HYPERBARIC OXYGEN THERAPY IN DELAYED WOUND HEALING

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PLAIN LANGUAGE SUMMARY

HYPERBARIC OXYGEN THERAPY IN DELAYED WOUND HEALING

BACKGROUND

Problem wound is that with one or more local complicating factors (such as exudate, infection) or systemic comorbidities, polypharmacy, etc. Delayed wound healing usually refers to wounds that take a long time to heal (longer than 4 to 6 weeks216), heal by secondary intention, do not heal or recur. Wounds are a major source of morbidity to patients and a major cost to hospital and community healthcare providers. Hyperbaric oxygen therapy (HBOT) is a treatment designed to increase the supply of oxygen to wounds that are not responding to other treatments. HBOT involves people breathing pure oxygen in a specially designed compression chamber

REVIEW QUESTION

Does HBOT increase the rate of healing of people with delayed wounds healing?

WHAT WE FOUND

We included nine randomised trials, Case series and Case report (290 participants) in this updated review. It is reported that in delayed non diabetic wound healing (as well Venous Leg Ulcer and Mixed Arterial, Venous and Lymphatic wounds) and in recurrent multiple non-healing vasculitic wounds (especially those who have not responded to immunosuppressive therapy) HBOT may improve the rate of the healing (reduces the average healing time) by increasing the Nitric Oxide (NO) level and the number of Endothelial Progenitor Cells (EPCs). HBOT may help pain relief. No trial was identified to confirm or refute any effects of HBOT, as monotherapy, in arterial, thermal burn and pressure wounds.
This plain language summary is up-to-date as of 30 March 2016

BACKGROUND

Delayed wound healing is an indication for HBOT which is internationally approved (UHMS, ECHM), although in the US there are increasing difficulties in obtaining reimbursement from the third party.
The Cochrane Collaboration® has recently published a review of the literature from 1946 to 2015 on "Hyperbaric oxygen therapy for chronic wounds"[1]. In contrast, in the present review the literature has been assessed from the 7th European Consensus Conference on Hyperbaric Medicine of Lille (France), 2004 to March 2016 (twelve years). Diabetic Foot Ulcers (DFU), Compromised Skin Grafts and Flaps, acute surgical wounds (class IV) with Surgical Site Infections (SSI) and acute infected traumatic wounds[26-27] were excluded because these topics are discussed elsewhere in the 10th ECHM Consensus Conference on Hyperbaric Medicine in Lille (France) on April 15th-16th, 2016. Besides, some critical topics about HBOT in wound care are focused, too.

DEFINITION

"Problem wound" is one that has one or more local complicating factors, such as exudate, infection and systemic comorbidities, polypharmacy, etc. "Delayed wound healing" usually refers to wounds that take a long time to heal (longer than 4 to 6 weeks), heal by secondary intention, do not heal or recur[2].

Wounds are a major source of morbidity to patients and a major cost to hospital and community healthcare providers.

Venous Leg Ulcer (VLU) is a chronic and recurrent condition most commonly caused by venous hypertension. Unless the aetiology is corrected, recurrence is common and an ulcer may remain unhealed for years. In most of the studies prevalence was higher in women and increased with age.

Currently, there appears to be a knowledge deficit on how to adequately manage problem wounds, given the low healing rates reported; for example, 50% of VLUs remaining unhealed after 1 year of treatment[3].

Prior to the application of HBOT in selected problem wounds, there must have been some attempts at treatment by other means.

INCIDENCE

Chronic wounds of the lower limb constitute a significant health problem. They are commonly occurring and reduce the quality of life of those affected. The true incidence and impact are difficult to assess accurately given the wide range of disease, the fact that much care is delivered at home and that many wound care products are purchased directly in some countries. The prevalence of chronic wounds ranges from 355-370 per 100.000 population[4]. Actually, it's important to separate the open wounds from the total of open and healed wounds: the estimated prevalence of open wounds ranges from 240 to 370 per 100.000 population[5]. Applying these rates to the adult population of Europe (492 million aged over 14)[6] suggests that between 1.2 and 1.8 million of patients have an open wound at any one time (Table 1).

The prevalence per type of wound is: Lower Limb Ulcer between 120 and 320 per 100.000 population[5].

The estimated prevalence of Venous Leg Ulcer (VLU) ranges between 40 and 300 per 100.000 population[4,15]. The rate increases with age to about 2000 VLU per 100.000 people aged over 80 years[15].

Pressure Ulcers [16-17] can be viewed as typical complications in all healthcare settings with a prevalence of 8,9% to 24,6% among hospital patients in the Europe, with a mean of 1.5 – 1.9 ulcers per patient[4,7,18]. 13-39% of ulcers were EPUAP grade 3 o 4 and 52% of patients had had their ulcer for more than one month [4,18]

Table 1: prevalence of Open Wounds and annual incidence and cost of Venous Leg Ulcers in Europe[4]

	Range	Total EU Countries
Adult population (2015)		492 million [6,9]
Population aged over 65 (2015)		84 million[9]
Open Wounds		
• Prevalence (adults)	240-370/100.000[5]	1,180,800-1,820,400
• Incidence (age over 65)	1.16% (venous only)[2]	980,000 (venous only)
• Cost per episode	€ 2,500-10,800[10,4]	€ 6,650 (on average)
• Indicative annual cost (only new cases /year)		€ 6,5 billion (venous only)

Although most patients are treated in the community, the majority of wound care costs arise in the hospital sector: 27-30% of acute hospital beds are likely to be occupied on any day by patients with a wound[4]. Most surgical wounds heal by primary intention, but any wound is at risk of infection that is a major issue because of its effect on patient morbidity and treatment costs. The standardized infection ratio [SIR] ranges from 1.7 to 3.6 for 100 operative procedures. The risk of Surgical-Site Infections (SSIs) depend on factors including the type of operation and the patient's age. SSIs are twice as common in patients over 64 (risk ratio 1.6; 95% confidence interval 1.2-2.3)[11] An acute hospital performing 10,000 surgical procedures annually may have 300-400 surgical infections at a cost of 3300-4400 excess bed-days or 1.74-2.32 million Euros (Table 2)

Table 2: Surgical site infection: estimated impact on an acute hospital performing 10,000 operations annually [4]

	Central estimate	Hospital Impact (annual)
Patients with surgical wound infection	3-4% of surgical procedures[12]	300-400 patients
Attributable length of stay	11 days[13]	3300-4400 bed days
Attributable cost per episode	€5800[14]	€1.74-2.32 million

CLINICAL PRESENTATION

Chronic wounds are often associated with poor blood circulation as for diabetes, arterial or venous disease. One characteristic of chronic wounds is that the wound tissues are hypoxic.
Normal wound healing proceeds through an orderly sequence of steps involving control of contamination and infection, resolution of inflammation, regeneration of the connective tissue matrix, angiogenesis and resurfacing. Several of these steps are critically dependent upon adequate perfusion and oxygen availability. The end result of this process is sustained restoration of anatomical continuity and functional integrity. Problem or chronic wounds are wounds that have failed to proceed through this orderly sequence of events and have failed to establish a sustained anatomic and functional result [19]. This failure of wound healing is usually the result of one or more local wound or systemic host factors inhibiting the normal

tissue response to injury. These factors include persistent infection, malperfusion and hypoxia, cellular failure, and unrelieved pressure or recurrent trauma[20]

The hypoxic nature of all wounds has been demonstrated[21] and the hypoxia, when pathologically increased, correlates with impaired wound healing and increased rates of wound infection. Local oxygen tensions in the vicinity of the wound are approximately half the values observed in normal, non-wounded tissue. The rate at which normal wounds heal has been shown to be oxygen dependent. Fibroblast replication, collagen deposition, angiogenesis[22], resistance to infection and intracellular leukocyte bacterial killing are oxygen sensitive responses essential to normal wound healing. However, if the periwound tissue is normally perfused, steep oxygen gradients from the periphery to the hypoxic wound centre support a normal wound healing response . Peripheral Arterial Occlusive Disease (PAOD) is a common co-morbidity that frequently complicates the management of both venous leg and diabetic foot non-healing ulcers[23].

STANDARD MANAGEMENT AND OUTCOME (HBO EXCLUDED)

'Chronic leg ulcer (CLU)' also known as chronic lower limb ulcer is a chronic wound of the leg that shows no tendency to heal after 3 months of appropriate treatment or is still not fully healed at 12 months. Leg ulcers are debilitating and greatly reduce patients' quality of life. The common causes are venous disease, arterial disease, and neuropathy. Less common causes are metabolic disorders, hematological disorders, and infective diseases. As many factors lead to chronic lower leg ulceration, an interdisciplinary approach to the systematic assessment of the patient is required, in order to ascertain the pathogenesis, definitive diagnosis and optimal treatment. A correct diagnosis is essential to avoid inappropriate treatment that may cause deterioration of the wound, delay wound healing or harm the patient[2].

'Arterial Ulcers' are wounds developing in the presence of demonstrated Peripheral Arterial Occlusive Disease (PAOD). Therapeutic measures would aim to improve ischaemia in the limb in order to promote healing, perhaps through bypass surgery when technically possible[23-24]

'Venous Leg ulcers (VLU)' are associated with venous insufficiency. Standard treatment for VLUs consists of sustained compression with bandages or stockings, together with a simple, non-adherent dressing and specific measures directed towards the cause of the wound [15].

"Diabetic Foot Ulcers (DFU)" occur as a result of various factors, such as mechanical changes in conformation of the bony architecture of the foot, peripheral neuropathy and PAOD, all of which occur with higher frequency and intensity in the diabetic population.

« Pressure Ulcer » is a localized injury to the skin and/or underlying tissue, usually over a bony prominence, as a result of pressure, or pressure in combination with shear (eg, sacrum, calcaneus, ischium). The superficial skin is less susceptible to pressure-induced damage than deeper tissues, and thus, the external appearance may underestimate the extent of pressure-related injury. Pressure ulcers are typically related to immobility (ie, bed-bound or chair-bound individual), but can also result from poorly fitting casts or other medical equipment [25]

« Acute surgical and traumatic wounds » occur as a result of a trauma or surgical procedures and whilst many heal uneventfully, sometimes poor local blood supply, infection, damage to the blood vessels, or a combination of factors result in these acute wounds taking longer to heal [26,27]

Problem wounds require strategies which include treatment of the underlying pathology (e.g. optimal diabetes care with blood glucose control, vein surgery, arterial reconstruction), systemic treatment and local treatment aimed at improving the local wound environment (e.g. pressure-relieving mattresses, Negative Pressure Wound Therapy, Growth factor therapies

that modulate processes in the proliferative phase of wound healing and acellular and cell-based tissue-engineered Dermal substitutes are most promising)[28]
In practice, wound management is often a sequential search for a successful combined approach. Kranke P and others[1] for their review about HBOT for chronic wounds, accepted as comparator group versus HBOT, any standard treatment regimen designed to promote wound healing. The salient feature of the comparison group was that these measures had failed before enrolment in the trials. They planned subgroup analysis to evaluate the impact of different comparator strategies.

RATIONALE FOR HBOT USE

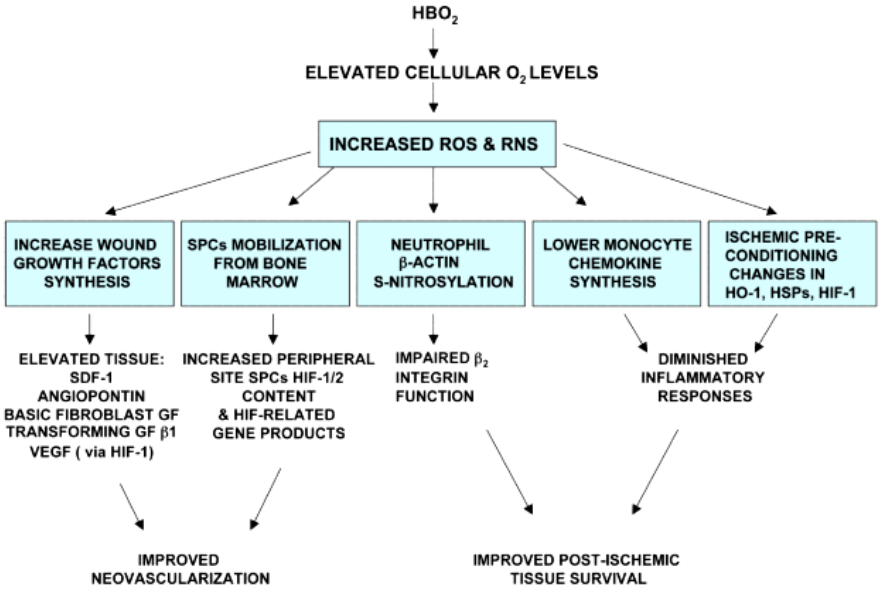
Hyperbaric Oxygen Therapy (HBOT) is a treatment designed to increase the supply of oxygen to wounds that are not responding to other treatments. HBOT involves people breathing pure oxygen in a specially designed compression chamber.

