Other parameters studied over time were total intake, total output, arterial oxygen saturation, arterial systolic blood pressure, height, daily weight, glucose level, nutritional status parameters—albumin, total protein, and total bilirubin. Other predictive parameters were length of stay in bed and comorbidities concerning immobilization, such as spinal cord injuries and severe fractures. Other predictive factors were focused on local PUs assessment in correlation to the Braden score—sensory perception, moisture, activity, mobility, nutrition, and friction shear. At present, one of the main topics in the theoretical research on wound healing is the role of oxidative stress in various phases of the healing process [27]. In our further presented analyses, we did not find any parameters of oxidative stress identification. We can say, however, that it is still understandable, as although it is widely believed that the amount of oxygen/nitrogen radicals might be crucial for further direction of a healing process, there are several systematic studies presenting detailed insights into reactive oxygen species (ROS)/nitrogen species (RNS). However, their role in particular phases of wound healing is still limited. On the other hand, the parameters mentioned above are mostly clinically significant and well known in clinical practice.

This paper is unique in applying machine learning methods to pressure ulcer prediction in modular critical care data, utilizing the Medical Information Mart for Intensive Care (MIMIC-IV) database in particular. Rare instances of related work are discussed in the following sections of this manuscript and mainly concern qualitatively different databases, limited sample sizes, and different architectures of the machine learning algorithms.

The structure of the database, data selection criteria, and qualitative aspects of the healthcare data are described in Section 2. Machine learning algorithms and their application in medical research are detailed in Section 3. The results are discussed in terms of performance measures of selected classifiers, correlation and importance of input parameters, and confusion matrix terms.

The main concern of this paper is to address both theoretical and practical aspects related to the application of machine learning-based classification models to pressure ulcers, while utilizing one of the largest available healthcare datasets.

2. Materials and Methods

Pressure ulcers are statistically associated with different risk factors and preventive measures. The successful utilization of ML-based PU prediction models requires consistent reporting of clinical variable selections, data pre-processing, and model specifications. Ideally, ML models should be interpretable to allow clinicians to understand and improve model performance; however, according to a review from 2021 [28], only 2 out of 62 analyzed studies concerning the MIMIC dataset and the application of ML techniques in various ICU settings resorted to visualization-based interpretations. Traditional ML models can be more easily interpreted when compared to deep learning models with many levels of features and hidden layers. In [29], a multi-scale deep convolutional architecture has been proposed to tackle the problem of mortality prediction inside the ICU while offering interpretable predictions, i.e., predictions accompanied by explanations and/or justifications which make for a more transparent decision process. Here, not only dataset-level but also patient-level interpretability is provided, working with raw features instead of pre-processed ones; however, this study is focused on a more general topic of mortality prediction inside the ICU, when compared to the PU prediction.

As the predictor importance may differ significantly in time for any given patient, the sensitivity analysis of input features is nontrivial. Logistic regression can be used in combination with time-window averaging to identify important patient features; however, different resulting importance rankings represent an artifact of the selected time window.

In this study, time-varying patient features were averaged within a week-long time window (due to lack of data) before the first record of the PU for the PU group. For the non-PU group, this averaging was based on the first week after admission, in order to utilize this model in the future for objective assessments of special care requirements during admission.

Despite the increasingly available scientific computing clusters, the size of a typical medical database is prohibitive in terms of deep unsupervised learning, i.e., multivariate analysis of the entire database is not computationally feasible. This is due to not only memory requirements, but also data quality, as healthcare data are no longer small, structured, and collected exclusively in electronic health records.

Worldwide digital healthcare data is estimated to currently equal between 25 exa-bytes (25×10^{18} bytes) [30] and 35 zeta-bytes (35×10^{21} bytes) [30], with an annual increase of between 1.2 and 2.4 exabytes per year [30]. Such a huge amount of patient data is generated by a variety of lab systems and health information systems (e.g., EHRs, CPOE, PACS, CDSS).

According to Rehman et al. [31], the quality of healthcare data is a cause of concern for four reasons: incompleteness, inconsistency, inaccuracy, heterogeneity, and data fragmentation. A variety of techniques are required to analyze data quality, such as data standardization, verification, validation, monitoring, profiling, and matching. The problem of "dirty" data is mostly related to missing values, duplication, outliers, and stale records.

Due to the above-mentioned challenges, full-sensitivity and parametric studies are rarely conducted and input variables (patient features) as well as parameters (such as time windows) cannot be objectively (automatically) identified.

Dataset

The data source for the presented study is the MIMIC-IV relational database, which represents the entire patient journey through a hospital, including performed procedures, medications given, laboratory values taken, and image analyses conducted [32]. This database is sourced from two in-hospital database systems, a custom hospital-wide electronic health record (HER) and an ICU-specific clinical information system. When creating the MIMIC-IV database, during the preparation process, data cleaning steps were not performed to ensure the data reflected a real-world clinical dataset. De-identifying results in date and time records random shifting into the future using an offset in days. Data for single patients are internally consistent; however, distinct patients are not temporally comparable [32].

A custom database for PU prediction has been extracted from MIMIC-IV, with 4652 patients with PU and a randomly sampled control group of the same size. Note that, due to the required normalization of the input variables, units are not relevant for the ML classification model.

Here, the time-invariant patient information includes age, gender, ethnicity, date of death, total intake (intravenous and fluid inputs), total output (patient outputs), and length of hospital stay.

The time-variant charted information includes arterial oxygen saturation, systolic arterial blood pressure, height, daily weight, and glucose (whole blood). The Braden scale [33] risk factors are also included sensory perception, moisture, activity, mobility, nutrition, and friction and shear. The nutritional assessment further includes albumin, total protein, and total bilirubin.

The patient information relating to fracture is a Boolean OR function that will result in TRUE if either one or more of the ICD-9 diagnosis codes related to fracture is present: fatigue fracture of vertebra; collapsed vertebra in diseases classified elsewhere; osteoporosis with pathological fracture; stress fracture, not elsewhere classified; pathological fracture, not elsewhere classified; fracture of bone in neoplastic disease; fracture of bone following insertion of orthopedic implant, joint prosthesis, or bone plate; fracture of skull and facial bones; fracture of neck; fracture of rib(s), sternum, and thoracic spine; fracture of lumbar

spine and pelvis; fracture of shoulder and upper arm; fracture of forearm; fracture at wrist and hand level; fracture of femur; fracture of lower leg, including ankle; fracture of foot, except ankle; fractures involving multiple body regions; fracture of spine, level unspecified; fracture of upper limb, level unspecified; fracture of lower limb, level unspecified; and fracture of unspecified body region.

Feature importance is computed as the mean and standard deviation of accumulation of the impurity decrease within each tree [34]. It is available both as an absolute value (FI) and a relative position (FI rank) in Table 1, together with a basic characterization of the input parameters, including the total count of PU patients and control group, their ratio, mean values, and variable type. The 4652 records of PU patients could not be used for the analysis due to the application of exclusion criteria. Patients had to be excluded if they died during hospital stay had an unrecorded PU date or had a majority of missing or null values in the selected input parameters. Debiasing [35] was used to tackle the sparsely populated data in included patients. As can be seen in Table 1, most patient features were not complete. Histograms of non-debiased input parameters before normalization are depicted in Figure 1. Correlation matrix (assuming linear relationship) for the input variables can be seen in Figure 2.

Error minimization is the usual goal of supervised machine learning classifiers while the choice of error evaluation metric is subjected to continuous debate in research and industry for several decades. A number of criteria need to be considered when choosing such a metric, e.g., interpretability, computational cost, differentiability, or popularity in a specific field.

Table 1. Characterization of input parameters and their importance for best performing RF model.

Parameter	Count dec	Count ndec	Ratio ndec/dec	Mean dec	Mean ndec	Data Type	FI	FI Rank
age	1979	4497	2.27	n/a	n/a	int64	9.41×10^{-3}	12
gender	1979	4497	2.27	n/a	n/a	category	3.64×10^{-4}	21
ethnicity	1979	4497	2.27	n/a	n/a	category	1.01×10^{-3}	19
ICU length	1979	4497	2.27	0.37	0.25	float64	2.72×10^{-1}	1
input	1979	793	0.40	2.69×10^{3}	4615.48	float64	1.27×10^{-1}	3
output	1952	784	0.40	1.38×10^{3}	3543.23	float64	1.73×10^{-1}	2
height	1356	442	0.33	168.33	170.02	float64	1.59×10^{-2}	11
weight	1472	475	0.32	84.25	85.23	float64	3.09×10^{-2}	9
blood pressure	413	185	0.45	118.23	119.24	float64	2.60×10^{-3}	15
glucose	311	149	0.48	159.25	148.07	float64	2.84×10^{-3}	13
o2sat	224	112	0.50	95.35	96.39	float64	1.27×10^{-3}	17
Braden sensory	1519	456	0.30	2.73	3.31	float64	3.37×10^{-2}	8
Braden moisture	1518	456	0.30	3.36	3.66	float64	4.33×10^{-2}	7
Braden activity	1518	456	0.30	1.21	1.65	float64	1.17×10^{-1}	4
Braden mobility	1517	456	0.30	2.28	2.85	float64	6.28×10^{-2}	6
Braden nutrition	1517	456	0.30	2.16	2.51	float64	7.85×10^{-2}	5
Braden friction	1513	456	0.30	1.80	2.36	float64	2.13×10^{-2}	10
albumin	344	138	0.40	2.88	3.27	float64	2.60×10^{-3}	16
protein	23	9	0.39	5.65	5.81	float64	2.88×10^{-4}	22
bilirubin	491	188	0.38	1.74	1.06	float64	2.71×10^{-3}	14
diag. spinal injury	1979	4497	2.27	n/a	n/a	bool	7.30×10^{-5}	23
diag. diarrhea	1979	4497	2.27	n/a	n/a	bool	1.04×10^{-3}	18
diag. fracture	1979	4497	2.27	n/a	n/a	bool	5.97×10^{-4}	20

FI, feature importance; dec, patients with PU; ndec, patients without PU.

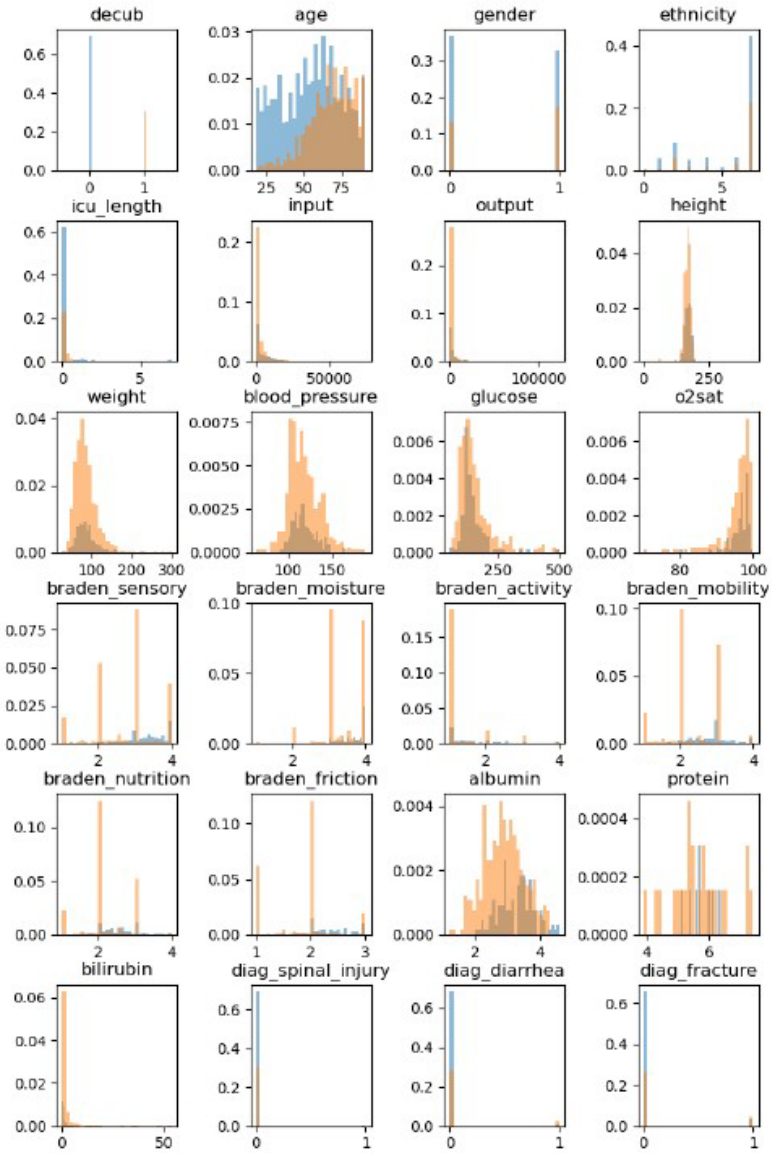


Figure 1. Histograms of non-debiased input parameters before normalization. The PU group is represented by blue color, while the orange color represents non-PU group.

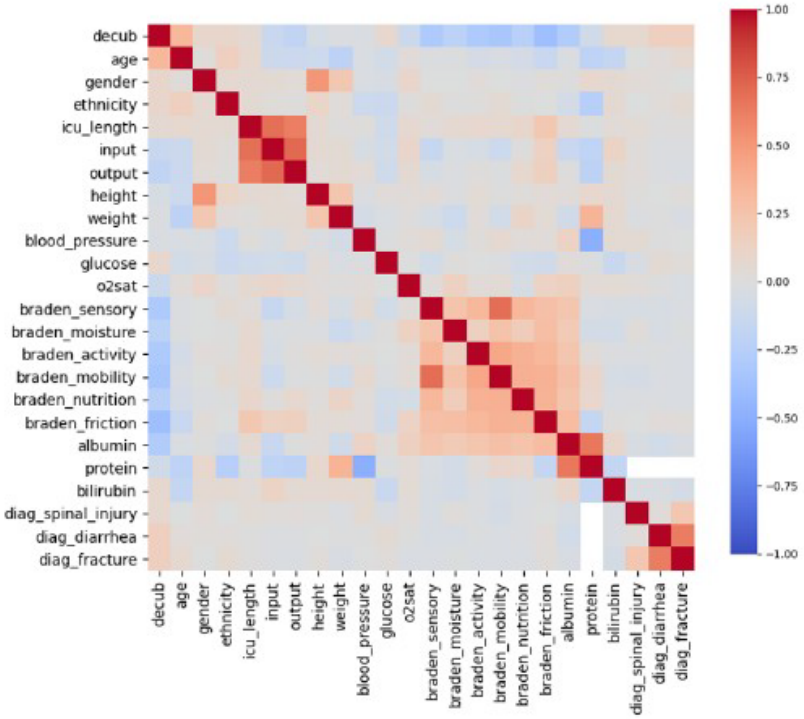


Figure 2. Correlation matrix of non-debiased input parameters.