PATHOPHYSIOLOGY OF HBOT IN WOUND HEALING

Specialized programs have emerged designed to identify and manage chronic wounds using standardized protocols and a variety of new technologies to improve outcomes. HBOT has been increasingly utilized in an adjunctive role in many of these wounds coinciding with optimized patient and local wound care.
Regardless of the primary etiology of problem wounds, a basic pathway to non-healing is the interplay between tissue hypoperfusion, resulting hypoxia, and infection. A large body of evidence exists which demonstrates that intermittent oxygenation of hypo-perfused wound beds, a process only achievable in selected patients by exposing them to hyperbaric oxygen treatment, mitigates many of these impediments and sets into motion a cascade of events that leads to wound healing[29].
Tissues contain a variety of cell types and HBOT may influence each in different ways. Physiologically, HBOT produces a directly proportional increase in the plasma volume fraction of transported oxygen that is readily available for cellular metabolism. Proper oxygenation of the vascularized connective tissue compartment is crucial to the efficient initiation of the wound repair process and becomes an important rate-limiting factor for the cellular functions associated with several aspects of wound healing. Availability of substrate for oxygen dependent enzymatic reactions critical to repair and resistance to infection is even more important than normalization of metabolic rate.
Suppression of synthesis of many bacterial toxins[30] occurs when tissue PO_2 values are sufficiently elevated during treatment. Neutrophils, fibroblasts, macrophages, and osteoclasts are all dependent upon an environment in which oxygen is not deficient in order to carry out their specific inflammatory or repair functions. Improved leukocyte function of bacterial killing and antibiotic potentiation have been demonstrated [30-31].
An event associated with chronic wounds (especially in post-ischemic tissue reperfusion) is adherence of circulating neutrophils to vascular endothelium by β2 integrins. Thom S.R. [32] has explained that in many tissues HBOT, *unlike Normobaric Oxygen (NBO)*, temporarily inhibits adherence/sequestration of neutrophils by inhibiting β_2 integrin function; induces antioxidant enzymes and anti-inflammatory proteins [32,33]. Exposure to HBOT *(and not to NBO)* inhibits neutrophil β2 integrin function because hyperoxia increases synthesis of reactive species derived from iNOS and myeloperoxidase, leading to excessive S-nitrosylation of cytoskeletal β actin[32]. This modification increases the concentration of short, non-cross-linked filamentous (F)-actin which alters F-actin distribution within the cell. HBOT does not reduce neutrophil viability and functions such as degranulation, phagocytosis and oxidative burst in response to chemoattractants remain intact[32]. **(Figure 1)**

A separate anti-inflammatory pathway for HBOT *(and not for NBO)* involves impaired pro-inflammatory cytokine production by monocyte-macrophages. This action has been shown in animal models and human beings[34]. The effect on monocyte/macrophages may be the basis for reduced levels of circulating pro-inflammatory cytokines under stress conditions[35]. The molecular mechanism is unknown, but could be related to HBOT *(and not NBO)*- mediated enhancement of heme oxygenase-1 and Heat Shock Proteins [*e.g.* HSP 70] [36]
When HBOT *(not normobaric oxygen, NBO)* is used in a prophylactic manner to induce ischemic tolerance, its mechanism appears related to up-regulation of HIF-1 and at least one of its target genes, erythropoietin (EPO)[35]. In case of non wounded tissue NBO may have similar effect on EPO[128]

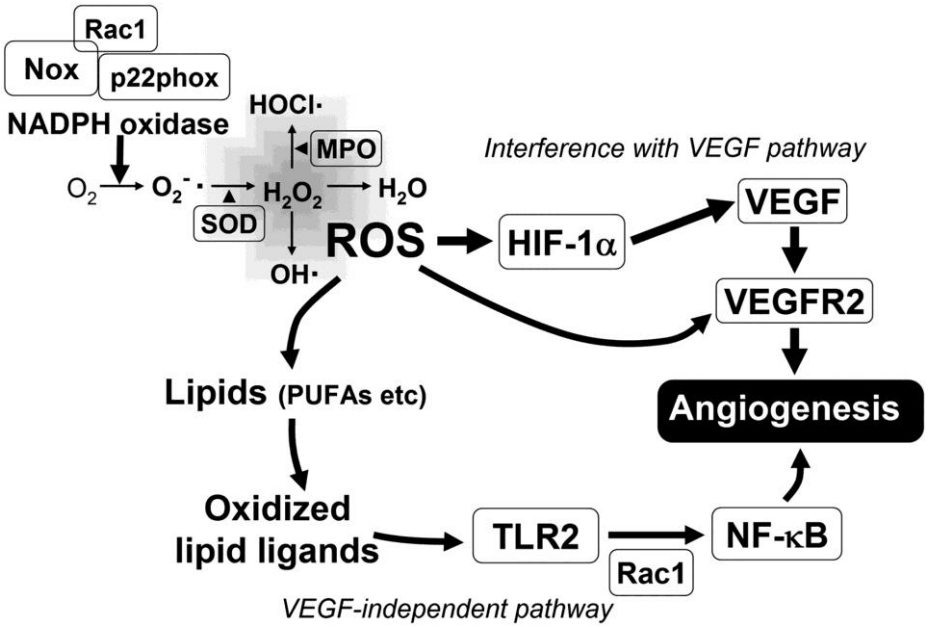
Figure 1. Overview on therapeutic mechanisms of HBOT related to elevations of tissue oxygen tensions. The figure outlines initial effects (denoted by boxes) that occur due to increased production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and their consequences. *Other abbreviations: GF=growth factor, VEGF=vascular endothelial growth factor, HIF= hypoxia inducible factor, SPCs=stem/progenitor cells, HO-1 =heme_oxygenase-1, HSPs=heat shock proteins. From:* Stephen R. Thom[35].



Besides, as part of their anti-microbial defense, Neutrophils form Extracellular Traps (NETs) by releasing decondensed chromatin lined with cytotoxic proteins[37]. NETs, however, can also induce tissue damage. If the injury involves skin repair, NETs will hinder the repair process, particularly in diabetes, in which neutrophils are more susceptible to NETosis. Inhibiting NETosis or cleaving NETs may improve wound healing and reduce NET-driven chronic inflammation in diabetes. It is possible to speed up wound healing by keeping neutrophils from producing bacteria-trapping neutrophil extracellular traps (NETs), as shown by Wong SL[38] in diabetic mice.
Blunting of systemic inflammatory responses[31] and prevention of leukocyte activation and adhesion following ischemic reperfusion [23] are effects that may persist even after completion of hyperbaric oxygen treatment.
Stimulation of tissue growth supporting wound healing has also been demonstrated by a variety of mechanisms.

1) Wound healing process should be seen as ‘‘waves’’ of ROS, lactate and Nitric Oxide (NO) production. A persistent increases in NO in wound fluid in diabetic ulcers associated with increased granulation tissue formation and wound closure when patients are exposed to 20 HBOTs at 2.0 ATA for 90 minutes has been demonstrated[39] In a Case series[39] the NO level was significantly elevated at 1 and 4 weeks after HBOT (2.0 ATA, 90 minutes, 20 sessions) and this was significantly correlated with the reduction in the wound area.
2) Oxidants appear to be among the most important signals that control the healing process, and this may be another mechanism for the benefits of HBOT in hypoxic wounds. It has been gradually established that oxidative stress plays a positive role during angiogenesis. The main mechanism of oxidative stress-induced angiogenesis involves Hypoxia-Inducible Factor (HIF)/Vascular Endothelial Growth Factor (VEGF) signaling, recent studies have identified several pathways that are VEGF-independent[40]. Figure 2

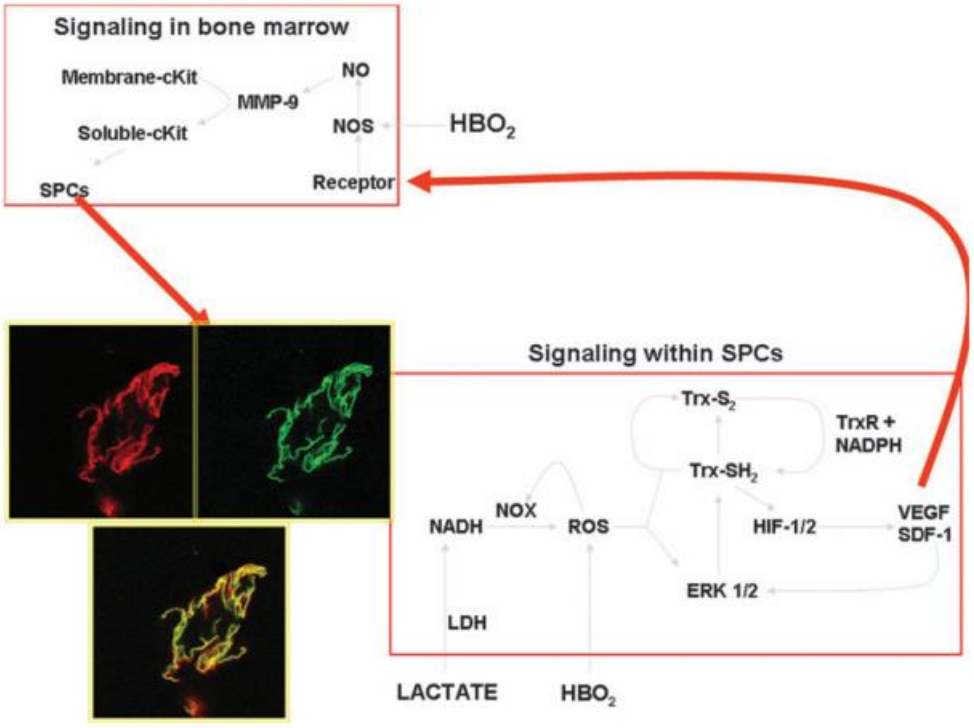
Figure 2: Schematic representation of ROS generation and its effect on angiogenesis[40]. Two main mechanisms are shown: ROS effect on known components of HIF-VEGF/VEGFR2 signalling pathway and VEGF-independent mechanism involving generation of lipid oxidation products. Abbreviations: NADH = Nicotinamide Adenine Dinucleotide Phosphate; MPO = myeloperoxidase; SOD = SuperOxide Dismutase; ROS = Reactive Oxygen Species; HIF = Hypoxia-Inducible Factor; VEGF = Vascular Endothelial Growth Factor; VEGFR2 = Vascular endothelial growth factor receptor 2; TLR2 = Toll-like receptor 2 (membrane protein); Rac1 = small (~21 kDa) signalling protein that appear to regulate cellular events, including the control of cell growth, cytoskeletal reorganization and the activation of protein kinases (proposed to be necessary for maintaining epidermal stem cells); NF-kB = nuclear factor kappa-light-chain-enhancer of activated B cells. (Kim YW, Byzova TV ©2014 by American Society of Hematology)



3) HBOT and Lipoid Acid supplementation downregulates the chronic inflammatory state, changing the protease/anti-protease levels within the wound microenvironment. Decrease in the MMP9 expression and MMP2 upregulation, together with increased levels of Platelet derived growth factor (PDGF-BB), contribute significantly to acceleration of the dermal wound repair process [41].

4) HBOT stimulates synthesis of basic fibroblast growth factor (bFGF) and transforming growth factor beta1 (TGF-beta1) by human dermal fibroblasts[127]
5) HBOT augments stem/progenitor cell (SPCs) release from bone marrow through a nitric oxide dependent mechanism associated with the wound repair process[42,43-47] (Figure 3). The population of CD34 cells in peripheral circulation doubled in response to single HBOT (2 ATA, 120 mins). Over course of 20 treatments circulating CD34 cells increased 8 fold[48]. With regard to this process, it is important to stress that - contrary to many of the traditional agents which increase SPCs - HBOT does not concomitantly elevate the circulating leukocyte count, which may be thrombogenic[43]. Newly mobilized SPCs appear to have greater content of HIF-1, HIF-2 and thioredoxin, which in the murine model exhibit improved neovascularization[44-46]. The assays of SPCs during the first weeks of care in patients with DFUs can provide insight into how well wounds will respond and may aid with decisions on the use of adjunctive measures[49]
   In randomized, single-center, placebo-controlled clinical trial[43] the number of Endothelial progenitor cell (EPC) was positively correlated with wound healing in the HBOT group (correlation coefficient 0.84; $P < 0.01$).

Figure 3: Summary of stem cell and peripheral wound site events impacted by HBOT[42]. Images in lower left are confocal microscope images that demonstrate vasculogenesis in a Matrigel implant placed in a mouse that was exposed to HBOT. They show CD34 + SPCs (green) and Nile red beads (red) injected via the heart to demonstrate functional blood vessels. The overlay between $CD34^+$ cells and beads is shown in yellow. Trx-S2 = oxidized thioredoxin; Trx-SH2 = reduced thioredoxin. (Fosen KM, Thom SR..© Mary Ann Liebert, Inc.)



The net result of serial HBOT exposures is improved local host immune response, clearance of infection, enhanced tissue growth and angiogenesis leading to progressive improvement in local tissue oxygenation and healing of hypoxic wounds.

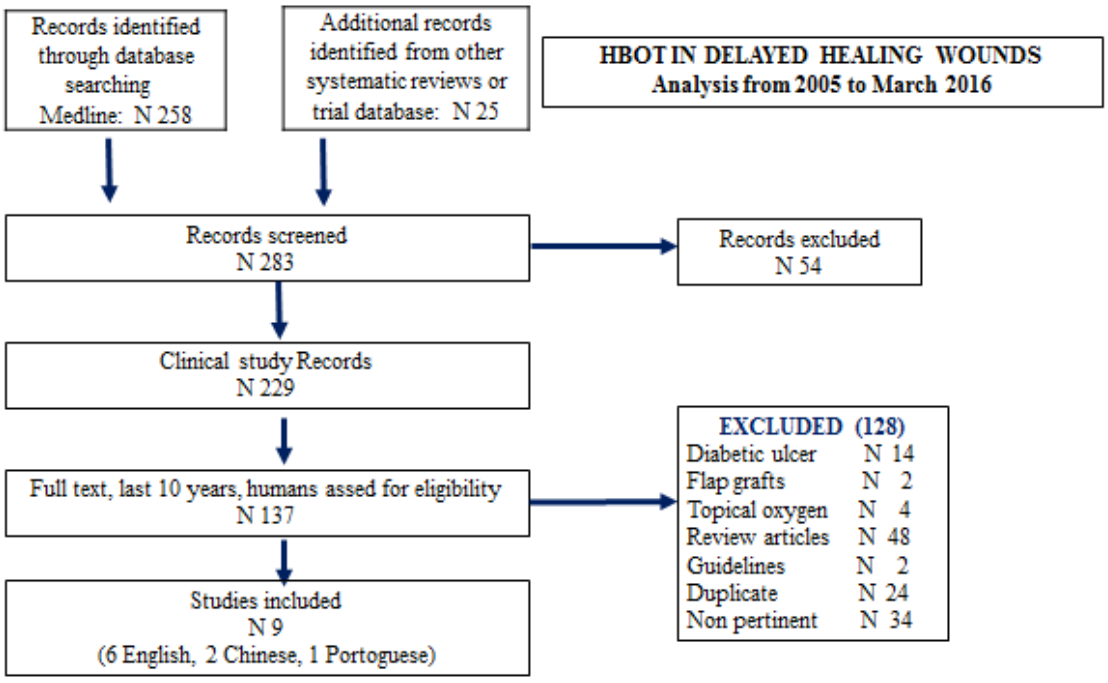
Patients with wounds which are potentially appropriate for adjunctive HBOT should be evaluated for likelihood of benefit. HBOT offers an intriguing opportunity to maximize oxygen delivery and ultimately to increase wound blood flow via neovascularization in the setting of minimal or insufficiently corrected blood flow.
Hypoxia (i.e. wound PO_2 < 40 mmHg) generally best defines wounds appropriate for HBOT— or rather, lack of hypoxia (i.e. wound PO_2 >40-50 mmHg) defines wounds potentially not appropriate for HBOT. Breathing 100% oxygen at 1 ATA or under hyperbaric conditions can improve the accuracy of $PtcO_2$ measurement in predicting successful healing with adjunctive HBOT [50].

EVIDENCE – BASED REVIEW OF HBOT USE

Kranke P and others[1] in an review on Hyperbaric Oxygen Therapy for chronic wounds for The Cochrane Collaboration, updated from 1946 to February 2015, searched for randomised controlled trials (RCTs) that compare the effect on chronic wound healing of treatment with HBOT compared with no HBOT. They included twelve randomised trials (577 participants). Randomised controlled trials (RCTs) comparing the effect on chronic wound healing of therapeutic regimens which include HBOT with those that exclude HBOT (with or without sham therapy) were selected. As results, pooled data of five trials with 205 participants showed an increase in the rate of wound healing (Risk Ratio – RR - 2.35, 95% Confidence Interval – CI - 1.19 to 4.62; P = 0.01) with HBOT at six weeks but this benefit was not evident at longer-term follow-up at one year. There was no statistically significant difference in major amputation rate (pooled data of five trials with 312 participants, RR 0.36, 95% CI 0.11 to 1.18). Most of the included trials studied foot ulcers in people with diabetes (10 trials, 531 participants). However, in one Randomised control trial (1994) [51] of 16 patients with Venous Leg Ulcers, data at six weeks (wound size reduction) and 18 weeks (wound size reduction and number of wounds healed) suggested a significant benefit of HBOT in terms of reduction in wound area only at six weeks (Mean Difference – MD - 33.00%, 95% CI 18.97 to 47.03, P < 0.00001). Kranke and others included a trial[52] (2012), which enrolled patients with non-healing lower limb wounds (as well Mixed Arterial, Venous and Lymphatic wounds) that is analysed in our review, too. No trial was identified, by Kranke and others, that considered arterial and pressure ulcers.
In contrast, in the present review the literature has been assessed from the 7th European Consensus Conference on Hyperbaric Medicine of Lille (France), 2004 to March 2016. 289 records were searched for that compare the effect on chronic wound healing of treatment with HBOT. Diabetic Foot Ulcers (DFU), Compromised Skin Grafts and Flaps, acute surgical wounds (class IV) with Surgical Site Infections (SSI) and acute infected traumatic wounds[26-27] were excluded because these topics are discussed in different reviews. Three Randomised controlled trials (RCTs), four Case series and two Case reports (290 participants) were included in the final analysis.
The MEDLINE was searched with the query ("Wound Healing"[Mesh] OR "Skin Ulcer"[Mesh]) NOT "Skin Transplantation"[Mesh]) AND "Hyperbaric Oxygenation"[Mesh] AND ("2004/01/01"[PDAT]:"3000/12/31"[PDAT]). The output included 258 records, which have been extended by 25 records not included in the Medline and found in the reference list of the above mentioned papers (283 records as total). A first selection was made applying exclusion criteria as [systematic reviews] and [medical genetics]. In the remaining 229 records a second selection was made applying the inclusion criteria as [humans], [full text in English] and [last 10 years]. From the 137 remaining records we excluded 131 papers as they presented studies about diabetic foot (N 14), skin flap/grafts (N 2), topical oxygen (N 4). Furthermore, we excluded the duplicate papers (N 24), reviews articles (N 48), guidelines (2)

and papers non-pertinent concerning HBO treatment in chronic wounds including surgical and traumatic wounds (N 34). For the final analysis, we included 9 papers reporting 9 studies (290 partecipants) enlisted in the Table 3. The list includes papers in English (N 6), Chinese (N 2) and in Portuguese (N 1). Figure 4.

Figure 4: Literature analysis for Hyperbaric Oxygen Therapy in Delayed Wound Healing (except Diabetic Foot Ulcer, Compromised Skin Grafts and Flaps) between 2005 and 2016 (assessed in March 2016)



One RCT[52] enrolled 30 adult patients (15 HBOT group vs 15 conventional therapy). with non - healing mixed wounds, as well Mixed Arterial, Venous and Lymphatic wounds treated for 30 days. In the HBOT group (2.5 ATA, 90 min, daily, 6 days a week, for a total of 30 sessions). For these "mixed wounds" there was a significant benefit of HBOT in terms of reduction in wound area at the end of treatment (30 days) (MD 61.88%, 95% CI 41.91 to 81.85, $P < 0.00001$). No statistically significant reduction in the amputation rate with the application of HBOT occurred (RR 0.2, 95%CI 0.03 to 1.51, $P = 0.12$).

In a case series[53] on Livedoid vasculopathy with recurrent multiple non-healing ulcers, involving feet and ankles and severe pain, HBOT (2.5ATA, 60 minutes, daily, six days a week, the number of sessions was 25 and 30 for the two patients treated) significantly improved the wound healing, the pain relief and quality of life

In randomized, single-center, placebo-controlled clinical trial[43] in 97 HBOT patient (vs 22 patients on hyperbaric air therapy as control group) the wound size significantly decreased at the 4-week end point (62.7% ± 22.3% in the HBOT group vs 34.4% ± 20.6% in the control group, $P < 0.05$). The number of Endothelial progenitor cell (EPC) was positively correlated with wound healing in the HBOT group (correlation coefficient 0.84; $P < 0.01$). HBOT protocol (monoplace chamber): 120 min, 90-min, daily, 5 days a week for 4 weeks (20 sessions). The pressure treatment is not indicated.

In a RCT[54] HBOT (2 ATA, 110 minutes, daily, for 5 to 10 days) significantly improved the delayed wound healing as result of pharyngeal and laryngeal carcinomas surgery

(pharyngolaryngectomy). HBOT group (versus control group) had a better average healing time of the wounds caused by necrosectomy of the myocutaneous flap or forearm flap (27.50 vs 45.00 days, $P < 0.01$; in the pharyngeal fistula (8. 50 vs 14.09 days, $P < 0.01$) and in the infected or fluid-filled wound (5.93 vs 8.62 days, $P < 0.01$)

In a retrospective case series[55,56] HBOT (2 ATA, 120 min, the number of sessions ranged between 29 and 41) significantly improved, over an average of 6 week, the healing of wounds in 11 (out of 12) patients suffering for calcific uraemic arteriolopathy and skin wound in end-stage of renal disease. The average duration of survival following successful treatment was 25.5 months (range 1.5–82).

In a Case series[39] the NO levels were significantly elevated at 1 and 4 weeks after HBOT (2.0 ATA, 90 minutes, 20 sessions) and correlated with the reduction in the wound area

In a Case report[57] HBOT (2.5 ATA, 90 minutes, daily, 10 sessions) was effective for complete wound healing, relief of the pain and improving the quality of life in a 15-year-old female with a Pyoderma Gangrenosum with multiple wounds in inguinal and suprapubic region, as well as on the right upper limb.

In a Case report[58] HBOT (2.6 ATA, 90 min, 16 sessions) favoured the wound healing in a 14 year old girl suffering for Systemic Lupus Erythematosus (SLE) and refractory vasculitic wound of the toe, with a delayed healing for more than 3 months.

In a Case series[59] of 36 patients aged ≥ 18 years with severe, non-healing, vasculitis-induced wounds that had not improved following immunosuppressive therapy, HBOT (2 ATA, 90 min, daily, 20 sessions) demonstrated complete healing in 28 patients (80%), partial healing in 4 (11.4%), no improvement in 3 (8.6%).

No trial was identified that considered HBOT, as monotherapy, effective in arterial[129], thermal burn[60] and pressure wounds[129]. We point out that, the Wound Healing Society clinical practice guideline for arterial insufficiency ulcers published in 2006[129] in Guideline #6.B.1a states: "In patients with non-reconstructable anatomy or whose ulcer is not healing despite revascularization, hyperbaric oxygen therapy (HBOT) should be considered as an adjuvant therapy. Selection criteria include ulcers that are hypoxic (due to ischemia) and the hypoxia is reversible by hyperbaric oxygenation" and gives hyperbaric oxygen a level of evidence determination of IIIB. In Guideline #6.B.1b states "HBOT should be investigated in the treatment of ischemia-reperfusion injury after revascularization ». In thermal burn and pressure, the HBOT appears to be effective to treat infections and/or in facilitating the plastic surgery[60,129].

All of these findings are subject to a potential publication bias. While we have made every effort to locate further unpublished data, it remains possible that this review is subject to a positive publication bias, with generally favorable trials more likely to achieve reporting.