3. Machine Learning Methods

It is well accepted that no classification method is universally better than any other [36]. Clearly, there are classes of target functions for which a method is best suited, and therefore, a cross-section of popular machine learning techniques has been chosen in order to predict the presence of pressure ulcers from a number of demographics and observed and measured patient features, with some characteristics unequally sampled in time (see Table 1). The medical data have been retrospectively collected within the MIMIC project [32].

Among the considered ML techniques are regression algorithms (logistic regression), instance-based algorithms (*k*-nearest neighbors and support vector machines), ensemble algorithms (random forest), artificial neural network algorithms (multi-layer perceptron), and Bayesian algorithms (naïve Bayes).

3.1. Regression Algorithms

Logistic regression (LR) is frequently used in medical research, as it estimates the relationship between one or more independent variables and a binary (dichotomous) outcome variable, such as "presence versus absence of pressure ulcer", "dead versus alive", or "positive versus negative for hypoxemia". An example of multivariate logistic regression application to identify pressure ulcer risk factors can be found in [37].

The LR classification model assumes L2 regularization, also known as ridge regression. This technique is used to prevent overfitting by introducing a regularization term into the optimization problem. Tolerance is set to 10^{-4}, the inverse of regularization strength (C) is set to 1.0, and the maximum number of iterations is limited to 100.

3.2. Instance-Based Algorithms

Space–time clusters of health events and their interactions are often investigated using the k-nearest neighbors (KNN) statistic, which is the number of case pairs that are k-nearest neighbors in both space and time, and is evaluated under the null hypothesis of independent space and time nearest neighbor relationships. Example applications can be found, e.g., in [38], where an adaptive-weighted k-nearest neighbors algorithm for the imputation of the first three months of screening visits has been developed.

The KNN model assumes a k parameter equal to 5 (based on heuristic technique), as larger values reduce the effect of noise on the classification, but make boundaries between classes less distinct. Additionally, the accuracy of KNN can be severely degraded if noisy or irrelevant features are present, or if the feature scales do not match their importance. Therefore, all input variables (patient features) were transformed to Gaussian distributions with zero mean value and unit standard deviation for all ML methods considered in this paper, assuming the central limit theorem.

According to [39], support vector machine (SVM)- and artificial neural network (ANN)-based classifiers have been the most useful artificial intelligence techniques to classify cancer. In particular, a study on liver biopsy images using a probabilistic neural network (PNN) has been presented, e.g., in [40]. An ANN classifier has also been used for breast cancer classification in the Wisconsin Breast Cancer Database (WBCD) [41], where a neural network with a feed-forward back-propagation algorithm was used to classify cancerous tumors from a symptom that causes the breast cancer disease. ANN classifiers are also used for successful lung cancer detection; in [42], a 16 descriptive attributes yield reported an accuracy of 97%. Based on various studies on cancer detection, SVM has the highest capability to classify datasets with a smaller number of input features, while ANN has better performance of accuracy in classifying datasets with a larger number of input features [39].

3.3. Artificial Neural Network Algorithms

The difference between ANN and SVM mainly concerns the classification of non-linear data, where SVM utilizes non-linear mapping to make the data linear separable, and therefore, the selection of the kernel function is the key. ANN, however, employs multi-layer connection and various activation functions in order to solve non-linear problems. Moreover, the more data is fed into the network, the better the generalization; thus, fewer errors can be expected from ANN. Conversely, SVM and random forest (RF) require significantly fewer input data.

The SVM model assumes a linear kernel with C equal to 2.0 and tolerance 10^{-3}. The multi-layer perceptron (MLP) neural network model assumes two hidden layers (100 and 20), a rectified linear unit (ReLU) activation function (default activation function of many types of neural networks), and an Adam optimizer, which is invariant to diagonal rescales of the gradients and is appropriate for problems with noisy and sparse gradients [43]. The learning rate for MLP is set to 10^{-3} and the number of complete passes through the training dataset (epochs) is set to 300.

3.4. Bayesian Algorithms

A naïve Bayes (NB) classifier is used in [44] to detect cardiovascular disease and identify its risk level, consisting of a training set of tuples and their associated class labels. Here, the probability for a particular (cardiovascular) disease, given its symptoms, can be estimated using the Bayesian conditional probability model. In [45], a disease prediction system based on NB is presented, including typhoid, malaria, jaundice, tuberculosis, and gastroenteritis. NB is known for its limitation stemming from the assumption of independent predictors, which are almost absent in real-life scenarios; however, as a simple and fast method, NB is useful for real-time predictions, multi-class predictions, or recommendation systems in general.

3.5. Ensemble Algorithms

A random forest classifier has been successfully applied in healthcare monitoring systems in combination with the Internet of Things (IoT) in [46] to identify fraudulent behaviors in healthcare claims [47], or in evaluations of patient safety culture [48]. An RF model assumes 100 estimators and a maximal depth equal to 6, i.e., the number of trees in the forest and the maximal number of levels in each decision tree. According to [49], RF has the best accuracy in pressure ulcer prediction when compared to SVM, ANN, and decision tree (DT) models. This is in line with the conclusion of this paper, despite that the origin of the patients and the selected features are different.

4. Results and Discussion

Among the commonly used performance measures of classifiers based on machine learning methods are the receiver operating characteristic (ROC) curves and area under the ROC curve (AUC); see Figure 3. The raw data produced by a classification scheme during testing are counts of the correct and incorrect classifications from each class. This information is typically displayed in a *confusion matrix* (Table 2), which is a form of contingency table showing the differences between the true and predicted classes for a set of labelled examples [50].

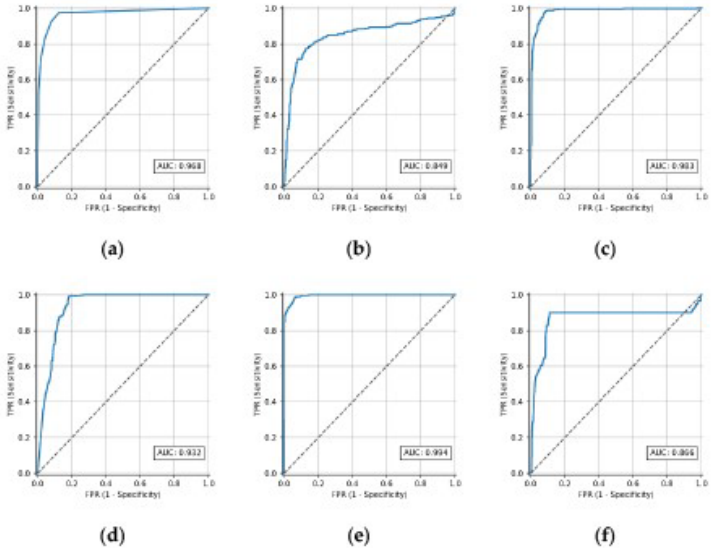


Figure 3. Performance of the 6 classification models considered at all classification thresholds (ROC curves): (**a**) *k*-nearest neighbors, (**b**) logistic regression, (**c**) multi-layer perceptron, (**d**) naïve Bayes, (**e**) random forest and (**f**) support vector machines.

Table 2. Evaluation of machine learning algorithms: scalar performance measures and confusion matrix terms. Values are color-coded on a green (favorable values)-to-red (adverse values) scale.

	Accuracy	Precision	Recall	F1-Score	AUC	Time [s]				
Model		PPV	TPR				TPR	TNR	FPR	FNR
Random Forest	0.960	0.946	0.916	0.930	0.947	0.437	0.92	0.98	0.02	0.08
Multi-layer Perceptron	0.944	0.899	0.911	0.905	0.934	24,130	0.91	0.96	0.04	0.09
k-Nearest Neighbors	0.921	0.890	0.832	0.860	0.895	0.001	0.83	0.96	0.04	0.17
SVM (linear kernel)	0.873	0.785	0.779	0.782	0.845	7.825	0.78	0.91	0.09	0.22
Naïve Bayes	0.851	0.752	0.734	0.743	0.817	0.004	0.73	0.90	0.10	0.27
Logistic Regression	0.842	0.816	0.595	0.688	0.770	0.042	0.59	0.94	0.06	0.41

Based on 80:20 split and fixed seed. PPV, positive predictive value; TPR, true positive rate; TNR, true negative rate; FPR, false positive rate; FNR, false negative rate.

While the ROC curve, which has been long used in conjunction with the Neyman–Pearson method [51] in signal detection theory, is a good visualization of a classifier's performance; e.g., as a decision threshold or suitable operating point, often it is desirable to obtain a scalar measure, especially for cross-validated estimates of a classifier's overall accuracy, i.e., the probability of a correct response. Such a single-figure estimate could be based on the area under the curve (AUC), or other popular metrics such as accuracy, precision, recall and F1-score; however, such measures are often insufficient, as they fail to characterize the complexity in model behavior, which has risen sharply over the last decade. For more thorough evaluation of classification models by probabilistic extension of the widely used threshold-based metrics, refer to [52].

Table 2 compares the above-mentioned metrics for the six considered ML methods and includes the average training times. The metrics are evaluated by standard binary classification with 0.5 threshold, i.e., accuracy is the fraction of correctly classified samples to total number of samples. Precision is the ratio of samples correctly classified to a particular class *c* to samples classified as class *c*, while recall is the fraction of samples in class *c* that are correctly retrieved. F1-score is an indicator quantifying the accuracy of a dichotomous model and it assumes both precision and recall of classification, i.e., it can be considered as a weighted average of model precision and recall.

The selection of the RF model and its accuracy corresponds to a study from a Chinese hospital [49], where slightly fewer patients (85%) were included in the study, which also differed in a number of additional aspects. The RF model is also recommended in a similar study from the USA [53], where 39% of patients were included and the performance (AUC) reached 79%, when compared to results presented in this paper; however, stage I and stage II pressure ulcers were distinguished in the prediction, which surely resulted in the lower AUC.

A comprehensive review of the scientific literature concerning the use of ML algorithms for PU prevention has recently been published by [54], where the best-performing technique for the prediction of surgery-related pressure ulcers is ANN, with an accuracy of 81.5%.

This paper is unique in addressing both theoretical and practical aspects related to the application of ML models to pressure ulcers, while utilizing one of the largest available Medical Information Mart for Intensive Care (MIMIC) datasets. Given the size of the database, a big data approach is necessary and overfitting remains a challenge, given the high-dimensionality of the problem, as the number of available parameters, some of which are non-uniformly distributed (sampled) in time, is often equal to or greater than the number of patients, which can be included. This leads to the subjective choices regarding inclusion and exclusion criteria, which has to be realistically assessed given the available (and missing) data and the flexibility of the ML models.

Future work will include a distinction between more pressure ulcer groups and ML-based image processing and pattern recognition, towards automated and objective pressure ulcer classification.

In order to succeed, in general, the lag between data collection and processing has to be addressed, as well as the issues of ownership, governance, and standards. Moreover, health care data is rarely standardized, often fragmented, and is generated in legacy IT systems. This represents a major barrier in front of real-time big data analytics in performance-based healthcare systems.

5. Conclusions

The presented paper concerns the machine learning approach to pressure ulcer prediction based on a number of demographics and observed and measured patient features, retrospectively collected within the MIMIC project.

A cross-section of popular learning algorithms has been selected such that it represents various approaches to supervised ML, as up to the current date, there has been no classification method universally better than any other.

The best-performing approach among the considered ML techniques, which include regression algorithms, instance-based algorithms, ensemble algorithms, artificial neural network algorithms, and Bayesian algorithms, is random forest, yielding an accuracy of 96%.

The predictor importance differs significantly in time for any given patient and based on the sensitivity analysis of the input features of the best performing RF model. The most important patient features are ICU length of stay, total intake (intravenous and fluid inputs), and total output, i.e., time-invariant patient information that is independent from the time-window averaging scheme.

Author Contributions: Conceptualization, J.P., P.Š. and A.H.; ML algorithms and validation, J.P. and R.K.; investigation, N.M., A.H. and P.Š.; data curation, A.H. and R.K.; writing, J.P., A.H. and P.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Ethical review and approval were waived for this study due to the fact that this article is focused only on data and information collected from database MIMIC IV (Medical Information Mart for Intensive Care).

Informed Consent Statement: Not applicable. No patients were involved in the design of the study. According to Czech law, there is no special ethical approval for the research purposes analyses if the authorized person analyses the data.

Data Availability Statement: This article is focused on anonymous data and information collection from EHR (Electronic Health Records) from database MIMIC IV (Medical Information Mart for Intensive Care). This database is open only for person, who has finished the Collaborative Institutional Training Initiative examination in Human Research-Data or Specimenens Only Research (Certification number 43354586 for corresponding author).