Table 3 Literature analysis for Hyperbaric Oxygen Therapy in Delayed Wound Healing (except Diabetic Foot Ulcer, Compromised Skin Grafts and Flaps, surgical and traumatic wounds) between 2007 and 2016 (assessed in March 2016)

DELAYED WOUND HEALING
Updated from 2004 to March 2016

Study (authors, year)	Type	Nb patients	Aim(s) / Evaluation criteria	Inclusion / Exclusion criteria	HBO protocol (pressure, time, nb of session)	Results	Conclusion / comment
Kaur [52] 2012	Randomised controlled trials	30 adult patients (15 HBOT vs 15 conventional therapy)	Reduction ulcer area; amputation rate	Non - healing ulcer (mixed ulcer), despite conventional therapy of more than 4 weeks duration	conventional treatment plus HBOT 2.5 ATA, 90 min, daily, 6 days a week, for a total of 30 sessions	Reduction in wound area in the HBOT group of 59.27% compared with -2.61% in the control group (MD 61.88%, 95% CI 41.91 to 81.85, P < 0.00001) No statistically significant reduction in the amputation rate with the application of HBOT (RR 0.2, 95%CI 0.03 to 1.51, P = 0.12)	Favours HBOT for reduction ulcer area No statistically significant for limb salvage
Bhutani [53] 2012	Case series	2	wound healing, relief pain, and quality of life.	Livedoid vasculopathy with recurrent multiple non-healing ulcers involving feet and ankles and severe pain	2.5 ATA, 60 min, daily, 6 days/week, no. of session 25 and 30	Healing of the ulcers, pain relief	Favours HBOT
Ma YH [43] 2011	randomized, single-center, placebo-controlled clinical trial	119 (97 patients on long-term HBOT vs 22 patients on hyperbaric air therapy as control group)	wound healing through increasing CD34+ Endothelial Progenitor Cells (EPCs)	chronic wound in lower extremities lasting > 3 months	120 min, 90-min, daily, 5 days a week for 4 weeks (20 sessions) *monoplace chamber: the pressure treatment is not indicated	The wound size decreased at the 4-week end point (62.7% ± 22.3% in the HBOT group vs 34.4% ± 20.6% in the control group, P < 0.05) The number of Endothelial Progenitor Cells (EPCs) positively correlated with wound healing in (correlation coefficient 0.84; P < 0.01	Favours HBOT

Jiang W [54] 2011	Randomized controlled study	83 (HBOT group (n = 48) and without HBOT as control group (n = 35) by random	wound healing in pharyngeal and laryngeal carcinomas surgery	late healed wounds after pharyngolaryngectomy	2 ATA, 110 minutes, daily, from 5 to 10 sessions	HBOT group (versus control group) had a better average healing time of the wounds caused by necrosectomy of the myocutaneous flap or forearm flap (27.50 vs 45.00 days, $P < 0.01$); in the pharyngeal fistula (8. 50 vs 14.09 days, $P < 0.01$) and in the infected or fluid-filled wound (5.93 vs 8.62 days, $P < 0.01$)	Randomized controlled study Favours HBOT
Rogers NM [55] 2008	Retrospective Case series	12	wound healing	Patients with Calcific uraemic arteriolopathy and skin ulcer, in end-stage renal disease	2 ATA, 120 min, number of sessions ranged between 29 and 41	11/12 demonstrated healing of wounds over an average of 6 week. The average duration of survival following successful treatment was 25.5 months (range 1.5–82);	Favours HBOT
Boykin, Jr [39] 2007	Case series	6	Increasing local wound NO levels for wound healing		2.0 ATA, 90 minutes, 20 treatments	NO levels were significantly elevated at 1 and 4 weeks after therapy, and correlated with reductions in wound area	Favours HBOT, small series low level evidence
Vieira WA [57] 2011	Case report	1	wound healing, relief pain, and quality of life.	15-year-old female with a Pyoderma Gangrenosum, multiple ulcers in inguinal and suprapubic region, as well as on the right upper limb	2.5 ATA, 90 minutes, daily, 10 sessions	Complete healing	Favours HBOT
Olivieri [58] 2010	Case report	1	wound healing	14 year old girl suffered Systemic Lupus Erythematosus (SLE) + Refractory vasculitic ulcer of the toe for over 3 months	2.6 ATA, 90 min, 16 session (5 per week)	ulcer healed after 16 sessions	Favours HBOT

Efrati S [59] 2007	Case series	36	wound healing	patients aged >or= 18 years with severe, non-healing, vasculitis-induced ulcers that had not improved following immunosuppressive therapy	2 ATA, 90 min, daily, (5 per week), 4 weeks (20 sessions).	28 patients (80%) demonstrated complete healing, 4 (11.4%) had partial healing and 3 (8.6%) had no improvement.	Favours HBOT Moderate

PATIENTS SELECTION FOR HBOT

ASSESSMENT

HBOT will not accelerate tissue repair in wounds with normal oxygen tensions. It is essential in clinical practice to demonstrate and evaluate critical tissue ischemia before considering the use of HBOT[50]. Even before HBOT is considered, in the absence of infection, is it reasonable to provide documentation of vascular screening or documented endovascular or surgical correction of the Peripheral Arterial Occlusive Disease (PAOD) [50,61,62,63].

The use of transcutaneous oximetry (TCOM) is the golden standard to predict whether or not a patient suffering from non-healing lower extremity wound might receive benefit from HBOT[64,65]. Assessment of TCOM is a simple, reliable non-invasive diagnostic technique that provides an objective assessment of local tissue perfusion and oxygenation. It can be used for serial assessment of the soft tissue envelope surrounding the problem wound.

In normal conditions, the synthesis of nitric oxide requires a partial pressure of oxygen of 35-40 mmHg (or 50 uM of oxygen). When normobaric oximetry values are less than 35 to 40 mm Hg, a 100% normobaric oxygen challenge should be given via a non-rebreathing face mask. If the abnormally low TCOM values rise to 100 mm Hg or more, the patient will likely benefit from HBOT, especially when the patient has different impairment factors (as the cigarette smoking; rheumatic diseases; anemia; diabetes; respiratory, liver and/or kidney disease diseases). In part this is because the effect of hyperoxia on catalytic activity is reflected by values for the apparent Michaelis-Menten constant (apparent K_m) for oxygen and it differs among the three Nitric Oxide Synthase (NOS) isoforms This depends on the fact that the enzyme activity is constrained by ferric-ferrous conversion at the active site [35,47]. (Table 4).In the presence of impairment factors, nitric oxide synthesis is slowed or inhibited because of the decreased « apparent » affinity of the substrate (oxygen) to the binding site (Nitric Oxide Synthetase). The change in Michaelis-Menten constant (apparent K_m) can be overcome by increasing the substrate concentration (that is the ppO_2), in which case the substrate will outcompete the inhibitor in binding to the enzyme (NOS).

The neuronal isoform (nNOS or NOS1) is involved in the development of nervous system. It functions as a retrograde neurotransmitter important in long term potentiation and hence is likely to be important in memory and learning. nNOS has many other physiological functions, including regulation of cardiac function and peristalsis and sexual arousal in males and females. An alternatively spliced form of nNOS is a major muscle protein that produces signals in response to calcium release from the SR. nNOS in the heart protects against cardiac arrhythmia induced by myocardial infarction. HBO inhibits pain in rats with chronic constriction injury (CCI) through the regulation of spinal nNOS expression.[66]

High-output of inducible NOS (iNOS or NOS2) usually occurs in an oxidative environment and thus high levels of NO have the opportunity to react with superoxide leading to peroxynitrite formation and cell toxicity. These properties may define the roles of iNOS in host immunity, enabling its participation in anti-microbial and anti-tumor activities as part of the oxidative burst of macrophages[67].

Endothelial NOS (eNOS), also known as nitric oxide synthase 3 (NOS3), generates NO in blood vessels and is involved with regulating vascular function.[68]

Table 4 The effect of hyperoxia on catalytic activity is reflected by values for the apparent Michaelis-Menten constant (apparent K_m) for oxygen and it differs among the three Nitric Oxide Synthase (NOS) isoforms[35,47]

Nitric Oxide Synthase (NOS) isoforms	ppO_2 needed to normalize the apparent Michaelis-Menten constant (apparent K_m)	
Neuronal NOS (nNOS or NOS1)	~ 490 mmHg	350 μM
Inducible NOS (iNOS or NOS2)	~ 130 mmHg	190 μM
Endothelial NOS (eNOS or NOS3 or cNOS)	~ 38 mmHg	53 μM

Even if the TCOM values rise to 100 mmHg or more, during HBOT, this does not excuse the patient from a vascular workup, as many patients have multifactorial components to tissue hypoxia[52].

Impairments in eNOS function are related to hyperglycemia, insulin resistance, impaired enzyme synthesis, disordered caveolin associations and enhanced protein kinase C activity. Production of superoxide free radical ($O2^{-}$), is augmented in diabetes and this will reduce bioavailability of ·NO because the two radicals react rapidly to generate alternative RNS. Disordered balance between ($O2^{-}$) and ·NO is reflected by elevated levels of nitrotyrosine in plasma of type II diabetics[69]. Data from diabetic animals and humans indicate that HBOT can overcome some aspects of eNOS inhibition[70].

The use of the various oxygen monitoring systems to predict the HBOT effectiveness in healing wounds, within expected times, is analyzed below.

CURRENT PROTOCOL

Treatment involves placing the patient in a compression chamber, increasing the environmental pressure within the chamber and administering 100% oxygen for respiration. In this way, it is possible to deliver a greatly increased partial pressure of oxygen to the tissues. Different protocols are used for delivery of oxygen between several trials[1].

In Venous Leg Ulcers (VLU), Hammarlund[71] used a treatment session of 2.4 ATA for 90 minutes to a total of 30 sessions over six weeks.

In Mixed Ulcer group, Kaur[52] delivered HBOT at 2.5 ATA for 90min, 6 days a week to a total of 30 sessions over five weeks.

The therapeutic dose of HBOT (pressure, time and length of treatment course) should be made more specific in relation to the type of chronic wound. The most widely used HBOT protocol for delayed wound healing is: pressure of 2.0 ATA (range: 2-2,6 ATA); total time of 90 minutes (range: 90-120 minutes); daily, 5-6 days a week; total number of 30 sessions (range 10-40 sessions). Utilization review is required to half of the prescribed HBOT sessions in order to decide on the appropriateness of continuing the treatment or any necessary corrective measures to improve the result[130].

In the presence of limb threatening infection after debridement or incompletely corrected peripheral arterial occlusive disease, patients may require twice-daily treatments. Once stabilized, treatment frequency may decrease to once daily[130].

Note. Since the multiplace hyperbaric chamber is compressed by air and the patient breathes oxygen through a mask, it would be reasonable to measure the Fraction of Inspired Oxygen (FiO_2) in the mask which must be higher than 90% otherwise it must be adopted a corrective measure to improve the FiO_2 (as well as increase the absolute pressure; choose a mask that fit better to the face or a more effective ventilation mode).

ADVERSE EVENTS

HBOT is associated with some risk of adverse effects including damage to the ears, sinuses and lungs from the effects of pressure, temporary worsening of short-sightedness, claustrophobia and oxygen poisoning[72]. Although serious adverse events are rare, HBOT cannot be regarded as an entirely benign intervention. Kranke and others[1] could not assess safety as none of the trials included in their review reported whether there were any major adverse events.

COST IMPACT

The cost of wound care was estimated to be 2.5 – 3.9 million of Euros per 100.000 population[4].

For patients with an Surgical site infection (SSI), compared with patients without an SSI in Retrospective study of data from January 1, 2007 to December 31, 2010 [13], the daily total charges were USD 7.493 vs USD 7.924 (P = .99); the mean length of stay was 10.56 days vs 5.64 days (P < .001) and 30-day readmission rate was 51.94 vs 8.19 readmissions per 100 procedures (P < .001). The change in profit due SSIs was USD 2.268.589. The data suggest that hospitals have a financial incentive to reduce SSIs, but hospitals should expect to see an increase in both cost and revenue when SSIs are reduced.

Average annual treatment cost per patient with Venous Leg Ulcer (VLU) treated with compression bandaging (the gold standard) ranges from 2.459 to 10.800 Euros[4]. (Table 1)

It is estimated that there are up to 190,000 people with VLUs in the UK, incurring an estimated annual cost to the NHS of approximately GBP 168–198 million[10]. In UK, community nursing services account for a great proportion of the healthcare costs associated with VLUs[10]. It was estimated that district nurses devote between 25% and 50% of their time to the care of people with wounds.

Approximately 50% of VLUs will not have healed at 1 year, however, even with compression therapy. The cost of treating such wounds has been estimated to be up to three times greater than that of treating healing wounds[73].

The addition of HBOT may improve the proportion of wounds that achieve healing and thereby enhance the quality of life in such selected participants[74].

While amputations have declined over the last 15 to 20 years, there are still 65,000-70,000 amputations performed annually[75,76] and in 54 to 73 percent of these cases, there is no angiogram performed despite the fact that angiograms can reduce the odds of amputation by 90 percent[75,76,77]. In 60 to 71 percent of lower extremity amputations, revascularization is not attempted prior to amputation and amputation is frequently used as the first and only treatment for Critical Limb Ischemia, CLI[75,76,77]. Identifying wounds most likely to benefit, measuring Wound Hypoxia, is paramount for cost effective application of HBOT. If clinicians utilize diagnostic imaging technology efficiently, it may help them choose more effective treatment to enhance wound healing in these patients and help prevent amputations. Detecting microvascular disease early and utilizing HBOT can decrease the risk of major amputation from 9 percent (1 in 10) to 31 percent (1 in 3) [78]

Amputation is less cost-effective than conservative treatment (including bypass or endovascular intervention and HBOT). The patient cost for amputation includes lost wages, co-payments and deductibles, and modifications for disabled living, which doesn't include other negative patient outcomes such as poor ambulation (60%-80% are unable to walk) [79], depression (35%)[76,80], high 2-year mortality rates (30%-50%)[81,82], contralateral amputation (36%- 50%)[70,80,81], hospital readmissions (22% at 30 days)[83], lengthy healing process[84,85], reduced quality of life[84,85], and chronic pain (95%)[85].

For pressure ulcer an estimate of the costs of treating pressure ulcers in the UK, at August 2011 price, varies from GBP 1.214 (category 1) to GBP 14.108 (category IV). Costs increase with ulcer severity because the time to heal is longer and the incidence of complications is higher in more severe cases[7,86].