Acknowledgments: This work was supported by the Ministry of Health of the Czech Republic under grant no. NU21-09-00541, "The role of oxidative stress in pressure ulcers treatment in a patient with spinal injury". All rights reserved. The grant no. TJ04000232 by the Technology Agency of the Czech Republic is also acknowledged.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Sen, C.K.; Gordillo, G.M.; Roy, S.; Kirsner, R.; Lambert, L.; Hunt, T.K.; Gottrup, F.; Gurtner, G.C.; Longaker, M.T. Human Skin Wounds: A Major and Snowballing Threat to Public Health and the Economy. *Wound Repair Regen.* **2009**, *17*, 763–771. [CrossRef] [PubMed]
2. Haesler, E.; European Pressure Ulcer Advisory Panel (EPUAP); National Pressure Injury Advisory Panel (NPIAP); Pan Pacific Pressure Injury Alliance (PPPIA). Prevention and Treatment of Pressure Ulcers/Injuries: Clinical Practice Guideline. Available online: https://www.internationalguideline.com/ (accessed on 27 March 2022).
3. Pokorná, A.; Leaper, D. Assessment and Documentation of Non-Healing, Chronic Wounds in Inpatient Health Care Facilities in the Czech Republic: An Evaluation Study. *Int. Wound J.* **2015**, *12*, 224–231. [CrossRef] [PubMed]
4. Hiser, B.; Rochette, J.; Philbin, S.; Lowerhouse, N.; Terburgh, C.; Pietsch, C. Implementing a Pressure Ulcer Prevention Program and Enhancing the Role of the CWOCN: Impact on Outcomes. *Ostomy. Wound Manag.* **2006**, *52*, 48–59.
5. Samaniego, I.A. A Sore Spot in Pediatrics: Risk Factors for Pressure Ulcers. *Pediatr. Nurs.* **2003**, *29*, 278.
6. Tubaishat, A.; Papanikolaou, P.; Anthony, D.; Habiballah, L. Pressure Ulcers Prevalence in the Acute Care Setting: A Systematic Review, 2000–2015. *Clin. Nurs. Res.* **2018**, *27*, 643–659. [CrossRef] [PubMed]
7. Al Mutairi, K.B.; Hendrie, D. Global Incidence and Prevalence of Pressure Injuries in Public Hospitals: A Systematic Review. *Wound Med.* **2018**, *22*, 23–31. [CrossRef]
8. Moore, Z.; Cowman, S. Pressure Ulcer Prevalence and Prevention Practices in Care of the Older Person in the Republic of Ireland. *J. Clin. Nurs.* **2012**, *21*, 362–371. [CrossRef]
9. Russo, C.A.; Steiner, C.; Spector, W. *Hospitalizations Related to Pressure Ulcers among Adults 18 Years and Older, 2006: Statistical Brief# 64*; Agency for Healthcare Research and Quality: Rockville, MD, USA, 2006.
10. Pokorná, A.; Benešová, K.; Mužík, J.; Jarkovský, J.; Dušek, L. Data Sources for Monitoring of Non-Healing Wounds in a National Health Information System–Epidemiology of Non-Healing Wounds–Analysis of the National Register of Hospitalized Patients in 2007–2015. *Čes. Slov. Neurol. Neurochir.* **2017**, *80*, S8–S17. [CrossRef]
11. Pokorná, A.; Benešová, K.; Jarkovský, J.; Mužík, J.; Beeckman, D. Pressure Injuries in Inpatient Care Facilities in the Czech Republic. *J. Wound. Ostomy Cont. Nurs.* **2017**, *44*, 331–335. [CrossRef]
12. Pokorná, A.; Štrombachová, V.; Mužík, J.; Dolanová, D.; Búřilová, P.; Pospíšil, M.; Kučerová, J.; Gregor, J.; Komenda, M.; Dušek, L. SHNU—Úvod. Available online: https://shnu.uzis.cz/ (accessed on 23 February 2022).
13. Búřilová, P.; Dolanová, D.; Saibertová, S.; Benešová, K.; Jarkovský, J.; Krupová, L.; Pokorná, A. Základní Epidemiologická Analýza Pacientů s Dekubity v Národním Zdravotnickém Informačním Systému. *Léčba Ran* **2021**, *8*, 6–7.
14. Kottner, J.; Cuddigan, J.; Carville, K.; Balzer, K.; Berlowitz, D.; Law, S.; Litchford, M.; Mitchell, P.; Moore, Z.; Pittman, J.; et al. Pressure Ulcer/Injury Classification Today: An International Perspective. *J. Tissue Viability* **2020**, *29*, 197–203. [CrossRef] [PubMed]
15. Hess, C.T. Classification of Pressure Injuries. *Adv. Skin Wound Care* **2020**, *33*, 558–559. [CrossRef] [PubMed]
16. Chan, W.S.; Pang, S.M.C.; Kwong, E.W.Y. Assessing Predictive Validity of the Modified Braden Scale for Prediction of Pressure Ulcer Risk of Orthopaedic Patients in an Acute Care Setting. *J. Clin. Nurs.* **2009**, *18*, 1565–1573. [CrossRef] [PubMed]
17. Šáteková, L.; Žiaková, K.; Zeleníková, R. Predikčná Validita Bradenovej Škály, Nortonovej Škály a Waterlowej Škály v Slovenskej Republike. *Cent. Eur. J. Nurs. Midwifery* **2015**, *6*, 283–290. [CrossRef]
18. Becker, D.; Tozo, T.C.; Batista, S.S.; Mattos, A.L.; Silva, M.C.B.; Rigon, S.; Laynes, R.L.; Salomão, E.C.; Hubner, K.D.G.; Sorbara, S.G.B.; et al. Pressure Ulcers in ICU Patients: Incidence and Clinical and Epidemiological Features: A Multicenter Study in Southern Brazil. *Intensive Crit. Care Nurs.* **2017**, *42*, 55–61. [CrossRef]
19. Borghardt, A.T.; do Prado, T.N.; Bicudo, S.D.S.; de Castro, D.S.; Bringuente, M.E.d.O. Pressure Ulcers in Critically Ill Patients: Incidence and Associated Factors. *Rev. Bras. Enferm.* **2016**, *69*, 460–467. [CrossRef]
20. Bluestein, D.; Javaheri, A. Pressure Ulcers: Prevention, Evaluation, and Management. *Am. Fam. Physician* **2008**, *78*, 1186–1194.
21. Garcia, A.D.; Thomas, D.R. Assessment and Management of Chronic Pressure Ulcers in the Elderly. *Med. Clin.* **2006**, *90*, 925–944. [CrossRef]
22. Jaul, E. Assessment and Management of Pressure Ulcers in the Elderly. *Drugs Aging* **2010**, *27*, 311–325. [CrossRef]
23. Lichterfeld-Kottner, A.; Lahmann, N.; Kottner, J. Sex-Specific Differences in Prevention and Treatment of Institutional-Acquired Pressure Ulcers in Hospitals and Nursing Homes. *J. Tissue Viability* **2020**, *29*, 204–210. [CrossRef]
24. Kröger, K.; Niebel, W.; Maier, I.; Stausberg, J.; Gerber, V.; Schwarzkopf, A. Prevalence of Pressure Ulcers in Hospitalized Patients in Germany in 2005: Data from the Federal Statistical Office. *Gerontology* **2009**, *55*, 281–287. [PubMed]
25. Stotts, N.A.; Wu, H.-S. Hospital Recovery Is Facilitated by Prevention of Pressure Ulcers in Older Adults. *Crit. Care Nurs. Clin. N. Am.* **2007**, *19*, 269–275. [CrossRef] [PubMed]
26. Redelings, M.D.; Lee, N.E.; Sorvillo, F. Pressure Ulcers: More Lethal than We Thought? *Adv. Skin Wound Care* **2005**, *18*, 367–372. [CrossRef] [PubMed]
27. Hokynková, A.; Babula, P.; Pokorná, A.; Nováková, M.; Nártová, L.; Šín, P. Oxidative Stress in Wound Healing—Current Knowledge. *Ceska Slov. Neurol. Neurochir.* **2019**, *82*, 37–39. [CrossRef]
28. Syed, M.; Syed, S.; Sexton, K.; Syeda, H.B.; Garza, M.; Zozus, M.; Syed, F.; Begum, S.; Syed, A.U.; Sanford, J.; et al. Application of Machine Learning in Intensive Care Unit (ICU) Settings Using MIMIC Dataset: Systematic Review. *Informatics* **2021**, *8*, 16. [CrossRef]

29. Caicedo-Torres, W.; Gutierrez, J. ISeeU: Visually Interpretable Deep Learning for Mortality Prediction inside the ICU. *J. Biomed. Inform.* **2019**, *98*, 103269. [CrossRef]
30. Home—IEEE Big Data. Available online: https://bigdata.ieee.org/ (accessed on 1 February 2022).
31. Rehman, A.; Naz, S.; Razzak, I. Leveraging Big Data Analytics in Healthcare Enhancement: Trends, Challenges and Opportunities. *Multimed. Syst.* **2021**, 1–33. [CrossRef]
32. Johnson, A.; Bulgarelli, L.; Pollard, T.; Horng, S.; Celi, L.A.; Mark IV, R. Mimic-Iv (Version 0.4). *PhysioNet* **2022**. [CrossRef]
33. Kozier, B. *Fundamentals of Nursing: Concepts, Process and Practice*; Pearson Education: London, UK, 2008; ISBN 978-0-13-197653-5.
34. Scikit-Learn: Machine Learning in Python—Scikit-Learn 1.0.2 Documentation. Available online: https://scikit-learn.org/stable/ (accessed on 3 February 2022).
35. Sklearn.Decomposition.SparseCoder. Available online: https://scikit-learn/stable/modules/generated/sklearn.decomposition.SparseCoder.html (accessed on 3 February 2022).
36. Hastie, T.; Tibshirani, R.; Friedman, J.H.; Friedman, J.H. *The Elements of Statistical Learning: Data Mining, Inference, and Prediction*; Springer: Berlin/Heidelberg, Germany, 2009; Volume 2.
37. Nijs, N.; Toppets, A.; Defloor, T.; Bernaerts, K.; Milisen, K.; Van Den Berghe, G. Incidence and Risk Factors for Pressure Ulcers in the Intensive Care Unit. *J. Clin. Nurs.* **2009**, *18*, 1258–1266. [CrossRef]
38. Tavazzi, E.; Daberdaku, S.; Vasta, R.; Calvo, A.; Chiò, A.; Di Camillo, B. Exploiting Mutual Information for the Imputation of Static and Dynamic Mixed-Type Clinical Data with an Adaptive k-Nearest Neighbours Approach. *BMC Med. Inform. Decis. Mak.* **2020**, *20*, 174. [CrossRef]
39. Ubaidillah, S.H.S.A.; Sallehuddin, R.; Ali, N.A. Cancer Detection Using Aritifical Neural Network and Support Vector Machine: A Comparative Study. *J. Teknol.* **2013**, *65*. [CrossRef]
40. Pan, S.-M.; Lin, C.-H. Fractal Features Classification for Liver Biopsy Images Using Neural Network-Based Classifier. In Proceedings of the 2010 International Symposium on Computer, Communication, Control and Automation (3CA), Tainan, Taiwan, 5–7 May 2010; Volume 2, pp. 227–230.
41. Azmi, M.S.B.M.; Cob, Z.C. Breast Cancer Prediction Based on Backpropagation Algorithm. In Proceedings of the 2010 IEEE Student Conference on Research and Development (SCOReD), Kuala Lumpur, Malaysia, 13–14 December 2010; pp. 164–168.
42. Nasser, I.M.; Abu-Naser, S.S. *Lung Cancer Detection Using Artificial Neural Network*; Social Science Research Network: Rochester, NY, USA, 2019.
43. Kingma, D.P.; Ba, J. Adam: A Method for Stochastic Optimization. *arXiv* **2017**, arXiv:14126980.
44. Miranda, E.; Irwansyah, E.; Amelga, A.Y.; Maribondang, M.M.; Salim, M. Detection of Cardiovascular Disease Risk's Level for Adults Using Naive Bayes Classifier. *Healthc. Inform. Res.* **2016**, *22*, 196–205. [CrossRef] [PubMed]
45. Fernando, Z.T.; Trivedi, P.; Patni, A. DOCAID: Predictive Healthcare Analytics Using Naive Bayes Classification. In Proceedings of the Second Student Research Symposium (SRS), International Conference on Advances in Computing, Communications and Informatics (ICACCI'13), Mysore, India, 22–25 August 2013; pp. 1–5.
46. Kaur, P.; Kumar, R.; Kumar, M. A Healthcare Monitoring System Using Random Forest and Internet of Things (IoT). *Multimed. Tools Appl.* **2019**, *78*, 19905–19916. [CrossRef]
47. Jyothi, P.N.; Rajya, D.; Rama, N. Identifying Fraudulent Behaviors in Healthcare Claims Using Random Forest Classifier With SMOTEchnique. *Int. J. E-Collab.* **2020**, *16*, 30–47. [CrossRef]
48. Simsekler, M.C.E.; Qazi, A.; Alalami, M.A.; Ellahham, S.; Ozonoff, A. Evaluation of Patient Safety Culture Using a Random Forest Algorithm. *Reliab. Eng. Syst. Saf.* **2020**, *204*, 107186. [CrossRef]
49. Song, J.; Gao, Y.; Yin, P.; Li, Y.; Li, Y.; Zhang, J.; Su, Q.; Fu, X.; Pi, H. The Random Forest Model Has the Best Accuracy Among the Four Pressure Ulcer Prediction Models Using Machine Learning Algorithms. *Risk Manag. Healthc. Policy* **2021**, *14*, 1175–1187. [CrossRef]
50. Bradley, A.P. The Use of the Area under the ROC Curve in the Evaluation of Machine Learning Algorithms. *Pattern Recognit.* **1997**, *30*, 1145–1159. [CrossRef]
51. Therrien, C.W. *Decision, Estimation and Classification: An Introduction to Pattern Recognition and Related Topics*; International, Ed.; John Wiley and Sons Ltd.: New York, NY, USA, 1989; ISBN 978-0-471-50416-0.
52. Yacouby, R.; Axman, D. Probabilistic Extension of Precision, Recall, and F1 Score for More Thorough Evaluation of Classification Models. In Proceedings of the First Workshop on Evaluation and Comparison of NLP Systems, Online, November 2020; pp. 79–91. Available online: https://aclanthology.org/2020.eval4nlp-1.9 (accessed on 27 March 2022).
53. Alderden, J.; Pepper, G.A.; Wilson, A.; Whitney, J.D.; Richardson, S.; Butcher, R.; Jo, Y.; Cummins, M.R. Predicting Pressure Injury in Critical Care Patients: A Machine-Learning Model. *Am. J. Crit. Care* **2018**, *27*, 461–468. [CrossRef]
54. Ribeiro, F.; Fidalgo, F.; Silva, A.; Metrôlho, J.; Santos, O.; Dionisio, R. Literature Review of Machine-Learning Algorithms for Pressure Ulcer Prevention: Challenges and Opportunities. *Informatics* **2021**, *8*, 76. [CrossRef]

5. FUTURE PERSPECTIVE

The work carried out over the past 15 years provides a strong foundation for future advancements in the management and treatment of PUs. To build upon this foundation, it is essential to focus on key areas that enhance interdisciplinary collaboration, leverage advanced technologies, and create standardized approaches to improve patient outcomes.

A critical future step is strengthening collaboration between healthcare professionals, artificial intelligence (AI) experts, and software developers. This partnership is vital for developing innovative tools and systems that can predict risk factors for postoperative complications and optimize treatment planning. AI-driven solutions should be integrated seamlessly with clinical workflows, aiding healthcare providers in decision-making and risk assessment.

Standardized documentation and photo documentation of PUs are essential for ensuring consistency in clinical practice. By implementing uniform protocols for data collection, wound assessment, and image capture, clinicians can improve the quality of care and enable more accurate tracking of patient's treatment progress. A centralized clinical database of PUs could serve as a valuable resource for research, allowing for statistical evaluations of postoperative complications and detailed analyses of risk factors associated with poor outcomes.

To enhance surgical outcomes, future efforts should focus on risk factor analysis and prediction of postoperative complications using AI. By leveraging large datasets and advanced ML techniques, AI can identify patients at higher risk for complications and suggest tailored interventions to mitigate these risks. Additionally, developing technologies for precise measurement of wound surface areas will further standardize wound assessment and improve monitoring of healing progress.

The future of wound healing research must focus on developing standardized methods for quantifying wound surface area, particularly for chronic wounds like PUs or in another extensive acute or chronic skin and soft tissue defects. A key advancement in this area is the creation of a mathematical wound model that converts 3D missing tissue volumes into a 2D representation of "active wound surface area". This standardized metric offers a consistent framework for assessing wounds, ensuring comparability across studies, and

enabling objective evaluation of healing progress and treatment responses. Our mathematical model, which recalculates the 3D missing volume to a 2D wound surface, is planned for patent application.

One critical application of this model is investigating protein loss and its impact on wound healing. Protein loss through wound exudate can hinder tissue repair and worsen malnutrition. By analysing protein loss per square centimetre of an “active wound surface”, it becomes possible to determine precise nutritional needs, predict healing outcomes, and develop targeted biochemical and nutritional interventions. Beyond protein loss, this metric allows for studying inflammatory dynamics, metabolic changes, and the efficacy of treatments in relation to the wound's biochemical environment.