Regarding the choice of the setting of care more efficient, most patients suffering from chronic wounds can be treated as outpatients. This reduces costs associated with hospitalization. It's important to show that HBOT is synergistic with other therapy (NPWT, Cellular and / or Tissue-based Products – CTPs - for wounds) reducing the healing time compared to the benchmark.
Worldwide, it is spreading a diagnostic and therapeutic path that provides wound care in three different and progressive settings of care. The first level is represented by General Pratictioners and Nursing Home Care. The second level of treatment is for outpatients (Territorial Center of reference for Wound Care). The third level is Hospital based. HBOT, administered in an outpatient Hyperbaric and Wound Centre, allows the community to significantly reduce the number and cost of inappropriate admissions to hospital for Diagnosis Related Groups (DRGs) related with wound care[87].
In the USA, Medicare Outpatient Prospective Payment System (OPPS) was created to allow patients, who were not sick enough to warrant hospital admission, the opportunity to receive complex services as outpatient. Although Hospital based OutPatient wound care Departments (HOPDs) costs normally exceed those of services provided in a qualified healthcare professional's (QHP's) office, Centers for Medicare & Medicaid Services (CMS) sought to reduce overall Medicare costs and beneficiary coinsurance by preventing an even more expensive inpatient stay.
The results of a Today's Wound Clinic[88] reader survey support these data: HBOT represents 44% of the HOPDs revenue, followed by debridements at 37% and application of Cellular and/or Tissue-based Products (CTPs) for wounds at 8%.
Unfortunately, although HBOT seems effective for various acute and chronic wounds, currently there is little direct evidence on the cost-effectiveness of HBOT in the treatment of acute and chronic wounds[74]. Although there is some evidence suggesting effectiveness of HBOT, none of the included studies in this review measured utilities or expressed their health outcomes as QALYs. The lack of available evidence on economic endpoints is striking, given the fact that HBOT is widely applied in these settings and is reimbursed by insurance companies in Europe and the USA for the treatment of chronic wounds. Further studies should include economic outcomes in large clinical studies of strong methodological quality in order to make recommendations on the cost-effectiveness of applying HBOT in wound care.
When performing a cost-effectiveness analysis alongside a clinical trial, the most preferred approach should be taking all costs into account from a societal perspective. After this analysis, the perspective can be changed into the standpoint of, e.g., the government, the hospital or the patient.[89]
A cost-utility analysis is the preferred option when a study aims to determine the costs and efficacy of a treatment option, in which quality of life is an important factor. In such analyses, the outcome is often expressed as the effect on the quality-adjusted life years (QALY) that are lost or gained by the use of a specific therapy[89]. The International Society for Pharmacoeconomics and Outcome Research Task Force in Good Research Practices: randomized clinical trials-cost- effectiveness analysis (ISPOR RCT-CEA) has formulated recommendations for the design of economic analyses alongside clinical trials. An important

recommendation is that health utilities or QALYs should be measured directly from the study participants[90]

CRITICAL ASPECTS

A. HBOT treatment indications for delayed wound healing (page 20)
B. HBOT reduced healing times compared to benchmark healing times (page 22)
C. Oxygen monitoring systems. Prediction of effectiveness of HBOT in wounds healing within expected times (page 24)
D. Combined application of HBOT with other healing procedures in the treatment of wounds within expected times (page 29)
E. Differences between HBOT, topical tissue and other oxygenation procedures (page 31)
F. Creation of an European Wound Registry for the assessment of benefits of HBOT in wound care. (page 32)

A. HBOT TREATMENT INDICATIONS FOR DELAYED WOUND HEALING

Data indicate that basic science and insight into HBOT is improving, whereas there is still more to learn regarding the coordination of HBOT with other treatments and there remains a need for further clinical research. We believe that HBOT is appropriate in chronic wound when one or more risk factors are associated with the delayed healing.

Bradly speaking, there appears to be a knowledge deficit on how to adequately manage complex wounds, given the low healing rates reported; for example, 50% of Venous Leg Ulcers (VLUs) remaining unhealed after 1 year of treatment[3].

In part this is because there is no clear definition of a problem chronic wound. In practical terms it is a wound with one or more complicating factors, such as exudate, infection, comorbidity, polypharmacy, etc.

In Table 5 an example of chronic wound risk factors associated with delayed healing outcomes is shown.

Table 5. Example of chronic wound risk factors associated with delayed healing outcomes.

Diabetic Foot Ulcers (DFU) [91]	**Pressure Ulcers (PU)** [92]	**Venous Leg Ulcers (VU)** [92]
Patient renal impairment or male gender	Immobility	wound duration > 6 months
Loss of foot protective sensation	Loss of protective sensation	wound size > 5 cm^2
Local infection or probes to bone	Poor nutritional status	Wound devitalized tissue or slough
< 50% ulcer area ↓ in 4 weeks	< 40% ulcer area ↓ in 2 weeks	< 40% ulcer area ↓ in 4 weeks
Initial wound area > 2 cm^2	Full-thickness wound	Full-thickness wound

Any or all of these conditions can arrest wound healing and in fact can occur simultaneously:

1) ***Bacterial bioburden*** (responsible for the possible inflammatory response and evolution to infection, with local and systemic response of the organism to microbes);
2) Metalloprotease and other ***proteases*** that inhibit growth factors;
3) ***pH*** is an important indicator of the process of tissue repair. With acid pH the healing proceeds normally. The basic pH indicates a wound that does not heal because there could be an excess of proteases or infection;
4) ***Temperature***. A low temperature (hypothermia) indicates a poor blood circulation in the wound. A high temperature (hyperthermia) indicates an inflammatory process or an active infection;
5) ***Hypoxia and Oxidative stress***. Within certain physiological limits, oxidative stress promotes the healing because it induces the synthesis of Reactive Oxygen and Nitrogen Species (ROS, RNS) scavengers as well as growth factors (such as Vascular Endothelial Growth Factor, VEGF, which promotes angiogenesis). When oxidative stress exceeds the capacity of the scavengers to buffer the ROS and RNS, the healing process will be retarded.
6) ***Moisture***. Repair proceeds in humid environment. Excess moisture causes maceration and delays the healing process.

The correction of tissue ischemia and improved oxygen delivery has been clearly shown to improve wound healing[62] In patients with large vessel stenosis, this is accomplished by vascular bypass or by endovascular intervention[63]. However, in those areas where smaller vessels are damaged such as in radiation injury or diabetes, the HBOT by improving oxygen carrying capacity, increasing oxygen diffusion and correcting the localized ischemia at a cellular level may allow neovascularization and healing to occur in tissues that were previously unresponsive.
In the setting of tissue hypoxia, ppO_2 <30 mm Hg, such as in collagen vascular disease, renal insufficiency, protein energy malnutrition, the restoration of normal healing can be challenged. While the obvious solution to this problem seems to be the restoration of normal blood flow, in reality it is often much more complicated than this, as the patient's underlying medical condition(s) often prevents the complete reversal of the hypoxic process. In these cases, it is often advantageous to consider the use of HBOT to augment the normal healing process. In part this is because the effect of hyperoxia on catalytic activity is reflected by values for the apparent Michaelis-Menten constant (apparent K_m) for oxygen and it differs among the three Nitric Oxide Synthase (NOS) isoforms (Table 4).

In the presence of Peripheral Arterial Occlusive Disease, PAOD (after angioplasty or in case of impossibility for vascular surgery), HBOT can provide sufficient oxygen to support normal synthesis of nitric oxide (normalization of NOS activity in the presence of co-morbidities), as shown in Table 6

Table 6. Partial pressure of oxygen at several atmospheric pressure in normal and ischaemic tissues[35,50,61-63]

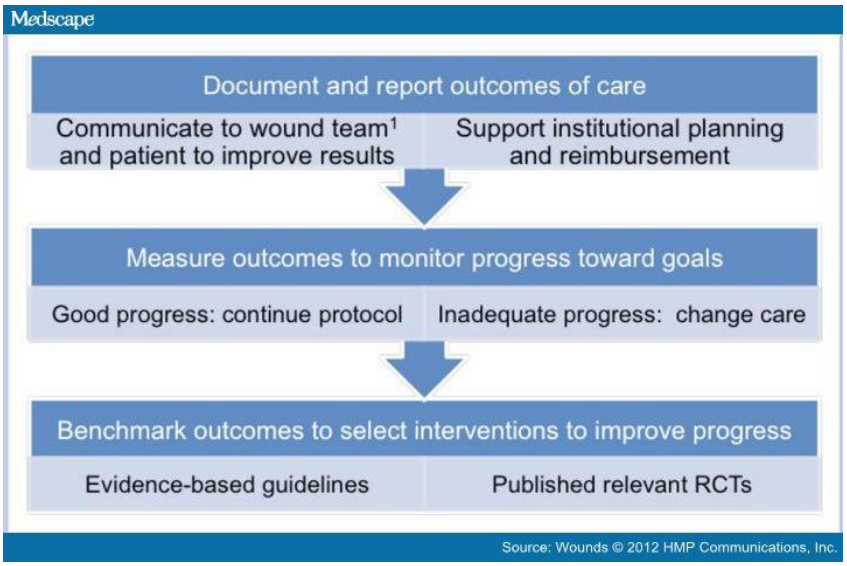
ppO_2 (bar)	PaO_2 (mmHg)	$PtcO_2$ (mmHg)	
		normal	PAOD
0,21	**90 ± 9**	**41 ± 10**	**20 ± 5**
1	**625 ± 23**	**76 ± 45**	**20 ± 8**
2	**1356 ± 28**	**280 ± 50**	**104 ± 20**
2,5	**1700**	**348**	**152**
2,8	**2100**	**451 ± 80**	**201 ± 40**

A Case study[93] showed that in a 58-year-old patient, suffering for several comorbidities, with a full thickness patella burns (from kneeling on hot concrete from 4 days earlier) the HBOT (2.4 ATA, 90 minutes, 15 sessions), in association with other treatments, was paramount in saving gastrocnemius flap and split thickness skin graft.
Diabetes mellitus (DM) was uncontrolled with a glycohemoglobin (HbA1c) of 11.2% (normal 4.2% to 6.4%). Arterial duplex confirmed 20% stenosis at femoral artery and >50% at bilateral dorsalis pedis (DP) and posterior tibial (PT). Transcutaneous Oximetry Measurement (TCOM) showed severe hypoxia with a tissue oxygen partial pressure (PtcO2) of 3 to 20 mmHg and 10 to 23 mmHg in the right knee (RK) and left knee (LK) respectively (normal>50 mmHg). In view of vascular comorbidities and good hyperbaric oxygen challenge response (PtcO2 >50 mmHg in both knees on 100% oxygen), HBOT was started with DM endocrine and dietitian optimisation; concurrent application of Negative Pressure Wound Therapy (NPWT); intravenous Piperacillin-Tazocin to treat multi-resistant Staphylococcus aureus and Pseudomonas aeruginosa (on wound culture).

B. HBOT REDUCED HEALING TIMES COMPARED TO BENCHMARK HEALING TIMES

In the wound care sector, benchmark outcomes are considered a powerful tools for improving practice and progress toward patient and wound goals [94,95,96,97]. Benchmarking one's outcomes compared to robust results reported in the literature can either identify opportunities for improving practices or justify adhering to current practice. Only if one's current healing outcomes for a relevant wound fall short of corresponding benchmarks is there reason to consider changing practice. It would be important and very useful to demonstrate that HBOT, when combined with other standard therapies, enhances the benchmark's outcomes. (Figure 5)

Figure 5. Ways professionals use benchmark outcomes to select interventions (as HBOT) to improve progress in managing wounds. Wounds© 2012 HMP Communications, Inc.



The processes involved in wound healing depend upon the interaction between many time-dependent components[95]. Understanding healing duration is vital in wound management: increased time to healing correlates with greater rates of infection, scarring and non-healing. The usefulness of wound-healing trajectories as predictors of efficacy of treatment for diabetic foot ulcers and venous stasis ulcers has been demonstrated and validated[97]. A wound-healing trajectory integrates the many time-dependent processes that are part of the healing process and is affected by systemic and local deterrents to healing (the presence of the local and systemic impediments to the wound healing seems to be where the HBOT appears to be more effective).

As the healing of open wounds follows an exponential curve, wound-healing trajectories (percentage of wound closure versus time) have been used to describe chronic wound healing[95]. Although wound healing trajectories were initially intended for acute wounds, they can also be used to evaluate the healing of chronic and complex wounds, such as diabetic foot ulcers, pressure ulcers, and venous stasis ulcers[96]. The trajectory curve, similar to the Gompertz growth curve for biological systems, is sigmoid-shaped, with time on the x-axis and percentage of wound closure on the y-axis, so that the rate of change in wound area decreases as the residual wound area approaches total closure [95].

In order to assess the appropriate time for wound healing, Bolton L.[98] published eight RCTs and one meta-analysis qualified as benchmark resources for DU, PU, and VU.. Three RCTs[99-101] and the same meta-analysis[102] applied to VU provided the benchmarks for VU. The others RCTs, published before 2004, represent healing benchmarks for DU and PU.

Illustrating use of a benchmarking tool for VU management, a specialized VU service engaging in evidence-based practice had increased VU 12-week healing from 12% in a community-managed cohort to 53% in a subsequent cohort diagnosed and managed in the clinic. The specialized clinic 12-week healing outcomes match the best reported for VU, highlighting the exemplary value of its multidisciplinary leg wound service. Using study summaries clinical staff can adjust expectations and resource management for the level of delayed healing risk experienced within their practice setting and recognize key areas on which to focus care for a specific patient to avert healing delays.

No single RCT with at least 100 subjects with a PU per study arm was found, so the only benchmark for PU was a meta-analysis of smaller controlled studies. Documented healing outcomes of 57% healed in 12 weeks for a cohort of 507 patients with partial- or full-thickness thickness (Stage II–Stage III) pressure ulcers were similar to the 61% reported for Stage II-III pressure ulcers in the meta-analysis for groups managed optimally with a hydrocolloid dressing[94]. Currently, this appears to be the best benchmark to aim for regarding PU. The cohort of patients with full-thickness (Stage III or Stage IV) PU experienced lower 12-week percent healed (36% of 373 full-thickness PU healed in 12 weeks) than the 61% of 134 partial-thickness Stage II PU reported as healed in 12 weeks from the same settings. Documenting full- and partial-thickness PU healing outcomes separately revealed a need to change future PU RCT designs, separating full- and partial-thickness PU outcome analyses to improve PU benchmarking. There appears to be no qualifying RCT benchmark for full-thickness PU percent healed after 12 weeks of care.

The Table 7 shows wound-healing trajectories (percentage of wound closure versus time) as an indicator of the total time of healing[103]

Table 7. percentage of wound area reduction by time as an indicator of the total time of healing[103]

Type of wound	% wound area reduction by time as an indicator of the total time of healing
Venous wound	**> 20% 4 weeks, complete healing in 24 weeks**
Diabetic Foot wound	**> 50% 4 weeks, complete healing in 12 weeks**
Surgical wound dehiscence	**50% to 2 weeks, complete healing in 3 weeks**
Pressure wound	**> 50% at 2 weeks, complete healing time related to severity**

In a RCT[54] HBOT group (versus control group) had a better average healing time of the wounds caused by necrosectomy of the myocutaneous flap or forearm flap (27.50 vs 45.00 days, $P < 0.01$; in the pharyngeal fistula (8. 50 vs 14.09 days, $P < 0.01$) and in the infected or fluid-filled wound (5.93 vs 8.62 days, $P < 0.01$)

Although published in 1994, a RCT shows that HBOT (2.5 ATA, 90 minutes, daily, 5 days per week, for a total of 30 treatments) may be used as a valuable adjunct to conventional therapies to have a better average healing time of the delayed non-diabetic wound healing. Sixteen otherwise healthy patients who had nondiabetic, chronic leg ulcers with no large vessel disease were included in a double-blind study. Patients were grouped according to age and then randomly assigned to two groups breathing either air or oxygen. At weeks 2 the mean decrease of the wound areas in the HBOT group were of the 6 percent (SD ± 14 , 4) versus 2.8 percent (SD ± 11) in the air group. At week 4, 22 percent (SD ± 13) versus 3.7 percent (SD ± 11) giving a p value less than 0.05 using the Mann-Whitney U test. At week 6, 35.7 percent (SD ± 17) versus 2.7 percent (SD ± 11) with a p value less than 0.001.

C. OXYGEN MONITORING SYSTEMS. PREDICTION OF EFFECTIVENESS OF HBOT IN WOUNDS HEALING WITHIN EXPECTED TIMES

The laboratory evidence for hypoxia playing a major role in wound healing failure is not in dispute. Clinical studies identifying the risks of wound or amputation flap healing failure define periwound hypoxia as a primary determinant of future healing failure. There is a level of oxygen below which a wound does not have the capacity to heal.

HBOT will not accelerate tissue repair in wounds with normal oxygen tensions. It is essential in clinical practice to demonstrate and evaluate critical tissue ischemia before considering the use of HBOT[50]. Even before HBOT is considered, in the absence of infection, is it reasonable to provide documentation of vascular screening or documented endovascular or surgical correction of the Peripheral Arterial Occlusive Disease (PAOD) [50,61,62,63].

The use of transcutaneous oximetry (TCOM) is the golden standard to predict whether or not a patient suffering from non-healing lower extremity wound might receive benefit from HBOT[64,65].

Kaur[52] reported after 30 days, periwound TCOM improved by 11.8 mmHg in the HBOT group (P = 0.01) and decreased by 5.7 mgHg from baseline value in the control group (P = 0.2). The baseline TCOM values were not statistically different between both groups (P = 0.407). The periwound transcutaneous oxygen tensions in the affected tissue were significantly higher in those participants who had received HBOT (HBOT 11.8 mmHg higher, 95% CI 5.7 to 17.8, P = 0.0002, I2 = 25.4%). (Table 8)

Table 8. Comparison 3 Mixed ulcer types, Outcome 3 Periwound transcutaneous oxygen tension at the end of treatment (study of Kaur[52] evaluated by Kranke P and others[1]). Copyright ©2015 The Cochrane Collaboration. Published by JohnWiley & Sons, Ltd.

Review: Hyperbaric oxygen therapy for chronic wounds

Comparison: 3 Mixed ulcers types

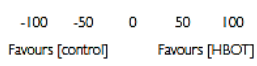
Outcome: 3 Periwound transcutaneous oxygen tensions at the end of treatment

Study or subgroup	HBOT N	Mean(SD)	Control N	Mean(SD)	Mean Difference IV,Fixed,95% CI	Weight	Mean Difference IV,Fixed,95% CI
Kaur 2012	15	34.3 (14.8)	15	21.4 (9.5)		100.0 %	12.90 [4.00, 21.80]
Total (95% CI)	**15**		**15**			**100.0 %**	**12.90 [4.00, 21.80]**

Heterogeneity: not applicable

Test for overall effect: Z = 2.84 (P = 0.0045)

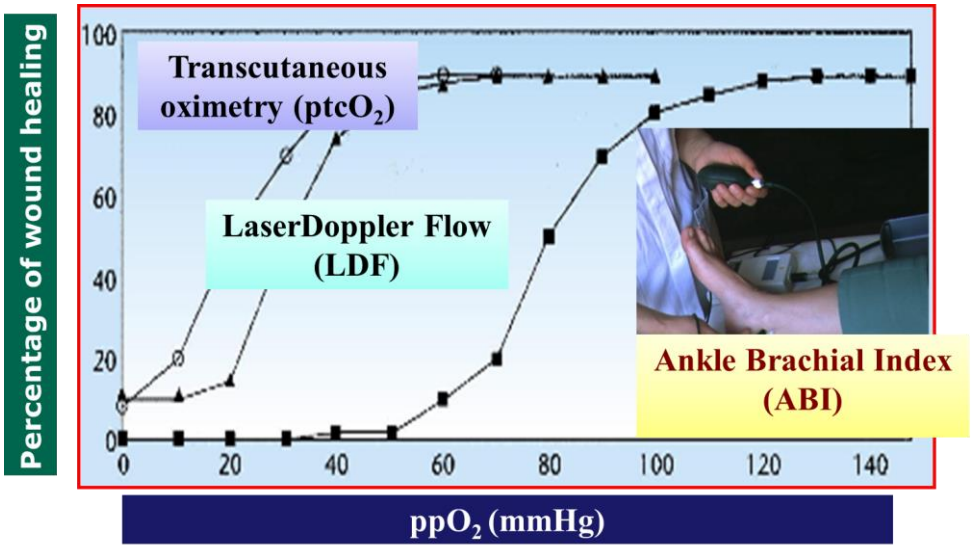
Test for subgroup differences: Not applicable



A Case study[93] showed that HBOT (2.4 ATA, 90 minutes, 15 sessions), in the association with other treatments, saved gastrocnemius flap and split thickness skin graft in a 58-year-old patient, suffering for several comorbidities, with a full thickness patella burns (from kneeling on hot concrete from 4 days earlier). HBOT was started In view of vascular comorbidities and good hyperbaric oxygen challenge response (tissue oxygen partial pressure, PtcO2 >50 mmHg in both knees on 100% oxygen). In the baseline evaluation, Transcutaneous Oximetry Measurement (TCOM) showed severe hypoxia with a (PtcO2) of 3 to 20 mmHg and 10 to 23 mmHg in the right knee (RK) and left knee (LK) respectively (normal>50 mmHg).

TCOM is non-linear with respect to blood flow, exhibiting a hyperbolic response to changes in blood flow that is more pronounced as flow rates decrease. There is some variability in $PtcO_2$ values obtained based upon the type of electrode and temperature used. In normal conditions, the synthesis of nitric oxide requires a partial pressure of oxygen of 35-40 mmHg (or 50 uM of oxygen). In general, values below 25-40 mmHg have been associated with poor healing of wound and amputation flaps with the lower the value the greater the degree of healing impairment. When normobaric oximetry values are less than 25 to 40 mm Hg, the addition of provocative testing with lower extremity elevation or dependency or following occlusion induced ischemia and recovery or with 100% normobaric oxygen challenge given via a non-rebreathing face mask may increase the sensitivity of the test as a screening tool for detecting occult lower extremity arterial insufficiency[104,63]. If the abnormally low TCOM values rise to 100 mm Hg or more, the patient will likely benefit from HBOT, especially when the patient has different impairment factors (as the cigarette smoking; rheumatic diseases; anemia; diabetes; respiratory, liver and/or kidney disease diseases).
Transcutaneous oximetry (TCOM) is generally accepted as a better predictor of failure than success and the most useful oxygen monitoring system for predicting failure to heal a wound without intervention, failure to heal a planned amputation, failure to respond to HBOT, as well as evaluating the success of revascularization. TCOM is better correlated to the Laser Doppler Flow and is a more accurate reflection of changes in perfusion than the measurement of Ankle Brachial Index[104]. (Figure 6)

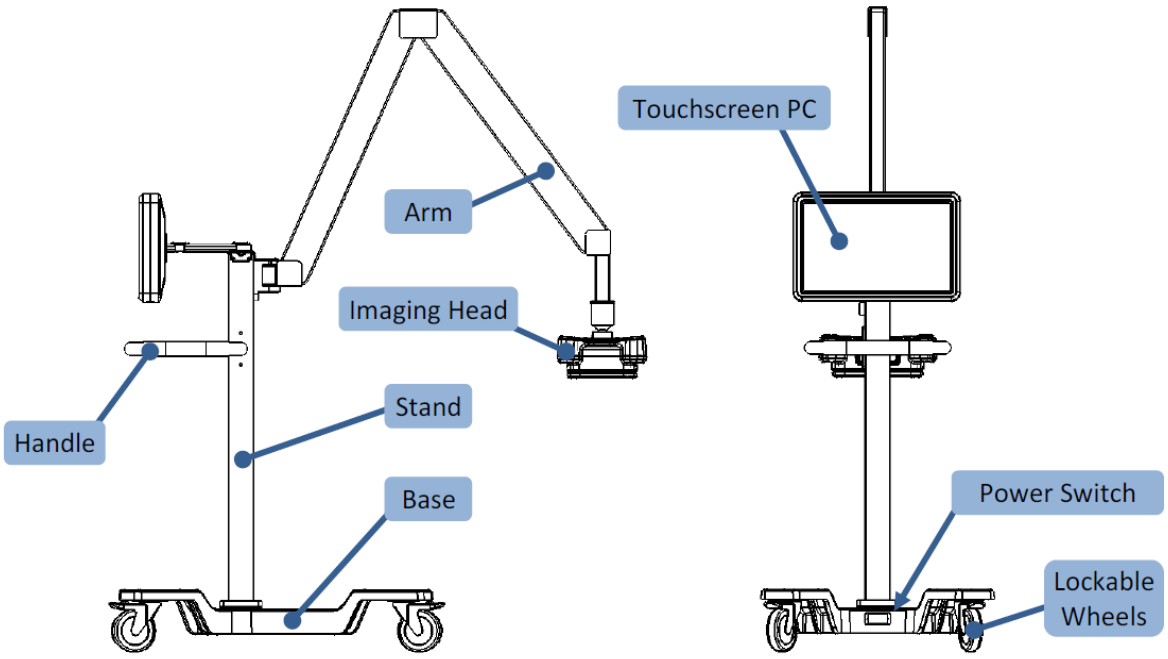
Figure 6: The values of the transcutaneous oximetry is better correlated to the Laser Doppler Flow value than to the Ankle Brachial Index



It was reported that obtaining objective data by transcutaneous oximetry monitoring could be sometimes challenge due to environmental variables such as room temperature and patient variables such as oedema or inflammation, caffeine or nicotine use[105]. With TCOM, only the periwound is measured and it is not possible to place a probe into the wound itself. In extremely thin patients, bony prominences limit scanning ability as well and interpreting that data is quite challenging.