Adopting this 2D wound surface area metric in clinical practice could enable personalized treatment planning, optimize surgical timing, and improve outcome prediction by linking biochemical activity to wound area stabilization. Integrating this model into practice requires multidisciplinary collaboration and advanced technologies, such as AI-driven analysis and real-time biochemical and nutritional monitoring. Establishing this approach as a standard could significantly enhance the understanding and treatment of chronic wounds, ultimately improving patient outcomes and quality of life.

A significant objective is the creation of comprehensive guidelines for timing and type of reconstructive procedures for PUs. These guidelines should be based on clinical evidence, integrating factors such as nutritional optimization, wound characteristics, and patient-specific needs to determine the most appropriate surgical approach. Statistical evaluations of postoperative complications and long-term outcomes should underpin these guidelines, ensuring they are both practical and evidence based.

In conclusion, the future of PUs management lies in fostering interdisciplinary collaboration, embracing advanced technologies, and establishing standardized clinical practices. By addressing these priorities, we can improve the quality of care, optimize surgical outcomes, and advance the field toward a more effective and patient-centred approach to treating PUs.

6. CONCLUSIONs

This habilitation thesis provides a broad perspective on the issue of pressure ulcers, but at the same time, it addresses several extremely particular characteristics that have the potential to affect the healing process of PUs and other hard-to-heal wounds that are rather difficult to cure. This thesis represents the culmination of 15 years of dedicated clinical practice and scientific research in the field of PUs management. Over this time, I have sought to bridge the gap between clinical care and research, addressing the multifaceted challenges posed by PUs, from prevention to advanced surgical management.

Through clinical practice, I have gained firsthand experience in the complexities of treating PUs in diverse patient populations, including critically ill individuals and those with long-term mobility impairments. This work has highlighted the importance of a multidisciplinary approach, where prevention, conservative therapy, surgical reconstruction, and nutritional support are integrated to achieve the best possible outcomes. My efforts have led to the development of internal clinical guidelines based on years of observation and practice, aiming to standardize care and reduce complications associated with surgical interventions. On the scientific front, my research has delved into various aspects of wound healing and PUs management. Initial studies explored the effects of dietary supplementation with polyunsaturated fatty acids on wound healing in animal models, providing foundational insights into the role of nutrition in tissue repair. This work evolved into clinical investigations of oxidative stress parameters in pressure ulcers and their association with different debridement techniques, further advancing our understanding of the biochemical environment of chronic wounds. In recent years, I have embraced innovative technologies, including artificial intelligence, that has shown significant potential in predicting the risk of PUs, enabling early interventions and personalized care strategies. Building on this foundation, our focus is expanding toward predicting post-surgical complications following PUs reconstruction. The aim is to reduce the risk of complications and improve long-term outcomes. However, a critical challenge remains: the absence of a comprehensive clinical database with standardized data specific to pressure ulcers. To address this gap, we are currently developing standardized protocols for documenting all relevant aspects of pressure ulcer management. These protocols include detailed patient history, laboratory investigations with established nutritional parameters and biomarkers, advanced imaging

methods such as X-ray, CT, and MRI, and standardized photo documentation. Additionally, we aim to incorporate precise wound measurement techniques, including calculations of the “active wound surface area”, to provide a more detailed and consistent understanding of wound dynamics. The creation of such a clinical database will not only facilitate the effective application of AI in risk prediction but also enhance the overall prevention and treatment strategies for PUs. This resource will provide a comprehensive framework for managing PU-related care, supporting both AI-driven tools and traditional clinical decision-making to improve outcomes in prevention, therapy, and post-surgical care.

The overarching goal of my work has been to address the complex interplay of factors that influence PUs development and healing, including malnutrition, inflammation, and surgical challenges. By combining clinical expertise with scientific aproach, I have sought to improve patient outcomes and contribute to the broader body of knowledge in this field.

While significant progress has been made, many challenges remain. The need for robust, evidence-based guidelines, particularly regarding the timing of surgical interventions and the role of nutritional optimization, is pressing. Future research must aim to validate clinical findings and translate them into standardized protocols to further enhance the quality of care for patients with PUs.

In conclusion, this thesis reflects a journey of continuous learning and innovation, driven by a commitment to improving the lives of patients affected by PUs. It is my hope that the insights gained, and contributions made through this work will serve as a foundation for ongoing advancements in this critical area of medicine.

7. List of references

1. Levine JM. Historical notes on pressure ulcers: the cure of Ambrose Pare. *Advances in Skin & Wound Care*. 1992;5(2):23-27.

2. Haesler E. European Pressure Ulcer Advisory Panel (EPUAP); National Pressure Injury Advisory Panel (NPIAP); Pan Pacific Pressure Injury Alliance (PPPIA). *Prevention and Treatment of Pressure Ulcers/Injuries: Clinical Practice Guideline International Guideline*. Published online 2019.

3. Emily H. European pressure ulcer advisory Panel, national pressure injury advisory Panel and Pan pacific pressure injury alliance. *Prevention and treatment of pressure ulcers/injuries: clinical practice guideline EPUAP/NPIAP/PPPIA*. Published online 2019.

4. Hiser B, Rochette J, Philbin S, Lowerhouse N, Terburgh C, Pietsch C. Implementing a pressure ulcer prevention program and enhancing the role of the CWOCN: impact on outcomes. *Ostomy/wound management*. 2006;52(2):48-59.

5. Samaniego IA. A sore spot in pediatrics: risk factors for pressure ulcers. *Pediatric Nursing*. 2003;29(4):278.

6. Tubaishat A, Papanikolaou P, Anthony D, Habiballah L. Pressure ulcers prevalence in the acute care setting: a systematic review, 2000-2015. *Clinical nursing research*. 2018;27(6):643-659.

7. Al Mutairi KB, Hendrie D. Global incidence and prevalence of pressure injuries in public hospitals: a systematic review. *Wound Medicine*. 2018;22:23-31.

8. Pokorná A, Benešová K, Mužík J, Jarkovský J, Dušek L. Data Sources for Monitoring of Non-healing Wounds in a National Health Information System - Epidemiology of Non-healing Wounds - Analysis of the National Register of Hospitalized Patients in 2007- 2015. *Datové zdroje pro sledování nehojících se ran v Národním zdravotnickém informačním systému - epidemiologie nehojících se ran v České republice - analýza Národního registru hospitalizovaných v letech 2007- 2015*. 2017;80:8-17.

9. Pokorna A, Muzik J, Burilova P, et al. Pressure lesion monitoring-data set validation after second pilot data collection. *CESKA A SLOVENSKA NEUROLOGIE A NEUROCHIRURGIE*. 2018;81:S6-S12.

10. Russo CA, Steiner C, Spector W. Hospitalizations related to pressure ulcers among adults 18 years and older, 2006: Statistical brief# 64. Published online 2011.

11. Moore Z, Cowman S, Conroy RM. A randomised controlled clinical trial of repositioning, using the 30 tilt, for the prevention of pressure ulcers. *Journal of clinical nursing*. 2011;20(17-18):2633-2644.

12. Stotts NA, Wu HS. Hospital recovery is facilitated by prevention of pressure ulcers in older adults. *Critical care nursing clinics of North America*. 2007;19(3):269-275.

13. Búřilová P, Dolanová D, Saibertová S, et al. Základní epidemiologická analýza pacientů s dekubity v Národním zdravotnickém informačním systému. *Léčba ran*. 2021;8(1):6-7.

14. Búřilová P, Pokorná A, Benešová K, et al. A ten-year follow-up of the prevalence of pressure injuries in the Czech Republic: Analysis of the National Registry. *International Wound Journal*. 2022;19(7):1870-1877.

15. Pokorná A, Dolanová D, Benešová K, et al. How the COVID-19 pandemic influences the prevalence of pressure injuries in the Czech Republic: A nationwide analysis of a health registry in 2020. *Journal of Tissue Viability*. 2022;31(3):424-430.

16. Mervis JS, Phillips TJ. Pressure ulcers: Pathophysiology, epidemiology, risk factors, and presentation. *Journal of the American Academy of Dermatology*. 2019;81(4):881-890.

17. Haesler E. National pressure ulcer advisory panel, european pressure ulcer advisory panel and pan pacific pressure injury alliance. *Prevention and treatment of pressure ulcers: quick reference guide*. Published online 2014:14-32.

18. Shoham N, Gefen A. Deformations, mechanical strains and stresses across the different hierarchical scales in weight-bearing soft tissues. *Journal of tissue viability*. 2012;21(2):39-46.

19. Kottner J, Black J, Call E, Gefen A, Santamaria N. Microclimate: a critical review in the context of pressure ulcer prevention. *Clinical Biomechanics*. 2018;59:62-70.

20. Kosiak M. Etiology of decubitus ulcers. *Arch phy Med Rehab*. 1961;42:19-29.

21. Loerakker S, Manders E, Strijkers GJ, et al. The effects of deformation, ischemia, and reperfusion on the development of muscle damage during prolonged loading. *Journal of applied physiology*. 2011;111(4):1168-1177.

22. Cui FF, Pan YY, Xie HH, et al. Pressure combined with ischemia/reperfusion injury induces deep tissue injury via endoplasmic reticulum stress in a rat pressure ulcer model. *International journal of molecular sciences*. 2016;17(3):284.

23. Slomka N, Gefen A. Relationship between strain levels and permeability of the plasma membrane in statically stretched myoblasts. *Annals of biomedical engineering*. 2012;40:606-618.

24. DeFloor T. The risk of pressure sores: a conceptual scheme. *Journal of clinical nursing*. 1999;8(2):206-216.

25. Slomka N, Or-Tzadikario S, Gefen A. Cellular Deformations under Compression in Cells Involved in Deep Tissue Injury. *Bioengineering Research of Chronic Wounds: A Multidisciplinary Study Approach*. Published online 2009:235-248.

26. Gefen A. How much time does it take to get a pressure ulcer? Integrated evidence from human, animal, and in vitro studies. *Ostomy Wound Manage*. 2008;54(10):26-28.

27. Scheel-Sailer A, Frotzler A, Mueller G, Annaheim S, Rossi RM, Derler S. Biophysical skin properties of grade 1 pressure ulcers and unaffected skin in spinal cord injured and able-bodied persons in the unloaded sacral region. *Journal of tissue viability*. 2017;26(2):89-94.

28. Berke CT. Pathology and clinical presentation of friction injuries: case series and literature review. *Journal of Wound Ostomy & Continence Nursing*. 2015;42(1):47-61.

29. Lyder CH. Pressure Ulcer Prevention and Management. *JAMA*. 2003;289(2):223-226. doi:10.1001/jama.289.2.223

30. Michel JM, Willebois S, Ribinik P, Barrois B, Colin D, Passadori Y. As of 2012, what are the key predictive risk factors for pressure ulcers? Developing French guidelines for clinical practice. *Annals of Physical and Rehabilitation Medicine*. 2012;55(7):454-465. doi:https://doi.org/10.1016/j.rehab.2012.08.003

31. Maklebust J, Sieggreen M. *Pressure Ulcers: Guidelines for Prevention and Management*. Lippincott Williams & Wilkins; 2001.

32. Vašíčková L, Siegelová J, Mašek M. Význam tlakové mapy (pres sure mapp ing system) pro pa cienty s mobilitou na vozíku. *Česká a Slovenská Neurologie a Neurochirurgie*. 2016;79(Supplementu1):S15-S19.

33. Minematsu T. Changes in tissue structure and barrier function in macerated skin. *J Jpn WOCM*. 2011;15:278.

34. Buckingham KW, Berg RW. Etiologic factors in diaper dermatitis: the role of feces. *Pediatric Dermatology*. 1986;3(2):107-112.

35. Li Y zhe, Quan Y shu, Zang L, et al. Transdermal delivery of insulin using trypsin as a biochemical enhancer. *Biological and Pharmaceutical Bulletin*. 2008;31(8):1574-1579.

36. Smith J, Ediss I, Mullinger M, Bogoch A. Fecal chymotrypsin and trypsin determinations. *Canadian Medical Association Journal*. 1971;104(8):691.

37. Li Y zhe, Quan Y shu, Zang L, et al. Trypsin as a novel potential absorption enhancer for improving the transdermal delivery of macromolecules. *Journal of Pharmacy and Pharmacology*. 2009;61(8):1005-1012.

38. Moore ZE, Patton D. Risk assessment tools for the prevention of pressure ulcers. *Cochrane Database of Systematic Reviews*. 2019;(1).

39. Lahmann NA, Tannen A, Kuntz S, et al. Mobility is the key! Trends and associations of common care problems in German long-term care facilities from 2008 to 2012. *International Journal of Nursing Studies*. 2015;52(1):167-174.

40. Narita H. Prevention of decubitus ulcer in patients with severe mental disorders. *[Kango gijutsu]:[Nursing technique]*. 1986;32(5):589-593.

41. Grand E. Decubitus ulcer as a symptom of depression. The prognosis is dependent on the form of depression and on its treatment. *Pflege Zeitschrift*. 1994;47(10):544-547.

42. Heilporn A. Psychological factors in the causation of pressure sores: case report. *Spinal Cord*. 1991;29(2):137-139.

43. Iizaka S, Okuwa M, Sugama J, Sanada H. The impact of malnutrition and nutrition-related factors on the development and severity of pressure ulcers in older patients receiving home care. *Clinical Nutrition*. 2010;29(1):47-53.

44. Litchford MD. Putting the 2019 nutrition recommendations for pressure injury prevention and treatment into practice. *Advances in Skin & Wound Care*. 2020;33(9):462-468.

45. Reed RL, Hepburn K, Adelson R, Center B, McKnight P. Low serum albumin levels, confusion, and fecal incontinence: Are these risk factors for pressure ulcers in mobility-impaired hospitalized adults? *Gerontology*. 2003;49(4):255-259. doi:10.1159/000070407

46. Lachenbruch C, Ribble D, Emmons K, VanGilder C. Pressure ulcer risk in the incontinent patient. *Journal of Wound, Ostomy and Continence Nursing*. 2016;43(3):235-241.

47. Kayser SA, VanGilder CA, Lachenbruch C. Predictors of superficial and severe hospital-acquired pressure injuries: A cross-sectional study using the International Pressure Ulcer Prevalence™ survey. *International journal of nursing studies*. 2019;89:46-52.

48. Kayser SA, Koloms K, Murray A, Khawar W, Gray M. Incontinence and incontinence-associated dermatitis in acute care: a retrospective analysis of total cost of care and patient outcomes from the premier healthcare database. *Journal of Wound, Ostomy, and Continence Nursing*. 2021;48(6):545.

49. Gerhardt LC, Strässle V, Lenz A, Spencer ND, Derler S. Influence of epidermal hydration on the friction of human skin against textiles. *Journal of the Royal Society Interface*. 2008;5(28):1317-1328.

50. Koloms K, Cox J, VanGilder CA, Edsberg LE. Incontinence Management and Pressure Injury Rates in US Acute Care Hospitals: Analysis of Data From the 2018-2019 International Pressure Injury Prevalence™(IPUP) Survey. *Journal of Wound, Ostomy and Continence Nursing*. 2022;49(5):405-415.

51. Cox J, Edsberg LE, Koloms K, VanGilder CA. Pressure injuries in critical care patients in US hospitals: results of the International Pressure Ulcer Prevalence Survey. *Journal of Wound, Ostomy, and Continence Nursing*. 2022;49(1):21.

52. Cox J, Schallom M. Pressure injuries in critical care patients: a conceptual schema. *Advances in skin & wound care*. 2021;34(3):124-131.

53. Dube A, Sidambe V, Verdon A, et al. Risk factors associated with heel pressure ulcer development in adult population: A systematic literature review. *Journal of Tissue Viability*. 2022;31(1):84-103.

54. Souza DMST de, Santos VLC de G. Risk factors for pressure ulcer development in institutionalized elderly. *Revista latino-americana de enfermagem*. 2007;15:958-964.

55. Pawlaczyk M, Lelonkiewicz M, Wieczorowski M. Age-dependent biomechanical properties of the skin. *Advances in Dermatology and Allergology/Postępy Dermatologii i Alergologii*. 2013;30(5):302-306.

56. Jaul E. Assessment and management of pressure ulcers in the elderly. *Drugs & aging*. 2010;27(4):311-325.

57. Gerstein AD, Phillips TJ, Rogers GS, Gilchrest BA. Wound healing and aging. *Dermatologic clinics*. 1993;11(4):749-757.

58. Van De Kerkhof P, Van Bergen B, Spruijt K, Kuiper J. Age-related changes in wound healing. *Clinical and Experimental Dermatology*. 1994;19(5):369-374.

59. Štrombachová V, Búřilová PP, Kučerová BMJ, Pospíšil MM, Macková MD, Kolková MA. Centrální systém hlášení nežádoucích událostí–Metodika Nežádoucí událost DEKUBITUS Plná verze metodiky.

60. Hokynková A, Šín P, Pokorná A, et al. Komplexní léčba dekubitů. Published online December 19, 2019. https://portal.med.muni.cz/clanek-696-komplexni-lecba-dekubitu.html. ISSN 1801-6103

61. Thompson D. An evaluation of the Waterlow pressure ulcer risk-assessment tool. *British Journal of Nursing*. 2005;14(8):455-459.

62. Anthony D, Parboteeah S, Saleh M, Papanikolaou P. Norton, Waterlow and Braden scores: a review of the literature and a comparison between the scores and clinical judgement. *Journal of clinical nursing*. 2008;17(5):646-653.

63. Chan WS, Pang SMC, Kwong EWY. Assessing predictive validity of the modified Braden scale for prediction of pressure ulcer risk of orthopaedic patients in an acute care setting. *Journal of clinical nursing*. 2009;18(11):1565-1573.

64. Bergstrom N, Braden B, Laguzza A, Holman V. The Braden Scale for predicting pressure sore risk. Nursing Research, 36 (4), 205-210. *Orthopaedic Nursing*. 1988;7(1):42.

65. Šáteková L, Žiaková K, Zeleníková R, others. Predikčná validita Bradenovej škály, Nortonovej škály a Waterlowej škály v Slovenskej republike. *Central European Journal of Nursing and Midwifery*. 2015;6(3):283-290.

66. Norton D, McLaren R, Exton-Smith A. An Investigation of Geriatric Nursing Problems in Hospital. London, England: National Corporation for the Care of Old People. Published online 1962.

67. Bell J. Are pressure ulcer grading and risk assessment tools useful? *WOUNDS UK*. 2005;1(2):62.

68. Waterlow J. A risk assessment card. *Nursing times*. 1985;81:51-55.

69. Shiffman MA, Low M, others. *Pressure Injury, Diabetes and Negative Pressure Wound Therapy*. Springer; 2020.

70. Šín P, Hokynková A, Marie N, Andrea P, Krč R, Podroužek J. Machine learning-based pressure ulcer prediction in modular critical care data. *Diagnostics*. 2022;12(4):850.

71. Ribeiro F, Fidalgo F, Silva A, Metrôlho J, Santos O, Dionisio R. Literature review of machine-learning algorithms for pressure ulcer prevention: Challenges and opportunities. In: *Informatics*. Vol 8. MDPI; 2021:76.

72. Deng X, Yu T, Hu A. Predicting the risk for hospital-acquired pressure ulcers in critical care patients. *Critical care nurse*. 2017;37(4):e1-e11.

73. Pokorná A, Štrombachová V, Mužík J, et al. SHNU - Úvod. National portal System for Reporting Adverse Events. Prague: Institute of Health Information of the Czech Republic. 2016. Accessed February 23, 2022. https://shnu.uzis.cz/

74. Kottner J, Kröger K, Gerber V, Schröder G, Dissemond J. Recognition and correct classification of pressure ulcers: a position paper. *Der Hautarzt*. 2018;69:839-847.

75. Edsberg LE, Black JM, Goldberg M, McNichol L, Moore L, Sieggreen M. Revised national pressure ulcer advisory panel pressure injury staging system: revised pressure injury staging system. *Journal of Wound, Ostomy, and Continence Nursing*. 2016;43(6):585.

76. Pokorná A, Mužík J, Búřilová P, et al. Monitoring tlakových lézí - validace datasetu po druhém pilotním sběru dat. *Pressure lesion monitoring - data set validation after second pilot data collection*. 2018;81:S6-12.

77. Kottner J, Cuddigan J, Carville K, et al. Prevention and treatment of pressure ulcers/injuries: The protocol for the second update of the international Clinical Practice Guideline 2019. *Journal of tissue viability*. 2019;28(2):51-58.

78. Halim A, Khoo T, Saad AM. Wound bed preparation from a clinical perspective. *Indian Journal of Plastic Surgery*. 2012;45(02):193-202.

79. De Francesco F, Ogawa R. From Time to Timer in Wound Healing Through the Regeneration. *Cell Biology and Translational Medicine, Volume 22: Stem Cell Driven Approaches in Regeneration and Repair*. Published online 2024:1-18.

80. Dumville JC, Webster J, Evans D, Land L. Negative pressure wound therapy for treating pressure ulcers. *Cochrane Database of Systematic Reviews*. 2015;(5).

81. Dwivedi M, Srivastava R, Bhagat A, et al. Pressure ulcer management in paraplegic patients with a novel negative pressure device: a randomised controlled trial. *Journal of wound care*. 2016;25(4):199-207.

82. Riebelová V, Válka J, Francu M. Trendy soudobé chirurgie. *Dekubity Praha: Galén*. Published online 2000.

83. Strohal R, Dissemond J, Jordan O'Brien J, et al. EWMA Document: Debridement: An updated overview and clarification of the principle role of debridement. *Journal of wound care*. 2013;22(Sup1):S1-S49.

84. Stryja J. Význam a možnosti chirurgického débridementu dekubitů. *Česká a Slovenská Neurologie a Neurochirurgie*. 2016;79(Supplementu1):S25-S27.

85. Hokynková A, Šín P, Černoch F, et al. Employment of flap surgery in pressure ulcers surgical treatment. *Ceska a Slovenska Neurologie a Neurochirurgie*. 2017;80. doi:10.14735/amcsnn2017S41

86. Hokynková A, Šín P, Černoch F, Adlerová T, others. Skin grafting in surgical treatment of pressure ulcers. Published online 2022.

87. Černoch F, Jelínková Z, Rotschein P. Využití svalového laloku v rekonstrukci recidivy ischiadického dekubitu. *Reconstruction of recurrent ischiadic pressure ulcer using turnover hamstring muscle flap*. 2019;82:15-18.

88. Clavien PA, Sanabria JR, Strasberg SM. Proposed classification of complications of surgery with examples of utility in cholecystectomy. *Surgery*. 1992;111(5):518-526.

89. Dindo D, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. *Annals of surgery*. 2004;240(2):205-213.

90. Lindqvist EK, Sommar P, Stenius M, Lagergren JF. Complications after pressure ulcer surgery–a study of 118 operations in spinal cord injured patients. *Journal of Plastic Surgery and Hand Surgery*. 2020;54(3):145-150.

91. Biglari B, Büchler A, Reitzel T, et al. A retrospective study on flap complications after pressure ulcer surgery in spinal cord-injured patients. *Spinal Cord*. 2014;52(1):80-83. doi:10.1038/sc.2013.130

92. Keys KA, Daniali LN, Warner KJ, Mathes DW. Multivariate Predictors of Failure after Flap Coverage of Pressure Ulcers. *Plastic & Reconstructive Surgery*. 2010;125(6):1725.

93. McCranie AS, Constantine RS, Lee N, Le EL, Gehring M, Iorio ML. Risk factors associated with pressure ulcer recurrence after reconstruction: Analysis of a national database. *Wound Repair and Regeneration*. 2023;31(5):641-646.

94. Paker N, Buğdaycı D, Gökşenoğlu G, Akbaş D, Korkut T. Recurrence rate after pressure ulcer reconstruction in patients with spinal cord injury in patients under control by a plastic surgery and physical medicine and rehabilitation team. *Turkish Journal of Physical Medicine and Rehabilitation*. 2018;64(4):322.

95. Labeau SO, Afonso E, Benbenishty J, et al. Correction to: Prevalence, associated factors and outcomes of pressure injuries in adult intensive care unit patients: the DecubICUs study. *Intensive care medicine*. 2021;47(4):503-520.

96. Lew CCH, Yandell R, Fraser RJ, Chua AP, Chong MFF, Miller M. Association between malnutrition and clinical outcomes in the intensive care unit: a systematic review. *Journal of Parenteral and Enteral Nutrition*. 2017;41(5):744-758.

97. Hardy G, Ridley EJ, Tatucu-Babet OA. Nutrition and pressure injury prevention in the intensive care unit: weighing the evidence. *Intensive and Critical Care Nursing*. 2024;81:103579.

98. Singer P, Blaser AR, Berger MM, et al. ESPEN practical and partially revised guideline: clinical nutrition in the intensive care unit. *Clinical Nutrition*. 2023;42(9):1671-1689.

99. Chen F, Wang X, Pan Y, Ni B, Wu J. The paradox of obesity in pressure ulcers of critically ill patients. *International Wound Journal*. 2023;20(7):2753-2763.

100. Zajac KK, Schubauer K, Simman R. The unavoidable pressure injury/ulcer: a review of skin failure in critically ill patients. *Journal of Wound Care*. 2024;33(Sup9):S18-S22.

101. Küçükdemirci Kaya P, Kaya A, Girgin N, Kahveci F, Akalın E, İşçimen R. Sepsis episodes caused by pressure injuries in critical illness: a retrospective observational cohort study. *Wound Management and Prevention*. 2023;69(4).

102. Exudate management: recent updates and practical guidelines. October 17, 2023. Accessed January 14, 2025. https://www.linkforwoundhealing.info/en-gb/scientific-content/2019/exudate-management-recent-updates-and-practical-guidelines

103. Kassym L, Zhetmekova Z, Kussainova A, et al. Pressure Ulcers and Nutrients: From Established Evidence to Gaps in Knowledge. *Current Medicinal Chemistry*. Published online 2024.

104. Norman K, Pichard C, Lochs H, Pirlich M. Prognostic impact of disease-related malnutrition. *Clinical nutrition*. 2008;27(1):5-15.

105. Gould LJ, Alderden J, Aslam R, et al. WHS guidelines for the treatment of pressure ulcers—2023 update. *Wound Repair and Regeneration*. 2024;32(1):6-33.

106. Williams JZ, Barbul A. Nutrition and wound healing. *Critical Care Nursing Clinics*. 2012;24(2):179-200.

107. Albina JE. Nutrition and wound healing. *Journal of Parenteral and Enteral Nutrition*. 1994;18(4):367-376.

108. Loftus TJ, Brown MP, Slish JH, Rosenthal MD. Serum levels of prealbumin and albumin for preoperative risk stratification. *Nutrition in Clinical Practice*. 2019;34(3):340-348.

109. Wild T, Rahbarnia A, Kellner M, Sobotka L, Eberlein T. Basics in nutrition and wound healing. *Nutrition*. 2010;26(9):862-866.

110. Chu Y, Kong J, Xu J, Han G, Yu W, Xu X. Role of nutritional support in nursing practice for improving surgical site wound healing in patients post-surgery with risk of pressure ulcers. *International Wound Journal*. 2024;21(4):e14855.

111. Mazzotta MY. Nutrition and wound healing. *Journal of the American Podiatric Medical Association*. 1994;84(9):456-462.

112. Sherman A, Barkley M. Nutrition and wound healing. *Journal of wound care*. 2011;20(8):357-367.

113. Wong JK, Amin K, Dumville JC. Reconstructive surgery for treating pressure ulcers. *Cochrane Database of Systematic Reviews*. 2016;(12).

114. Deng L, Du C, Song P, et al. The role of oxidative stress and antioxidants in diabetic wound healing. *Oxidative medicine and cellular longevity*. 2021;2021(1):8852759.

115. Dunnill C, Patton T, Brennan J, et al. Reactive oxygen species (ROS) and wound healing: the functional role of ROS and emerging ROS-modulating technologies for augmentation of the healing process. *International wound journal*. 2017;14(1):89-96.

116. Wang G, Yang F, Zhou W, Xiao N, Luo M, Tang Z. The initiation of oxidative stress and therapeutic strategies in wound healing. *Biomedicine & Pharmacotherapy*. 2023;157:114004.

117. Pizzino G, Irrera N, Cucinotta M, et al. Oxidative stress: harms and benefits for human health. *Oxidative medicine and cellular longevity*. 2017;2017(1):8416763.

118. Poprac P, Jomova K, Simunkova M, Kollar V, Rhodes CJ, Valko M. Targeting free radicals in oxidative stress-related human diseases. *Trends in pharmacological sciences*. 2017;38(7):592-607.

119. Krizanova O, Penesova A, Sokol J, Hokynkova A, Samadian A, Babula P. Signaling pathways in cutaneous wound healing. *Frontiers in Physiology*. 2022;13:2438.

120. Bryan N, Ahswin H, Smart N, Bayon Y, Wohlert S, Hunt JA. Reactive oxygen species (ROS)–a family of fate deciding molecules pivotal in constructive inflammation and wound healing. *Eur Cell Mater*. 2012;24(249):e65.

121. Muzumdar S, Hiebert H, Haertel E, et al. Nrf2-mediated expansion of pilosebaceous cells accelerates cutaneous wound healing. *The American journal of pathology*. 2019;189(3):568-579.

122. Dunnill C, Patton T, Brennan J, et al. Reactive oxygen species (ROS) and wound healing : the functional role of ROS and emerging ROS-modulating technologies for augmentation of the healing process. Published online 2015:89-96. doi:10.1111/iwj.12557

123. Hokynková A, Babula P, Pokorná A, et al. Oxidative stress in wound healing – current knowledge. *Ceska a Slovenska Neurologie a Neurochirurgie*. 2019;82. doi:10.14735/amcsnn2019S37

124. Sanchez MC, Lancel S, Boulanger E, Neviere R. Targeting oxidative stress and mitochondrial dysfunction in the treatment of impaired wound healing: a systematic review. *Antioxidants*. 2018;7(8):98.

125. Kunkemoeller B, Kyriakides TR. Redox signaling in diabetic wound healing regulates extracellular matrix deposition. *Antioxidants & redox signaling*. 2017;27(12):823-838.