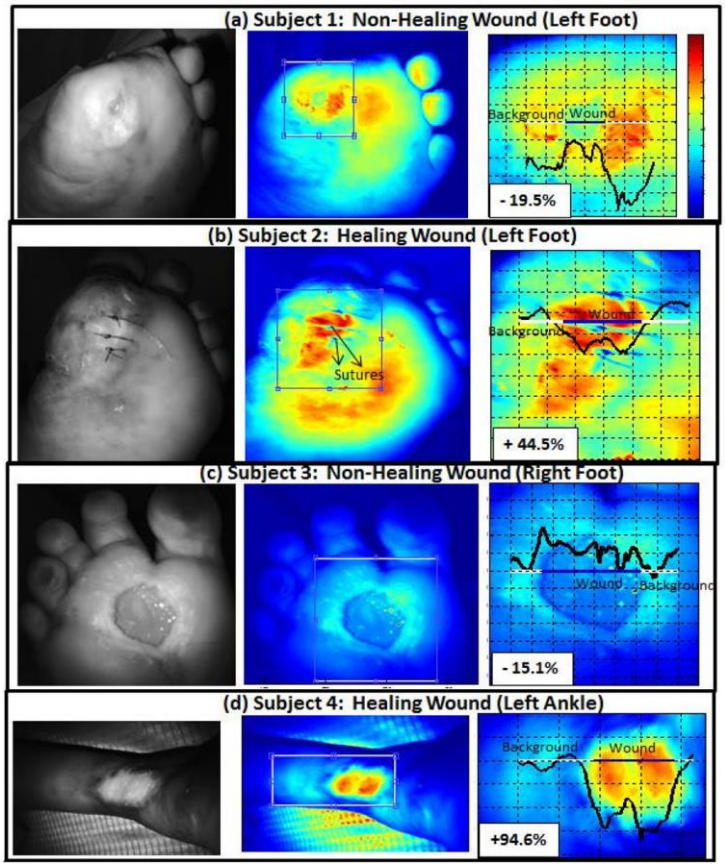
To address these limitations, there are two new diagnostic techniques - coupled with TCOM - can facilitate the measurement of real oxygenation of the wound: Near InfraRed Spectoscropy (NIRS) and LUNA Fluorescence Angiography System.
The Near InfraRed Spectroscopy (NIRS) has excellent potential to determinates oxygenation levels in superficial tissues for patients with potential circulatory compromise (Figure 7). The NIRS is a non-invasive tissue oxygenation measurement system that reports an approximate value of Oxygen saturation (StO_2), Oxyhemoglobin level (HbO_2), deoxyhemoglobin level (Hb) in superficial tissue (generally defined as Wound Bed Oxygen Saturation, StO_2)

Figure 7 The Kent Chamber for Near Infrared Spectroscopy (NIRS) used for research at the OutPatient Problem Wound Care and Hyperbaric Centre in Ravenna



NIRS images differences in blood flow beneath the surface of the wound with respect to its peripheries. It captures these differences from the detected NIR optical signals obtained by non-contact imaging of the entire region of interest in real time. Since it is very easy to detect the value of the wound bed oxygen saturation *(just a click on the touch screen to the desired point in the wound's image)* is better to evaluate a gradient between the value of the StO_2 in the centre of the lesion and on the healthy skin around the wound or respect the average of four readings on the edge of the lesion. The absorption of NIR light varies according to whether a wound is healing or otherwise. Non-healing wounds arrested in the inflammatory stage has increased absorption (or decreased reflection) of NIR light, as there is greater concentration of stagnated blood in the region. Conversely, a wound that is healing will progress from the inflammatory to the proliferation stage, followed by remodeling. With progression into proliferation, there is greater consumption of blood at the wound site, causing less absorption (or greater reflection) of NIR light in the region. Figure 8 shows examples from clinical studies performed on diabetic foot and/or leg ulcers, with healing and non-healing wounds[106,107]

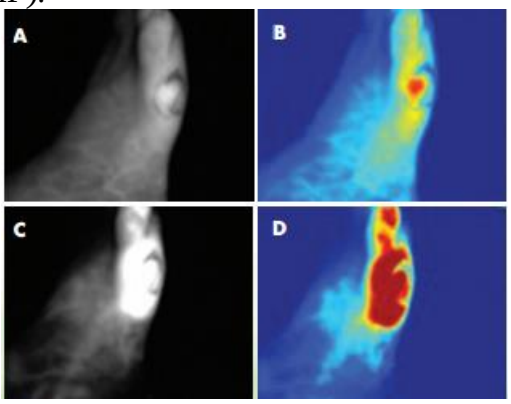
Figure 8. (a–d) Near-InfraRed Spectroscopy (NIRS) optical images of diabetic foot wounds. The plots in the third column on the right correspond to the zoomed-out region of interest, with the intensity profile at a chosen y plane. The intensity varies from maximum to minimum along the y axis. The plots show the scanner's ability to differentiate healing from non-healing wounds, as observed in a positive optical contrast in healing and a negative optical contrast in a non-healing wound. (Copyright© 2015 SPIE)



NIRS is useful to define the wound-healing trajectory (*percentage of wound closure versus time)* under a specific treatment (as HBOT) and predict chronic wound healing time.

The LUNA© Fluorescence Angiography System (Novadaq) is an emerging modality in assessing tissue perfusion in DFUs and chronic non-healing wounds using SPY Technology. SPY Technology utilizes fast, real-time imaging that allows clinicians to capture and review high-quality image sequences of blood flow in vessels and microvessels, tissue and organ perfusion. The LUNA© system utilizes an injectable dye, IndoCyanine Green (ICG), which absorbs and reflects light, resulting in fluorescence images that are visible on a computer monitor showing blood flow in vessels and perfusion in the area of the wound. Images are immediately visible and the procedure takes approximately five to 10 minutes. (Figure 9)

Figure 9. Fluorescence angiography shows flow in the wound on the side of a patient's foot. The wound has somewhat adequate blood flow, but it is decreased. *©2013 HMP Communications, LLC (HMP).*



D. COMBINED APPLICATION OF HBOT WITH OTHER HEALING PROCEDURES IN THE TREATMENT OF WOUNDS WITHIN EXPECTED TIMES

Over the past decade, there have been numerous advances in wound care. Despite these advances, it is important to realize that the causes of ulceration are often multifactorial and there is no one product that will heal all wounds. Caring for a patient with chronic ulceration is complex and necessitates multidisciplinary collaboration to achieve the goal of providing comprehensive wound care. The combined use of HBOT with other advanced wound healing modalities may be a useful synergy in the armamentarium of wound healing[108].
Schweyer MA (2008[109]) compared the data of hospitalized patients suffering from wounds in the next two years. In the second year (2007-2008) the standard therapy was associated with the Negative Pressure Wound Therapy (NPWT) and HBOT. The integration of these two therapies, versus the previous year, allowed to treat more patients (+ 11%); the total number of days of treatment and the total cost was reduced (-5%); the average lenght of hospitalization was also reduced (-11,6%). As shown in Table 9

Table 9. The addition of HBOT and Negative Pressure Wound Therapy (NPWT) to standard therapy has enabled a positive change for a comprehensive hospital-based Wound Care Center. Over the previous year, at constant budget, it increased the number of patients treated (+ 11%); the total number of days of treatment was reduced (-5%) and the average length of stay for patient was decreased (-11.6% days).

Period	Cost (€)	Number of patients	Days of treatment (total)	Average length of stay for patient (days)
Standard treatment (March 2006 – February 2007)	**292,042**	**642**	**6488**	**10,10**
Standard + HBOT + NWPT (March 2007 – February 2008)	**279,868**	**719 (+11%)**	**6435 (-5%)**	**8,95 (-11,6%)**

In a comparative analysis[110] looking at either compromised post-surgical wounds or wounds secondary to arterial insufficiency, the combination of NPWT with HBOT produced results

that exceeded those produced when either modality was used alone. In addition, the combined use of both therapies helped decrease the average number of HBOT treatments required.

A Case study[93] of a 58-year-old patient, suffering for several comorbidities, with a full thickness patella burns, showed the saving of gastrocnemius flap and split thickness skin graft by the association of HBOT (2.4 ATA, 90 minutes, 15 sessions); Diabetes Mellitus (DM) endocrine and dietitian optimisation ; two concurrent application of Negative Pressure Wound Therapy (V.A.C.®, Texas, USA) for a bio-occlusive environment removed excess exudates and promoted healing; intravenous Piperacillin-Tazocin to treat multi-resistant Staphylococcus aureus and Pseudomonas aeruginosa (on wound culture). At the tenth HBOT session, bilateral split thickness skin grafts were done. Due to early excessive mobility and poor wound care, the right knee graft broke down and required a local right gastrocnemius flap and split thickness skin graft. Patient returned to work 3 weeks later with no functional loss.

Akcali G[111] presented a patient with a recalcitrant wound caused by Cutaneous LeukocytoClastic Vasculitis (CLCV) who was successfully treated with HBOT and Cellular and / or Tissue-based Products – CTPs - for wounds.

The experience of the Out-patients Problem Wound Care and Hyperbaric Centre in Ravenna (Italy) shows the clinical efficacy of a multidisciplinary approach for the enhancement of healing in selected problem wounds[112,113]. In 2015, 416 patients for a total of 749 problem wounds were treated, for a total of 36.745 wound care procedures in 9021 access of the patients to the Centre. The choice of the association between the various therapies depends on the type and severity of the skin ulcer: mechanical ultrasound debridement; homologous Platelet gel *(*once per week for four times); Cellular and / or Tissue-based Products – CTPs - for wounds *(*once every two weeks for three times); HBOT (2,5 ATA in multiplace chamber with a FiO_2 in mask > 0.9, 90 minutes, daily, 5 days per week, for an average of 18 sessions) and Frequency Rhythmic Electrical Modulation System (20 sessions, 5 days per week). In Table 10 results at the time of the patient's discharge from Centre (usually within twelve weeks after that the out-patient is taken in charge, except for vasculitic wounds) are shown. Most of the wounds healed or improved *(reduced by 72% with a Falanga Wound Bed Score ≤ A2)*. Arterial (ischemic) leg ulcers had less benefit.

Table 10 Results at the time of the patient's discharge from Problem Wound Care and Hyperbaric Centre in Ravenna (Italy), usually within twelve weeks after that the out-patient is taken in charge, except for vasculitic wounds

Kind of skin wound	Number of patients (%)	Fully healed (%)	Improved (%)	Stationary/ worse (%)	Drop out /transferred to another setting of care (%)
Traumatic	117 (28)	75,6	8,4	5,8	10,20
Venous Leg Ulcer	116 (28)	80.5	12.2	4.9	2.4
Arterial (ischemic) Leg Ulcer	30 (7)	33.3	-	66.7	-
Mixed vascular (Lower Limb)	47 (11)	69.2	30.8	-	-
Diabetic Foot Ulcer	29 (7)	45,8	15,3	15,9	23
Rheumatic	47 (12)	72	24	-	4
Pressure	30 (7)	57.1	28.6	14.3	-

The Association for the Advancement of Wound Care Government and Regulatory Task Force[114] developed a content-validated Venous Leg Ulcer (VLU) guideline based on best available evidence supporting each aspect of VLU care in order to improve healing and reduce costs of care. National Guideline Clearinghouse (NGC) published the venous ulcer guideline of the Association for the Advancement of Wound Care (AAWC) (revised 2010 Dec 01)[115]. The items compiled from existing algorithms, level of best available evidence for each item, and corresponding Content Validity Index (CVI) values + standard deviation from the content validation study are presented in Table 11.

The Venous Ulcer Guideline containing all elements with A-level evidence plus those with a Content Validity Index (CVI) >0.75 resides on the AAWC and the Agency for Healthcare Research and Quality National Guideline Clearinghouse websites. Items with a CVI >0.75 supported by B- or C-level research present opportunities for further study. Based on two old reports[71, 116] the Evidence Level of Hyperbaric oxygen (HBOT) in Venous Leg Ulcer is C1 with a CVI of 0,38 (SD 2,3±1,21).

The latest guidelines for Venous Leg Ulcers, published by the Agency for Healthcare Research and Quality (AHRQ) in 2014, does not cite HBOT neither in favor nor against[117]

Table 11. This table is part Venous Ulcer Care Initiative (VUCI) algorithm of the Association for the Advancement of Wound Care (AAWC)[118]. Evidence ratings range between A= highest (eg. randomized controlled trials and C = lowest (eg. opinion-based). Items were deleted from the final Venous Ulcer Guideline unless supported by A-level evidence.