126. Cabello-Verrugio C, Ruiz-Ortega M, Mosqueira M, Simon F, others. Oxidative stress in disease and aging: mechanisms and therapies. *Oxidative Medicine and Cellular Longevity*. 2016;2016(2):8786564.

127. Cao L, Wang XL, Chu T, et al. Role of gasotransmitters in necroptosis. *Experimental Cell Research*. Published online 2024:114233.

128. Schneider A, Wang X, Kaplan D, Garlick J, Egles C. Biofunctionalized electrospun silk mats as a topical bioactive dressing for accelerated wound healing. *Acta biomaterialia*. 2009;5(7):2570-2578.

129. Lu M meng, Bai J, Shao D, et al. Antibacterial and biodegradable tissue nano-adhesives for rapid wound closure. *International Journal of Nanomedicine*. Published online 2018:5849-5863.

130. Wu H, Li F, Wang S, et al. Ceria nanocrystals decorated mesoporous silica nanoparticle based ROS-scavenging tissue adhesive for highly efficient regenerative wound healing. *Biomaterials*. 2018;151:66-77.

131. Samoili S, Cobo ML, Gómez E, De Prato G, Martínez-Plumed F, Delipetrev B. AI Watch. Defining Artificial Intelligence. Towards an operational definition and taxonomy of artificial intelligence. Published online 2020.

132. McCarthy J. What is artificial intelligence. Published online 2007.

133. Nilsson NJ. *Artificial Intelligence: A New Synthesis*. Morgan Kaufmann; 1998.

134. Neisser U, Boodoo G, Bouchard Jr TJ, et al. Intelligence: knowns and unknowns. *American psychologist*. 1996;51(2):77.

135. Kaplan J. *Artificial Intelligence: What Everyone Needs to knowR*. Oxford University Press; 2016.

136. McCarthy J. Human-Level Ai Is Harder Than It Seemed. Published online 1955.

137. Nilsson N. The quest for artificial intelligence: A history of ideas and achievements, The Quest for Artificial Intelligence: A History of Ideas and Achievements. doi: 10.1017. *CBO9780511819346*. Published online 2010.

138. Stone P, Brooks R, Brynjolfsson E, et al. Artificial Intelligence and Life in 2030: The One Hundred Year Study on Artificial Intelligence. Published online 2022.

139. Umělá inteligence: definice a využití | Zpravodajství | Evropský parlament. September 4, 2020. Accessed May 23, 2023. https://www.europarl.europa.eu/news/cs/headlines/society/20200827STO85804/umela-inteligence-definice-a-vyuziti

140. Yu KH, Beam AL, Kohane IS. Artificial intelligence in healthcare. *Nature biomedical engineering*. 2018;2(10):719-731.

141. Yang G, Ye Q, Xia J. Unbox the black-box for the medical explainable AI via multi-modal and multi-centre data fusion: A mini-review, two showcases and beyond. *Information Fusion*. 2022;77:29-52.

142. Hastie T, Tibshirani R, Friedman JH, Friedman JH. *The Elements of Statistical Learning: Data Mining, Inference, and Prediction*. Vol 2. Springer; 2009.

143. Abu-Mostafa YS, Magdon-Ismail M, Lin HT. *Learning from Data*. Vol 4. AMLBook New York; 2012.

144. Deo RC. Machine learning in medicine. *Circulation*. 2015;132(20):1920-1930.

145. Sidey-Gibbons JA, Sidey-Gibbons CJ. Machine learning in medicine: a practical introduction. *BMC medical research methodology*. 2019;19:1-18.

146. Patel B, Sengupta P. Machine learning for predicting cardiac events: what does the future hold? *Expert review of cardiovascular therapy*. 2020;18(2):77-84.

147. Hu S, Gao Y, Niu Z, et al. Weakly supervised deep learning for covid-19 infection detection and classification from ct images. *IEEE Access*. 2020;8:118869-118883.

148. Schmidhuber J. Deep learning in neural networks: An overview. *Neural Networks*. 2015;61:85-117. doi:10.1016/j.neunet.2014.09.003

149. Li S, Yin H, Fang L. Group-sparse representation with dictionary learning for medical image denoising and fusion. *IEEE Transactions on biomedical engineering*. 2012;59(12):3450-3459.

150. Li Q, Cai W, Wang X, Zhou Y, Feng DD, Chen M. Medical image classification with convolutional neural network. In: *2014 13th International Conference on Control Automation Robotics & Vision (ICARCV)*. IEEE; 2014:844-848.

151. John H. Holland. genetic algorithms. *Scientific american*. 1992;267(1):44-50.

152. Liu P, Basha MDE, Li Y, Xiao Y, Sanelli PC, Fang R. Deep Evolutionary Networks with Expedited Genetic Algorithms for Medical Image Denoising. *Medical Image Analysis*. 2019;54:306-315. doi:https://doi.org/10.1016/j.media.2019.03.004

153. Maulud D, Abdulazeez AM. A review on linear regression comprehensive in machine learning. *Journal of Applied Science and Technology Trends*. 2020;1(4):140-147.

154. Maalouf M. Logistic regression in data analysis: an overview. *International Journal of Data Analysis Techniques and Strategies*. 2011;3(3):281-299.

155. Blanco-Justicia A, Domingo-Ferrer J. Machine learning explainability through comprehensible decision trees. In: *Machine Learning and Knowledge Extraction: Third IFIP TC 5, TC 12, WG 8.4, WG 8.9, WG 12.9 International Cross-Domain Conference, CD-MAKE 2019, Canterbury, UK, August 26–29, 2019, Proceedings 3*. Springer; 2019:15-26.

156. Abdullah DM, Abdulazeez AM. Machine learning applications based on SVM classification a review. *Qubahan Academic Journal*. 2021;1(2):81-90.

157. Chandra B, Gupta M, Gupta M. Robust approach for estimating probabilities in Naive-Bayes classifier. In: *Pattern Recognition and Machine Intelligence: Second International Conference, PReMI 2007, Kolkata, India, December 18-22, 2007. Proceedings 2*. Springer; 2007:11-16.

158. Zhang Z. Introduction to machine learning: k-nearest neighbors. *Annals of translational medicine*. 2016;4(11).

159. Sinaga KP, Yang MS. Unsupervised K-means clustering algorithm. *IEEE access*. 2020;8:80716-80727.

160. Abdulkareem NM, Abdulazeez AM, others. Machine learning classification based on Radom Forest Algorithm: A review. *International Journal of Science and Business*. 2021;5(2):128-142.

161. Huang X, Wu L, Ye Y. A review on dimensionality reduction techniques. *International Journal of Pattern Recognition and Artificial Intelligence*. 2019;33(10):1950017.

162. Ray S. A quick review of machine learning algorithms. In: *2019 International Conference on Machine Learning, Big Data, Cloud and Parallel Computing (COMITCon)*. IEEE; 2019:35-39.

163. Jiang Y, Li X, Luo H, Yin S, Kaynak O. Quo vadis artificial intelligence? *Discover Artificial Intelligence*. 2022;2(1):4.

164. Rajpurkar P, Chen E, Banerjee O, Topol EJ. AI in health and medicine. *Nature Medicine*. 2022;28(1):31-38. doi:10.1038/s41591-021-01614-0

165. McKinney SM, Sieniek M, Godbole V, et al. International evaluation of an AI system for breast cancer screening. *Nature*. 2020;577(7788):89-94.

166. Ardila D, Kiraly AP, Bharadwaj S, et al. End-to-end lung cancer screening with three-dimensional deep learning on low-dose chest computed tomography. *Nature medicine*. 2019;25(6):954-961.

167. Huynh E, Hosny A, Guthier C, et al. Artificial intelligence in radiation oncology. *Nature Reviews Clinical Oncology*. 2020;17(12):771-781.

168. Campanella G, Hanna MG, Geneslaw L, et al. Clinical-grade computational pathology using weakly supervised deep learning on whole slide images. *Nature medicine*. 2019;25(8):1301-1309.

169. Fu Y, Jung AW, Torne RV, et al. Pan-cancer computational histopathology reveals mutations, tumor composition and prognosis. *Nature cancer*. 2020;1(8):800-810.

170. Zhou D, Tian F, Tian X, et al. Diagnostic evaluation of a deep learning model for optical diagnosis of colorectal cancer. *Nature communications*. 2020;11(1):2961.

171. Alley EC, Khimulya G, Biswas S, AlQuraishi M, Church GM. Unified rational protein engineering with sequence-based deep representation learning. *Nature methods*. 2019;16(12):1315-1322.

172. Chabon JJ, Hamilton EG, Kurtz DM, et al. Integrating genomic features for non-invasive early lung cancer detection. *Nature*. 2020;580(7802):245-251.

173. Blasiak A, Khong J, Kee T. CURATE. AI: optimizing personalized medicine with artificial intelligence. *SLAS technology*. 2020;25(2):95-105.

174. Portelli M, Bianco S, Bezzina T, Abela J. Virtual reality training compared with apprenticeship training in laparoscopic surgery: a meta-analysis. *The Annals of the Royal College of Surgeons of England*. 2020;102(9):672-684.

175. Gumbs AA, Perretta S, d'Allemagne B, Chouillard E. What is artificial intelligence surgery? *Artificial Intelligence Surgery*. 2021;1(1):1-10.

176. Hu Y, Zhang L, Li W, Yang GZ. Robotic sewing and knot tying for personalized stent graft manufacturing. In: *2018 IEEE/RSJ International Conference on Intelligent Robots and Systems (IROS)*. IEEE; 2018:754-760.

177. Hu Y, Li W, Zhang L, Yang GZ. Designing, prototyping, and testing a flexible suturing robot for transanal endoscopic microsurgery. *IEEE Robotics and Automation Letters*. 2019;4(2):1669-1675.

178. Zhou XY, Guo Y, Shen M, Yang GZ. Application of artificial intelligence in surgery. *Frontiers of Medicine*. 2020;14:417-430.

179. Thai MT, Phan PT, Tran HA, et al. Advanced soft robotic system for in situ 3D bioprinting and endoscopic surgery. *Advanced Science*. 2023;10(12):2205656.

180. Williams S, Layard Horsfall H, Funnell JP, et al. Artificial Intelligence in Brain Tumour Surgery—An Emerging Paradigm. *Cancers*. 2021;13(19):5010.

181. Patel V, Shah M. Artificial intelligence and machine learning in drug discovery and development. *Intelligent Medicine*. 2022;2(3):134-140.

182. Shaik T, Tao X, Higgins N, et al. Remote patient monitoring using artificial intelligence: Current state, applications, and challenges. *Wiley Interdisciplinary Reviews: Data Mining and Knowledge Discovery*. Published online 2023:e1485.

183. Jeddi Z, Bohr A. Remote patient monitoring using artificial intelligence. In: *Artificial Intelligence in Healthcare*. Elsevier; 2020:203-234.

184. Roosan D, Wu Y, Tatla V, et al. Framework to enable pharmacist access to health care data using Blockchain technology and artificial intelligence. *Journal of the American Pharmacists Association*. 2022;62(4):1124-1132.

185. Pham KT, Nabizadeh A, Selek S. Artificial intelligence and chatbots in psychiatry. *Psychiatric Quarterly*. 2022;93(1):249-253.

186. Kang WS, Chung H, Ko H, et al. Artificial intelligence to predict in-hospital mortality using novel anatomical injury score. *Scientific Reports*. 2021;11(1):23534.

187. Peng HT, Siddiqui MM, Rhind SG, Zhang J, Teodoro da Luz L, Beckett A. Artificial intelligence and machine learning for hemorrhagic trauma care. *Military Medical Research*. 2023;10(1):1-20.

188. Langerhuizen DW, Janssen SJ, Mallee WH, et al. What are the applications and limitations of artificial intelligence for fracture detection and classification in orthopaedic trauma imaging? A systematic review. *Clinical orthopaedics and related research*. 2019;477(11):2482.

189. Salvagno M, Taccone FS, Gerli AG, others. Can artificial intelligence help for scientific writing? *Critical care*. 2023;27(1):1-5.

190. Lavrač N. Selected techniques for data mining in medicine. *Artificial Intelligence in Medicine*. 1999;16(1):3-23. doi:https://doi.org/10.1016/S0933-3657(98)00062-1

191. Johnson A, Pollard T, Mark R. MIMIC-III clinical database (version 1.4). *PhysioNet*. 2016;10(C2XW26):2.

192. Johnson AE, Stone DJ, Celi LA, Pollard TJ. The MIMIC Code Repository: enabling reproducibility in critical care research. *Journal of the American Medical Informatics Association*. 2018;25(1):32-39.

193. Johnson A, Bulgarelli L, Pollard T, Celi LA, Mark R, Horng IV S. MIMIC-IV-ED. *PhysioNet*. Published online 2021.

194. Goldberger AL, Amaral LA, Glass L, et al. PhysioBank, PhysioToolkit, and PhysioNet: components of a new research resource for complex physiologic signals. *circulation*. 2000;101(23):e215-e220.

195. Reps JM, Schuemie MJ, Suchard MA, Ryan PB, Rijnbeek PR. Design and implementation of a standardized framework to generate and evaluate patient-level prediction models using observational healthcare data. *Journal of the American Medical Informatics Association*. 2018;25(8):969-975.

196. Reps JM, Williams RD, You SC, et al. Feasibility and evaluation of a large-scale external validation approach for patient-level prediction in an international data network: validation of models predicting stroke in female patients newly diagnosed with atrial fibrillation. *BMC medical research methodology*. 2020;20:1-10.

197. Sudlow C, Gallacher J, Allen N, et al. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS medicine*. 2015;12(3):e1001779.

198. Gunaseelan V, Kenney B, Lee JSJ, Hu HM. Databases for surgical health services research: Clinformatics Data Mart. *Surgery*. 2019;165(4):669-671.

199. SEER Search. SEER. Accessed June 12, 2023. https://seer.cancer.gov/search?q=cite&x=0&y=0

200. Biologic Specimen and Data Repository Information Coordinating Center (BioLINCC) | NHLBI, NIH. Accessed June 12, 2023. https://www.nhlbi.nih.gov/science/biologic-specimen-and-data-repository-information-coordinating-center-biolincc

201. Welcome to The Cancer Imaging Archive. The Cancer Imaging Archive (TCIA). Accessed June 12, 2023. https://www.cancerimagingarchive.net/

8. List of abbreviations

AI – artificial intelligence

BMI – body mass index

CITI PROGRAM – Collaborative Institutional Training Initiative

CO – carbon monoxide

CPG – Clinical Practice Guidelines for Prevention and Treatment of Pressure ulcers

DL – Deep Learning

EPUAP – European Pressure Ulcer Advisory Panel

FC – Fasciocutaneous flap

GA – Genetic Algorithms

H_2S – hydrogen sulphide

MC – Musculocutaneous flap

MIMIC – Medical Information Mart for Intensive Care

ML – Machine Learning

NN – Neural Networks

NO – nitric oxide

NPIAP – National Pressure Injury Advisory Panel

NPWT – Negative Pressure Wound Therapy

OMOP – Observational Medical Outcomes Partnership

OS – Oxidative Stress

PI – Pressure injuries

PU(s) – Pressure Ulcer(s)

ROS – Reactive Oxygen Species

SEER – Surveillance, Epidemiology, End-Results Monitoring

TCIA – The Cancer Imaging Archive

9. List of pictures

Figure 1: Pressure ulcers risk areas according to body position 19
Figure 2: Category I of pressure ulcer – non-blanchable erythema 20
Figure 3: Category II of pressure ulcer 21
Figure 4: Category III of pressure ulcer 22
Figure 5: Category IV of pressure ulcer 22
Figure 6: Unstageable category of pressure ulcer 23
Figure 7: Deep tissue injury 24
Figure 8: Relationship between computer science, AI and ML and further scope definition 101
Figure 9: Schematic of typical ML framework. Adopted from [141] 101
Figure 10: Semantic graph according to Jiang et al. Adopted from [163] 103
Figure 11: ML application in medical clinical practice. Adopted from [164] 105
Figure 12: Potential clinical impacts of AI in the neurosurgical management of brain tumours, in the pre-operative, intra-operative, and post-operative phase. Adopted from [180] 106

10. List of tables

Table 1: Braden scale risk of pressure ulcer. Modified and adapted from [65] 16
Table 2: Norton scale risk of pressure ulcer. Modified and adapted from [65] 17
Table 3: Flap plasties in reconstruction of sacral PUs. Modified, translated and adapted from [85] 29
Table 4: Flap plasties in reconstruction of trochanter PUs. Modified, translated and adapted from [85] 30
Table 5: Flap plasties in reconstruction of ischial PUs. Modified, translated and adapted from [85] 31
Table 6: Post-operative complications by Clavien-Dindo. Modified and adapted from[90]. 32

11. List of diagrams

Diagram 1: Factors influencing the susceptibility (tendency) of an individual to develop PUs. Modified and adapted from [59] 15

12. List of appendices

Appendix 1: "Guide to the Surgical Treatment of Pressure ulcers. Selected advice for patients, physicians, medical staff and lay caregivers" ("Průvodce chirurgickou léčbou dekubitů. Vybrané rady pro pacienty, lékaře, zdravotnický personál a laické pečovatele") 160
Appendix 2: CITI Program Certificate, ID: 43354586, Human Research, Data or Specimens Only Research, Massachusetts Institute Of Technology Affiliates 161
Appendix 3: Completion Report of Artificial Intelligence (AI) and Human Subject Protection 162
Appendix 4: Completion Report of the nine modules of the CITI programme 163

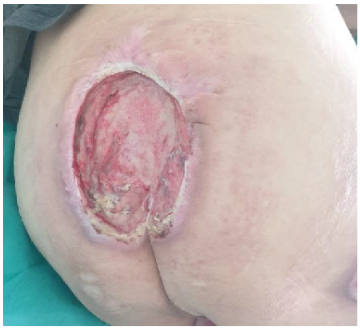
Průvodce chirurgickou léčbou dekubitů

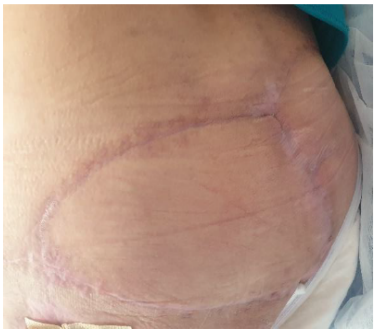
Vybrané rady pro pacienty, lékaře, zdravotnický personál a laické pečovatele

Hokynková A., Šín P., Krausová M. a kol.

Klinika popálenin a plastické chirurgie

Fakultní nemocnice Brno





Vážení pacienti,

připravili jsme pro Vás příručku, která by Vám měla být nápomocná v přípravě na chirurgickou léčbu dekubitů, nastínit průběh operace i pooperační péči v domácím prostředí. Našim cílem je poskytnout Vám co nejvíce informací tak, abyste byli k takovému operačnímu výkonu co nejlépe připraveni a zároveň věděli, jakým způsobem pečovat o rány v pooperačním období, a jak snížit riziko opětovného vzniku dekubitů.

MUDr. Alica Hokynková, Ph.D.

Co jsou dekubity?

Dekubity neboli proleženiny jsou místa poškozené tkáně (kůže, podkoží, svaly, kosti) zpravidla nad kostním výčnělkem způsobené tlakem kosti proti podložce. Jedná se o komplikovaně se hojící, někdy označované i jako rány chronické anebo nehojící se. Velmi často se vyskytují u pacientů po poranění míchy či s jinou poruchou citlivosti, kdy pacienti necítí tlak působící na tkáně a dochází k „proležení" či „prosezení". Samozřejmě se mohou vyskytnout i u pacientů pohyblivých tzv. chodících, kteří byli přechodně ve velmi těžkém stavu z různých příčin. Dekubity se nejčastěji nachází v oblasti křížové – sakrální dekubity, v oblasti výběžku stehenní kosti – trochanterické dekubity a v oblasti sedacích hrbolů – ischiadické dekubity. Samozřejmě mohou být i v jiných lokalizacích- např. v oblasti paty, kotníků, lokte, záhlaví a jinde při vynucených polohách.

Rozdělení a kategorie dekubitů

Dekubity I. kategorie jsou charakterizované neblednoucím[1] zarudnutím kůže, která není na svém povrchu porušená.

Dekubity II. kategorie mají obraz již porušeného kožního krytu ve formě puchýřů nebo povrchních vředů.

Dekubity III. kategorie se projevují úplnou ztrátou kůže, tedy jako otevřená rána, nejsou však postiženy hluboké struktury – svaly, šlachy či kosti.

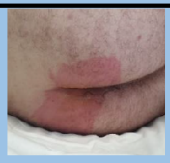
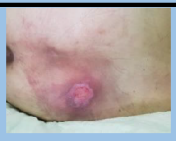
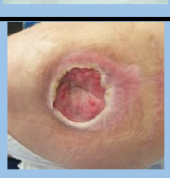
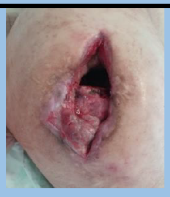
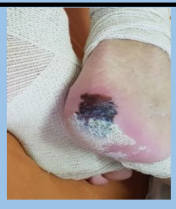
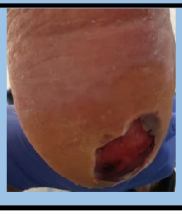
Dekubity IV. kategorie jsou charakterizovány kompletní ztrátou tkání zasahující ke svalům, šlachám, kloubům či kostem.

Zvláštními kategoriemi jsou:

Neklasifikovatelné dekubity jsou zpravidla kryty suchou černou nekrózou (laicky krustou, strupem) – tedy neživou tkání ve formě příškvaru, která znemožňuje vyšetření hloubky postižené tkáně.

Dekubity s podezřením na hluboké postižení tkání mohou být fialově nebo rudě zbarvené nebo také ve formě puchýřů naplněných krví, u kterých není jasně viditelná hloubka postižení tkání (typicky na patách).

[1] Kůže nezbledne ani po krátkém stlačení kůže (prstem) i po odstranění tlaku

Hloubka postižení	*Kategorie dekubitu*	*Popis*	*Klinický obraz*
POVRCHNÍ	I. kategorie	Neblednoucí zarudnutí	
	II. kategorie	Porušení kůže, puchýře	
HLUBOKÉ	III. kategorie	Úplná ztráta kůže	
	IV. kategorie	Postižení hlubokých struktur	
NEZNÁMÁ HLOUBKA DEFEKTU	Neklasifikovatelné	Suchá černá lpící nekróza	
	Podezření na hluboké postižení tkání	Fialově nebo červeně zbarvená kůže, puchýře naplněné krví	

Tabulka č. 1: Základní rozdělení dekubitů

Základní přehled předcházení a léčby dekubitů

1. PREVENCE – předcházení vzniku dekubitům

Základní postupy u všech typů dekubitů spočívá v preventivních opatřeních tykajících se zejména odlehčení tlaku v místě kostních výběžků proti podložce. Je tedy nutné pravidelné polohování a používání speciálních, antidekubitních pomůcek (podložky, náplasti, matrace, podsedáky, *viz Obr. č. 1, č. 2, č. 3*). Důležitou součástí antidekubitní prevence je i odstranění přebytečné vlhkosti (u inkontinence (samovolného, vůlí neovládaného odchodu) moči nebo stolice, při zvýšeném pocení atd).

Obr. č. 1, č. 2., č. 3: Příklad antidekubitních pomůcek (lokální materiály a polohovací pomůcky)

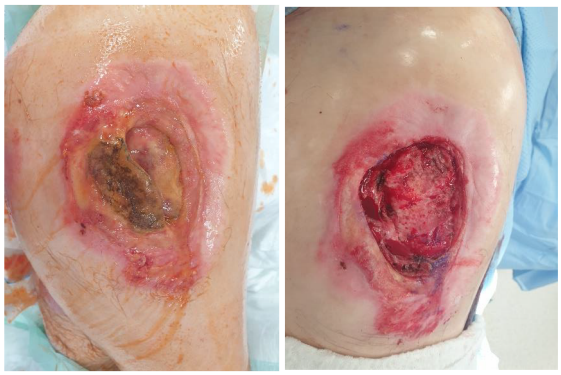
2. KONZERVATIVNÍ TERAPIE

Konzervativní terapie zahrnuje pravidelné převazy nehojících se nebo komplikovaně se hojících ran a uplatňuje se u všech kategoriích dekubitů, zejména však u dekubitů povrchových (I. a II. kategorie). Základem je dezinfekce

obnažených ploch oplachovými roztoky (např. Prontosan®, Octenisept® a jiné) s lokální aplikací různého krytí vlhké formy hojení, kterou indikuje lékař (praktický lékař, chirurg, specialista v oboru nehojících se ran, plastický chirurg, sestra-specialista na problematiku hojení ran atd.). Jejím cílem je snížení bakteriální zátěže rány, podpora hojení a vytvoření optimálních podmínek pro hojení.

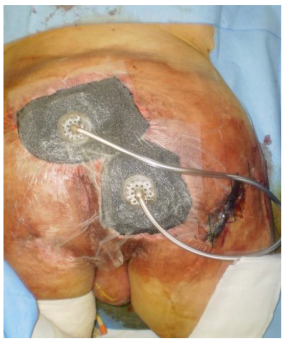
3. CHIRURGICKÁ TERAPIE

Chirurgická léčba dekubitů se uplatňuje především u hlubokých dekubitů (III. a IV. kategorie) a u dekubitů s neznámou hloubkou postižené tkáně. Probíhá zpravidla ve dvou fázích. V první fázi se provádí tzv. nekrektomie, tedy odstranění avitálních („mrtvých“) tkání, *viz Obr. č. 4*, včetně různých píštělí, chobotů (komunikující „chodby“ ve tkáních) nebo také odstranění zánětem změněných části kostí. Nekrektomii je možné provést na spádovém chirurgickém pracovišti.



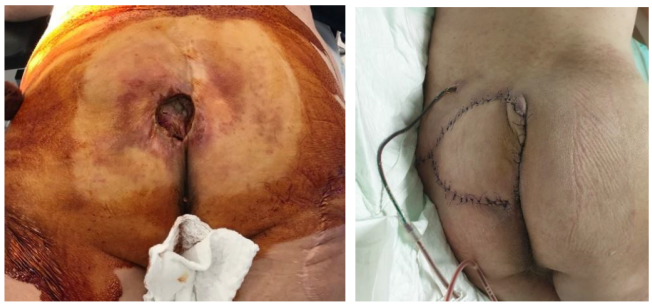
Obr. č. 4.: Trochanterický dekubitus před nekrektomií a po nekrektomii

Poté je o ránu pečováno konzervativní terapií, někdy i s využitím podtlakové terapie (izolovaně nebo v kombinaci s proplachem), která napomáhá hojení defektu, *Obr. č. 5.*

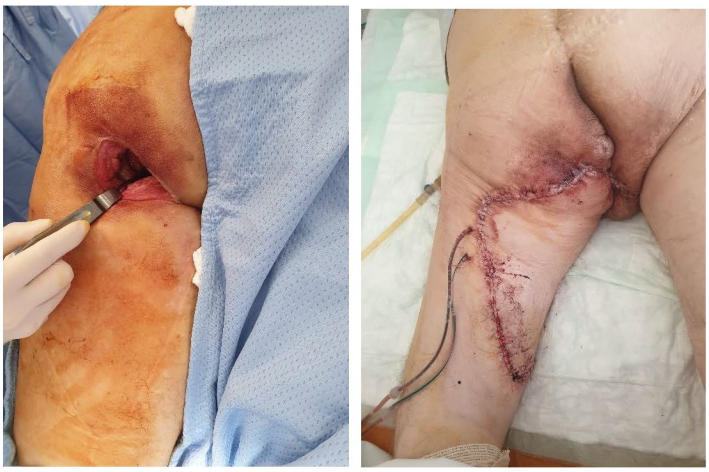


Obr. č. 5: Využití podtlakové terapie před operačním uzávěrem dekubitů

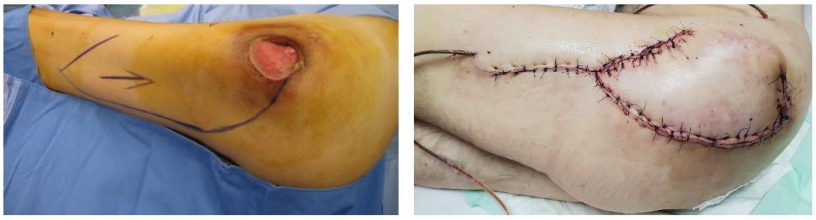
Zpravidla po 7 – 10 dnech, dle lokálního stavu rány, přistupujeme k tzv. rekonstrukci dekubitu. Jedná se o několikahodinový výkon na operačním sále většinou za přítomnosti anesteziologa. Rekonstrukce je prováděna tzv. lalokovou plastikou, kdy využíváme místní měkké tkáně v okolí defektu s posunem kůže, podkoží nebo svalové tkáně. Typ lalokové plastiky je závislý od lokalizace, hloubky defektu a jiných faktorů (*Obr. č. 6, č. 7, č. 8*).