Biophysical modalities	**Level of Best Available Evidence**	**Content Validity Index (Mean ± Standard Deviation of Ratings)**
Electric stimulation	A	0,81 (3,5±**0,81)**
Negative Pressure Wound Therapy (NPWT)	B	0,75 (3,1±**1,00)**
Warming therapy	C	0,44 **(2,3±1,26)**
Electromagnetic or radiofrequency stimulation	A	0,63 (2,8±**1,12)**
Laser stimulation	C	0,19 (2,0±**1,06)**
Infrared stimulation (eg. Monochromatic light)	C	0,56 (2.6±**1,06)**
Hyperbaric oxygen	C	0,38 (2,3±**1,21)**
Ultrasound stimulation	A	0,47 (2,5±**1,30)**
Whirlpool	C	0,44 (2,2±**1,35)**

E. DIFFERENCES BETWEEN HBOT, TOPICAL TISSUE AND OTHER OXYGENATION PROCEDURES

Topical tissue oxygenation procedures are designed to allow oxygen to enter the wound or skin via the external surface of the body rather than from capillaries within. These procedures include treatment methods that deliver pure gaseous oxygen, flushed in enclosures around a limb or wound site and devices that generate gaseous oxygen at the wound surface as a device (Natrox™) that, by electrolysis, produces pure (96.2%) humidified oxygen at ~15ml/hr (24 hours per day) increasing the oxygen concentration in the headspace above the wound[119].
This should not be confused with systemic HBOT, in which the patients breathe 100% oxygen while they are inside of a monoplace or multiplace chamber under greater-than-normal atmospheric pressure. Topical oxygen is frequently (and inappropriately) called "topical hyperbaric oxygen." Use of this term simply adds confusion and is a misapplication of the word "hyperbaric". According to The Centers for Medicare and Medicaid Services (CMS) and other third party carriers, breathing near 100% oxygen at 1 atmosphere of pressure or exposing isolated parts of the body to 100% oxygen does not constitute HBOT. The patient must breathe oxygen while inside a pressurized chamber typically 1.4 ATA or greater[120]. Center for Medicare & Medicaid Services (CMS) states that Topical Application of Oxygen does not meet the definition of HBOT as stated by CMV. Also, its clinical efficacy has not been established. Therefore, no Medicare reimbursement may be made for the topical application of oxygen[72]
Advocates claim that topical oxygen dissolves in tissue fluids, is bacteriostatic and stimulates angiogenesis and wound healing[121-123]. However, Randomized controlled trials are not yet reported[124].

F. CREATION OF AN EUROPEAN WOUND REGISTRY FOR THE ASSESSMENT OF BENEFITS OF HBOT IN WOUND CARE

According to the Agency for Healthcare Research and Quality (AHRQ)[125] and others[126], Wound Registries are an important source of benchmarking data. The strength of using registry results for benchmarking is in its compelling call for a change in practice if one's current healing outcomes for similar chronic wounds falls short of those displayed for similar wounds managed otherwise.

A limitation of registries may be variability in procedures of care and adherence to protocol, so those using registry results for benchmarking purposes may be less aware of the exact protocols followed to achieve a registry result. As registries expand, analyses of variables in care that affect outcomes will help clarify ideal care.

US Wound Registry (USWR) was issued to create an initial suite of 12 new quality measures for wound care and hyperbaric medicine as part of the USWR's Qualified Clinical Data Registry (QCDR). www.uswoundregistry.com/specifications.aspx

The activation of a European Register of wounds is suggested to collect information on the wound management and the use of HBOT in wound care in order to improve clinical practice.

CONCLUSION

"Problem wound" is one that has one or more local complicating factors, such as exudate, infection and systemic comorbidities, polypharmacy, etc. "Delayed wound healing" usually refers to wounds that take a long time to heal (longer than 4 to 6 weeks), heal by secondary intention, do not heal or recur[2].

Basic science and insight into HBOT in wound healing is improving, whereas there is still more to learn regarding the clinical application of HBOT in wound care.

HBOT should be recommended in the problem chronic wounds when there are comorbidities that inhibit the normal nitric oxide synthesis and the subsequent healing process. On the base of Randomized Clinical Trial, Case series and Case report, the HBOT seems to be effective in non - healing chronic lower limb wounds (as well Venous Leg Ulcer and Mixed Arterial, Venous and Lymphatic wounds); recurrent multiple non-healing vasculitic wound especially those who have not responded to immunosuppressive therapy.

No trial was identified that considered HBOT effective, as monotherapy, in arterial, thermal burn and pressure wounds. According to the Wound Healing Society[129], in patients with arterial wound wherever the anatomy is non-reconstructable or whose ulcer is not healing despite revascularization, HBOT should be considered as an adjuvant therapy (level of evidence IIIB), so for the treatment of ischemia-reperfusion injury after revascularization. In thermal burns and pressure wounds, HBOT appears to be effective to treat infections and in facilitating the plastic surgery[124, 60, 129].

In this review, Diabetic Foot Ulcers (DFU); Compromised Skin Grafts and Flaps; acute surgical wounds (class IV) with Surgical Site Infections (SSI) and acute infected traumatic wounds were excluded because presented elsewhere in the 10th ECHM Consensus Conference on Hyperbaric Medicine in Lille (France) on April 15th-16th, 2016.

When HBOT is prescribed, clear clinical targets should be defined, eg. reversion of hypoxia, anti-inflammatory effect, limb salvage, demarking of necrotic tissues before wound debridement or other.

Before HBOT, as physical exam is insufficient, it is reasonable to provide documentation of vascular screening or documented action to correct Peripheral Artery Occlusive Disease (PAOD) if present. The use of transcutaneous oximetry measurement (TCOM) is the golden standard for measuring the ppO_2. Other methods of measuring the perfusion and oxygenation of the wound bed could be the Laser Doppler Flow, Near InfraRed Spectroscopy (NIRS) and

the Fluorescence Angiography. The Ankle Brachial Index is not a reliable method for assessing perfusion in the Diabetic Foot Ulcer.
The therapeutic protocol of HBOT (pressure, time and length of treatment course) should be made more specific in relation to the type of chronic wound. In the published report, the most widely used HBOT protocol for delayed wound healing is: pressure of 2.0 ATA (range: 2-2,6 ATA); total time of 90 minutes (range: 90-120 minutes); daily, 5-6 days a week; total number of 30 sessions (range 10-40 sessions). Utilization review is required to half of the prescribed HBOT sessions in order to decide on the appropriateness of continuing the treatment or any necessary corrective measures to improve the result. In the presence of limb threatening infection after debridement or incompletely corrected peripheral arterial occlusive disease, patients may require twice-daily treatments. Once stabilized, treatment frequency may decrease to once daily[130].
The association of HBOT with other treatments, such as Negative Pressure Wound Therapy (NPWT), Platelet gel, Cellular and Tissue-based Products (CTPs), Skin Grafts and Flaps seems to be effective in improving healing and reducing the costs of care.
We suggest more trials to properly evaluate evaluate HBOT in combination with other interventions. (Type 1 recommendation; Level C evidence)
It seems useful to compare HBOT outcomes to robust results of other treatments *(benchmarking)* in order to identify opportunities for improving practices. Wound-healing trajectories *(percentage of wound closure versus time)* appear to show that therapeutic paths, including HBOT, facilitates the reduction of the total time of wound healing. Wound registries expand and report risk adjusted healing outcomes, thus related benchmarks will be clarified. The activation of a European Register of wounds is suggested to collect information on the wound management and the use of HBOT in wound care, in order to improve clinical practice.
There remains a need for further clinical research. Economic outcomes in large clinical studies of strong methodological quality should be included, in order to make recommendations on the cost-effectiveness and the effect on the Quality-Adjusted Life years (QALY) when the HBOT is applied in wound care.
Topical application of oxygen does not meet the definition of HBOT according to Center for Medicare & Medicaid Services (CMS). Furthermore, its clinical efficacy has not been established.

RECOMMENDATION

- We suggest HBOT in delayed non diabetic wound healing (as well Venous Leg Ulcer and Mixed Arterial, Venous and Lymphatic wounds) and in recurrent multiple non-healing vasculitic wounds (especially those who have not responded to immunosuppressive therapy). (Type 1 recommendation; Level B evidence).
- It has been reported that HBOT, in these kinds of wounds, may improve the rate of the healing (reduces the wound area and the average healing time) by increasing the Nitric Oxide (NO) level and the number of Endothelial Progenitor Cells (EPCs) which are correlated with the angiogenesis. HBOT may help pain relief. (Type 1 recommendation; Level B evidence).
- In delayed healing of arterial, thermal burn and pressure wound, no evidence to confirm or refute any effects of Hyperbaric Oxygen Therapy (HBOT) were found.
- HBOT should be recommended in delayed wound healing when there are comorbidities that inhibit the normal nitric oxide synthesis and the subsequent healing process. (Type 1 recommendation; Level C evidence).

- Prior to the application of HBOT in selected problem wounds, there must have been some attempt at treatment by other means. (Type 1 recommendation; Level A evidence)
- It would be reasonable to provide documentation of 30 days of standard wound care and - in Venous Leg Ulcer and mixed wounds - of vascular screening (physical exam is insufficient) or documented action to correct Peripheral Artery Occlusive Disease (PAOD) and Critical Limb Ischemia (CLI), if present. A subsequent vascular screening is needed to determine response to vascular intervention (if it is the case). Location of wound must be consistent. (Type 1 recommendation; Level B evidence).
- We recommend, as standard of care in non diabetic wounds, that HBOT should always be used as part of a multidisciplinary treatment plan with ongoing wound care on a regular basis and not simply as stand-alone therapy. (Type 1 recommendation; Level B evidence).
- It would be reasonable, in delayed wound healing, combining HBOT, Negative Pressure Wound Therapy (NPWT), Platelet gel, Cellular and / or Tissue-based Products (CTPs) and plastic surgery (as skin grafts and flaps) in order to improve wound healing and reduce the costs of care. (Type 1 recommendation; Level C evidence).
- We suggest more trials to properly evaluate HBOT in combination with other interventions (Type 1 recommendation; Level B evidence)
- The therapeutic dose of HBOT (pressure, time and length of treatment course) should be made more specific in relation to the type of chronic wound. (Type 1 recommendation; Level B evidence)
- Since the hyperbaric chamber is compressed by air and the patient breathes oxygen through a mask, it would be reasonable to measure the Fraction of Inspired Oxygen (FiO_2) in the mask which must be higher than 90%. Otherwise it must be adopted a corrective measure to improve the FiO_2 *(as well as increase the absolute pressure; choose a mask that fit better to the face or a more effective ventilation mode).* Type 1 recommendation; Level B evidence
- The use of transcutaneous oximetry measurement (TCOM) is recommended as golden standard for measuring the partial pressure of oxygen (ppO2). (Type 1 recommendation; Level A evidence)
- Other methods of measuring the perfusion and oxygenation of the wound bed include Laser Doppler Flow, Near InfraRed Spectroscopy (NIRS) and the Fluorescence Angiography. (Type 3 recommendation; Level C evidence)
- The mechanisms of action of HBOT are not valid for topical tissue oxygenation procedures. (Type 1 recommendation; Level B evidence)
- It could be reasonable to compare HBOT outcomes to robust results of other treatments *(benchmarking)* in order to identify opportunities for improving practices. (Type 1 recommendation; Level B evidence)
- It could be reasonable to create a European Wound Register to improve the healing outcomes for HBOT in wound care. (Type 1 recommendation; Level C evidence)
- We suggest more trials to properly evaluate HBOT in people with delayed wound healing. These trials must be adequately powered and designed to minimize all kinds of bias. (Type 1 recommendation; Level A evidence)
- New studies should use an appropriate comparator therapy or an effective sham therapy to assess the true additional effect of HBOT over standard treatment options. (Type 1 recommendation; Level A evidence)

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ABBREVIATIONS AND ACRONYMS

- ABI = Ankle Brachial Index
- AKA = Above Knee Amputation
- ATA = Absolute pressure (is the total ambient pressure on the system).
- BKA = Below Knee Amputation
- CLI = Critical Limb Ischemia
- CLU = Chronic leg ulcer
- CTPs = Cellular and/or Tissue-based Products
- DFU = Diabetic Foot Ulcer
- ECHM = European Committee for Hyperbaric Medicine
- eNOS = Endothelial Nitric Oxide Synthase
- EPC = Endothelial progenitor cell
- EPUAP = European Pressure Ulcer Advisory Panel
- FiO_2 = Fraction of Inspired Oxygen
- GBP = UK pound sterling (symbol: £; ISO code: GBP)
- HBO = Hyperbaric Oxygenation
- HBOT or HBO2T = Hyperbaric Oxygen Treatment
- HIF = Hypoxia-Inducible Factor
- HOPDs = Hospital based OutPatient wound care Departments (USA)
- IDDM = Insulin Dependent Diabetes Mellitus
- ISPOR RCT-CEA = The International Society for Pharmacoeconomics and Outcome Research Task Force in Good Research Practices: Randomized Clinical Trials-Cost-Effectiveness Analysis
- NIDDM = Non-Insulin Dependent Diabetes Mellitus
- NIRS = Near InfraRed Spectroscopy
- NBO = Normobaric Oxygen
- NO = Nitric Oxide
- NPWT = Negative Pressure Wound Therapy
- OPPS = Medicare Outpatient Prospective Payment System
- PAOD = Peripheral Arterial Occlusive Disease
- PDGF = Platelet Derived Growth Factor *(PDGF-BB: dimeric glycoprotein composed of two B,-BB, chains)*
- PO2 or ppO2= partial pressure of oxygen
- PtcO2 = Transcoutaneous Oxygen Tension
- QCDR = Qualified Clinical Data Registry
- RCT = Randomised controlled trials
- SIGN = Scottish Intercollegiate Guidelines Network
- SIR = Standardized Infection Ratio
- SSIs = Surgical-Site Infections
- SPCs = Stem/Progenitor cells

- TCOM = TransCutaneous Oximetry Measurement
- UHMS = Undersea and Hyperbaric Medical Society
- USWR = US Wound Registry
- VEGF = Vascular Endothelial Grow Factor
- VLU = Venous Leg Ulcer

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