Obr. č. 6: Příklad rekonstrukce sakrálního dekubitu



Obr. č. 7: Příklad rekonstrukce recidivujícího (opakujícího se) levostranného ischiadického dekubitu

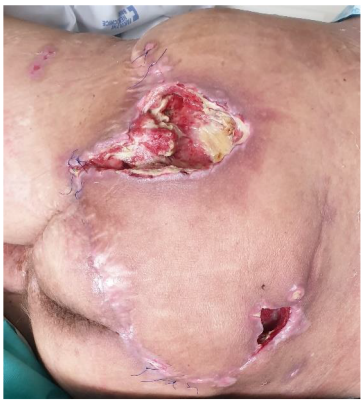


Obr. č. 8: Příklad rekonstrukce levostranného trochanterického dekubitu

Komplikace operační rekonstrukce

Mezi nejčastěji se vyskytující komplikace v brzkém pooperačním období patří krvácení z operační rány, infekce, později to může být např. tvorba tzv. seromu –

nahromadění tekutiny pod operační ránou, dehiscence – rozestup rány, prodloužené hojení, odumření části nebo celé lalokové plastiky, anebo také recidiva – znovuobjevení se dekubitu v místě operační rány, *Obr. č. 9.*



Obr. č. 9.: Recidiva sakrálního dekubitu po předchozím uzávěru lalokovou plastikou

Recidivující dekubity představují pro další operační řešení velký problém, jelikož se potýkáme s nedostatkem kůže a měkkých tkání v blízkosti defektu. Může se tedy stát, že techniky uzávěru jsou již vyčerpané a při opakujících se dekubitech je operační uzávěr někdy nemožný. Rána je pak ponechána ke konzervativní terapii jako nehojící se rána, mnohdy po zbytek života. Riziko komplikací spojených s operačním výkonem je u pacientů s dekubity významně vyšší než u pacientů s jiným typem operačních ran. Bývá to velmi často z důvodu malnutrice – podvýživy, základního onemocnění/poranění míchy, přidružené infekce, ale také z nedodržení nutné pooperační antidekubitní prevence či neadekvátní ošetřovatelské péče. Pečlivá předoperační příprava pacienta vede ke snížení rizika uvedených komplikací.

Předoperační příprava pacienta

Předoperační příprava pacienta k rekonstrukčnímu výkonu je zpravidla v režii praktického lékaře či internisty v závislosti na přidružených onemocněních.

Lokální péče o dekubity se opírá o konzervativní terapii s cílem snížení bakteriálního osídlení rány, které bývá mnohdy komplikováno zastoupením více druhů bakterií, ale také specifických, na některá antibiotika rezistentních bakterií (např. MRSA – Methicillin rezistentní Staphylococcus aureus), jež vyžadují v průběhu hospitalizace zvýšený hygienický, tzv. bariérový režim. Součástí přípravy je tedy i kultivační vyšetření – stěr z rány.

Základem komplexní předoperační přípravy jsou laboratorní vyšetření, která odhalí velmi často chudokrevnost – anémii. Ta by měla být dostatečně dlouho před operací korigována např. léky s obsahem železa a kyseliny listové. Dále se zaměřujeme na vyšetření krevní srážlivosti; v případě užívání léků „na ředění krve" je nutný převod na nízkomolekulární heparin. Mnohdy se v pooperačním období po rekonstrukci dekubitu neobejdeme bez podání krevních derivátů, proto je součástí základního screeningu i krevní skupina. Biochemické vyšetření se zaměřením se na hodnoty nutričních parametrů (albumin, prealbumin a celková bílkovina) pomáhají odhalit relativně častou podvýživu pacienta, která je spojována s poruchou hojení v pooperačním období. Korekce nižších hladin nutričních parametrů hraje stěžejní roli v rámci předoperační přípravy a opírá se substituci bílkovin jejich zvýšeným příjmem v potravě (maso, mléko, mléčné výrobky), ale také formou sippingu nebo bílkovinnými přídavky. V případě těžké formy podvýživy doporučujeme konzultaci nutričního specialisty. Součástí základního biochemického vyšetření je i hladina zánětlivého proteinu – CRP (C-reaktivní protein), který může odhalit přidružený infekt (velmi často v močovém ústrojí), jež by měl být léčen ještě před operací. Samozřejmostí je kompenzace diabetu či jiných interních nemocí. Doporučujeme také doplnit RTG

pánve u pacientů s dekubity lokalizovanými v této oblasti k vyloučení kostního postižení. Přehled základních předoperačních vyšetření shrnuje *Tabulka č. 2.*

Tabulka č. 2: Přehled základních předoperačních vyšetření před operačním uzávěrem dekubitů

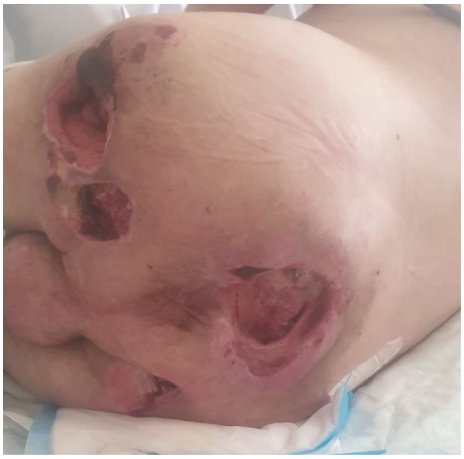
Předoperační vyšetření před operačním uzávěrem dekubitu			
Kultivační	*stěr z rány*	*ev. moč na kultivaci*	
Laboratorní	*krevní obraz, Hb>100g/L*	*základní koagulace*	*krevní skupina*
	biochemie	*urea, kreatinin, glykemie, Ca, Mg, P, K, Na, Cl, celková bílkovina>60g/L, albumin>30g/L, prealbumin>0,15g/L, CRP<50mg/L*	
		moč chemicky, sediment	
RTG pánve			
Interní předoperační vyšetření, RTG plic (věk nad 60 let), EKG			

Poznámka: Určeno zejména pro praktické lékaře a internisty připravující pacienta na operační výkon. Všechny parametry by měly být v mezích normálních hodnot nebo na její dolní hranici.

Pooperační péče

V průběhu operace a v pooperačním období je nutná důsledná antidekubitní prevence s využitím dostupných pomůcek a pravidelné polohování pacienta, ideálně každé 3 hodiny. Pokud je pacient v těžkém stavu, pak se doba mezi polohováním zkracuje. Podtlakové drény, které jsou nezbytnou součástí operace, jsou odstraňovány zpravidla nejdříve týden po operaci. Po operační rekonstrukci dekubitu je pacient sledován na naší klinice 7-14 dnů v závislosti na celkovém nebo lokálním stavu,

ale také na možnostech pooperačního sledování ve spádovém chirurgickém zařízení, dostupnosti domácí péče atd. Nadále je pokračováno v nutriční podpoře formou zvýšeného obsahu bílkovin v dietě. Po operačním uzávěru dekubitů jsou nutností pravidelné převazy – lokální péče o ránu, které je nutné provádět i po ukončení hospitalizace např. cestou domácí péče nebo instruovaným pečovatelem (blízkou osobou). Stehy jsou z rány odstraňovány nejdříve 3 týdny od operace v závislosti od lokálního nálezu. Operovaná oblast je ale odlehčená po dobu minimálně 6 týdnů od operace z důvodu možného poškození křehkých, hojících se tkání. Po 6 týdnech je možné začít operovanou oblast zlehka zatěžovat s postupným přidáváním zátěže. Při mnohočetných dekubitech (*Obr. č. 10*) je vhodné pečlivé načasování rekonstrukce jednotlivých typů dekubitu právě z důvodu nutnosti odlehčení operované oblasti. Většinou přistupujeme k etapovité rekonstrukci, nejdříve po 3 měsících od zhojení prvního rekonstruovaného defektu (dekubitu).



Obr. č. 10: Klinický obraz pacienta s mnohočetnými dekubity

Součástí pooperačního režimu jsou kontroly operatérem v naší ambulanci (pracovní dny mezi 8-11:00) Kliniky popálenin a plastické chirurgie – telefonický kontakt: 532 233 420, pavilon X, 3. patro, Fakultní nemocnice Brno, Jihlavská 20, 625 00 Brno.

Další užitečné informace najdete:

Dekubity | Portál věnovaný problematice proleženin/dekubitů.

Zahojime.cz | I malá rána může být velkou komplikací! Informace o prevenci a léčbě nehojících se ran.

European Pressure Ulcer Advisory Panel and National Pressure Ulcer Advisory Panel PPPIA. Prevention and Treatment of Pressure Ulcers : Quick Reference Guide. Clinical Practice Guideline. 2014. 1–75 s.

Použitá literatura:

1. Kottner J, Cuddigan J, Carville K, Balzer K, Berlowitz D, Law S, et al. Pressure ulcer/injury classification today: An international perspective. J Tissue Viability [Internet]. 1. srpen 2020;29(3):197–203. Dostupné z: http://10.0.3.248/j.jtv.2020.04.003
2. European Pressure Ulcer Advisory Panel and National Pressure Ulcer Advisory Panel PPPIA. Prevention and Treatment of Pressure Ulcers : Quick Reference Guide. Clinical Practice Guideline. 2014. 1–75 s.
3. Hokynková A. Komplexní léčba dekubitů. Portál LF MU Brno: Komplexní léčba dekubitů (muni.cz)
4. Pokorná A, Mužík J, Búřilová P, Saibertová S, Kelbich LKT, Koblihová E, et al. Monitor ing tlakových lézí - validace datasetu po druhém pilotním sběru dat. Press lesion Monit - data set Valid after Second Pilot data Collect [Internet]. 2. září 2018;81:S6-12. Dostupné z: http://ezproxy.muni.cz/login?url=https://search.ebscohost.com/login.aspx?direct=true&AuthType=ip,cookie,uid&db=asn&AN=133403886&lang=cs&site=eds-live&scope=site
5. Guttmann L. The problem of treatment of pressure sores in spinal paraplegics. Br J Plast Surg [Internet]. 1955;8:196–213. Dostupné z: https://www.sciencedirect.com/science/article/pii/S0007122655800379
6. SHEA JD. Pressure Sores Classification and Management. Clin Orthop Relat Res [Internet]. 1975;112. Dostupné z:

https://journals.lww.com/clinorthop/Fulltext/1975/10000/Pressure_Sores_Classification_and_Management.12.aspx

7. Hess CT. Classification of Pressure Injuries. Adv Skin Wound Care [Internet]. 2020;33(10). Dostupné z: https://journals.lww.com/aswcjournal/Fulltext/2020/10000/Classification_of_Pressure_Injuries.9.aspx

8. Geller CM, Seng SS. How to keep patients un-HAPI: Cardiac surgery and sacral pressure injuries: Invited expert opinion: Hospital-acquired pressure injuries. J Thorac Cardiovasc Surg [Internet]. 1. červenec 2020;160(1):158–63. Dostupné z: http://10.0.3.248/j.jtcvs.2019.12.108

Recenzent

Prof. PhDr. Andrea Pokorná, Ph.D.

Kontakt na autora:

MUDr. Alica Hokynková, Ph.D.

Klinika popálenin a plastické chirurgie

FN Brno, Jihlavská 20, 625 00 Brno

Hokynkova.Alica@fnbrno.cz

Tel: 00 420 532 232 610

Appendix 1: ***"Guide to the Surgical Treatment of Pressure ulcers. Selected advice for patients, physicians, medical staff and lay caregivers" ("Průvodce chirurgickou léčbou dekubitů. Vybrané rady pro pacienty, lékaře, zdravotnický personál a laické pečovatele")***

Appendix 2: ***CITI Program Certificate, ID: 43354586, Human Research, Data or Specimens Only Research, Massachusetts Institute Of Technology Affiliates***

COLLABORATIVE INSTITUTIONAL TRAINING INITIATIVE (CITI PROGRAM)

COMPLETION REPORT - PART 1 OF 2
COURSEWORK REQUIREMENTS*

* NOTE: Scores on this Requirements Report reflect quiz completions at the time all requirements for the course were met. See list below for details. See separate Transcript Report for more recent quiz scores, including those on optional (supplemental) course elements.

- **Name:** Alica Hokynková (ID: 10203727)
- **Institution Affiliation:** Independent Learner (ID: 569)
- **Phone:** +420777159961

- **Curriculum Group:** Webinars
- **Course Learner Group:** Artificial Intelligence (AI) and Human Subject Protections
- **Stage:** Stage 1 - Independent Learner

- **Record ID:** 43044113
- **Completion Date:** 11-Jun-2021
- **Expiration Date:** 11-Jun-2022
- **Minimum Passing:** 80
- **Reported Score*:** 80

REQUIRED AND ELECTIVE MODULES ONLY	DATE COMPLETED	SCORE
Artificial Intelligence (AI) and Human Subject Protections (ID: 20114)	11-Jun-2021	4/5 (80%)

For this Report to be valid, the learner identified above must have had a valid affiliation with the CITI Program subscribing institution identified above or have been a paid Independent Learner.

Verify at: www.citiprogram.org/verify/?kddf74dfe-b010-4d13-9ab1-d201e4983bac-43044113

Collaborative Institutional Training Initiative (CITI Program)
Email: support@citiprogram.org
Phone: 888-529-5929
Web: https://www.citiprogram.org

Appendix 3: *Completion Report of Artificial Intelligence (AI) and Human Subject Protection*

COLLABORATIVE INSTITUTIONAL TRAINING INITIATIVE (CITI PROGRAM)

COMPLETION REPORT - PART 1 OF 2
COURSEWORK REQUIREMENTS*

* NOTE: Scores on this Requirements Report reflect quiz completions at the time all requirements for the course were met. See list below for details. See separate Transcript Report for more recent quiz scores, including those on optional (supplemental) course elements.

- **Name:** Alica Holynková (ID: 10203727)
- **Institution Affiliation:** Massachusetts Institute of Technology Affiliates (ID: 1912)
- **Institution Email:** alicah@post.cz
- **Institution Unit:** Departments of burns and plastic surgery

- **Curriculum Group:** Human Research
- **Course Learner Group:** Data or Specimens Only Research
- **Stage:** Stage 1 - Basic Course

- **Record ID:** 43354586
- **Completion Date:** 30-Jun-2021
- **Expiration Date:** 29-Jun-2024
- **Minimum Passing:** 90
- **Reported Score*:** 94

REQUIRED AND ELECTIVE MODULES ONLY	DATE COMPLETED	SCORE
Belmont Report and Its Principles (ID: 1127)	30-Jun-2021	3/3 (100%)
History and Ethics of Human Subjects Research (ID: 498)	30-Jun-2021	4/5 (80%)
Basic Institutional Review Board (IRB) Regulations and Review Process (ID: 2)	30-Jun-2021	5/5 (100%)
Records-Based Research (ID: 5)	30-Jun-2021	3/3 (100%)
Genetic Research in Human Populations (ID: 6)	30-Jun-2021	4/5 (80%)
Populations in Research Requiring Additional Considerations and/or Protections (ID: 16680)	30-Jun-2021	5/5 (100%)
Research and HIPAA Privacy Protections (ID: 14)	30-Jun-2021	5/5 (100%)
Conflicts of Interest in Human Subjects Research (ID: 17464)	30-Jun-2021	5/5 (100%)
Massachusetts Institute of Technology (ID: 1290)	30-Jun-2021	No Quiz

For this Report to be valid, the learner identified above must have had a valid affiliation with the CITI Program subscribing institution identified above or have been a paid Independent Learner.

Verify at: www.citiprogram.org/verify/?k42805839-abfc-4e39-a2e7-728d2557ed96-43354586

Collaborative Institutional Training Initiative (CITI Program)
Email: support@citiprogram.org
Phone: 888-529-5929
Web: https://www.citiprogram.org

Appendix 4: *Completion Report of the nine modules of the CITI programme*