Hyperbaric oxygen therapy in the management of nonhealing wounds in patients with critical limb ischemia

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Critical limb ischemia is characterized with intolerable pain at rest and nonhealing wounds and/or gangrene. The treatment of nonhealing wounds in patients with critical limb ischemia calls for an extraordinary effort. However, major amputation is required in a significant number of patients. Hyperbaric oxygen therapy is one of the adjunctive treatments used in nonhealing wounds. Hyperbaric oxygen therapy enhances collagen synthesis and maturation, fibroblast proliferation, epithelialization, increases leukocyte bacterial-killing capacity and induces angiogenesis. Hyperbaric oxygen therapy also exerts an antibacterial effect on selected microorganisms and reduces wound infection. Hyperbaric oxygen therapy is not a miraculous treatment modality. It is a good adjunctive therapy that increases the healing rate of wounds in selected patients. Hyperbaric oxygen therapy should be instituted together with conventional treatments. Antibiotherapy, strict metabolic control, daily wound care and debridement should not be overlooked during hyperbaric oxygen therapy.

Critical limb ischemia (CLI) is a severe obstruction of the arteries of the lower extremities, which markedly decreases blood flow. CLI is characterized with intolerable pain at rest and nonhealing wounds and/or gangrene. An objective criterion for CLI includes an ankle systolic blood pressure of 50 mmHg or less and a toe systolic blood pressure of 30 mmHg or less [1]. However, since the arterial wall calcification can impair compression of vessels, systolic blood pressures are measured greater than the actual levels in diabetic patients. Therefore, hemodynamic parameters of CLI are less reliable in diabetics [2].

The most frequent reason of obstructive arterial disease leading to CLI is atherosclerosis associated with hypertension, hypercholesterolemia, smoking and diabetes mellitus. Nonhealing wounds usually develop in the areas of foot trauma caused by improperly fitting shoes or an injury [2]. The treatment of nonhealing wounds in patients with CLI calls for an extraordinary effort. The goal of the treatment in patients with critical limb ischemia should be limb preservation. However, amputation is required in a significant number of patients [3].

Hyperbaric oxygen therapy (HBOT) involves the intermittent inhalation of 100% oxygen at a pressure higher than 1 atm absolute (ATA). HBOT favorably increases the amount of oxygen dissolved in arterial blood and leads to hyperoxia even in poorly perfused tissues [4–6]. The physiological effects of HBOT are related to the hyperoxia it establishes in the tissues. Oxygen in the blood is carried by two means: by binding to hemoglobin in erythrocytes and in dissolved form in plasma. People breathing room air at sea level (1 ATA) generally have a hemoglobin (Hb) oxygen saturation of approximately 97%. Assuming normal hemoglobin level (14 g/dl), Hb-bound oxygen content is 18.2 ml O_2/dl blood. At Hb 15 g/dl, it would be 19.5 ml O_2/dl blood. Dissolved oxygen content is 0.3 ml O_2/dl blood. At Hb 14 g/dl, dissolved oxygen represents 1.6% of total content; at Hb 15 g/dl, dissolved oxygen represents 1.5% of total content. Since, Hb is already 97% saturated at room air (1 ATA) it is impossible to markedly increase the amount of oxygen carried with Hb, but you can make a large difference in dissolved oxygen. HBOT leads to a favorable increase in the amount of oxygen dissolved in plasma under Henry's Law, which states that the amount of gas dissolved in a solution is directly proportional to the partial pressure of that gas. HBOT theoretically increases the dissolved oxygen content in plasma from 0.3 ml/dl to 4.4 ml/dl at 2.0 ATA and to 5.6 ml/dl at 2.5 ATA.

HBOT is used as a primary therapy in arterial-gas embolism, severe carbon monoxide poisoning, gas gangrene and decompression sickness [7]. It is also employed as an adjunctive treatment in nonhealing wounds, necrotizing soft-tissue infections, refractory osteomyelitis, osteoradionecrosis, crush injury, compromised skin grafts and flaps and thermal burns [7]. The

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aim of this article is to highlight the role and effectiveness of HBOT in the management of nonhealing wounds in patients with critical limb ischemia.

Effects of HBOT on impaired wound healing

A good understanding of the role of HBOT on wound healing relies on a good understanding of the role of hypoxia and oxygen on wound healing. Wound healing, while being complex, is quite a systematic process involving the inflammatory, proliferative and tissue-remodeling phases. Tissue injury causes the disruption of blood vessels and injured parenchymal cells generate some vasoactive mediators and chemotactic factors. These mediators recruit inflammatory cells to the site of injury. The oxygen tension frequently decreases to 20 mmHg in the wound [8,9]. This decrease is not only due the damage to the vascular structure, but also because of the high oxygen consumption of the recruited inflammatory cells.

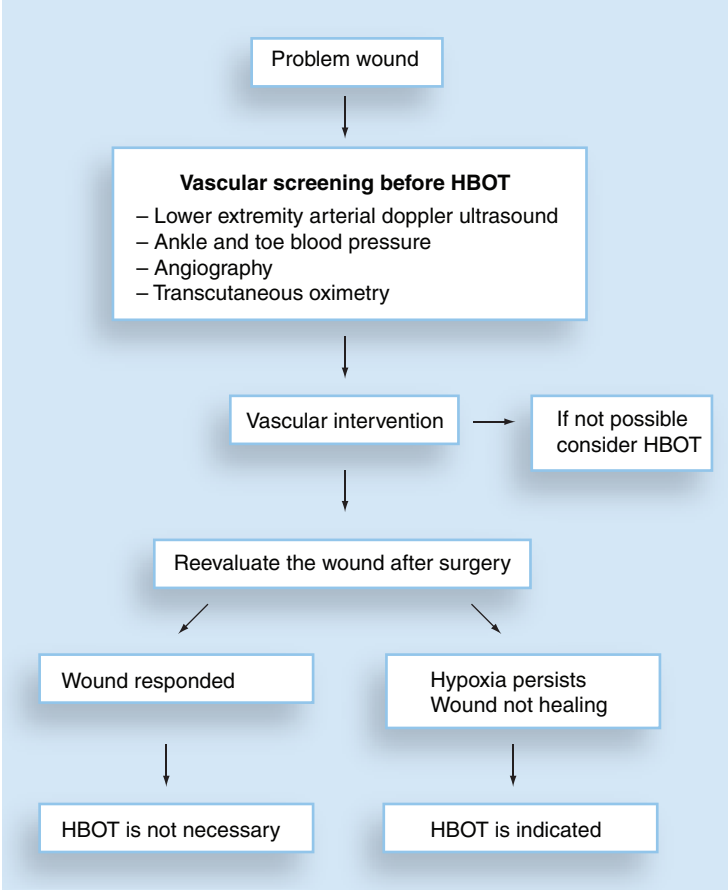
Many processes involved in wound repair such as fibroblast migration and proliferation, protein synthesis, production of growth factors and angiogenesis are actually stimulated by hypoxia [10–12]. Chronic hypoxia, on the contrary acts as an inhibitor on the process of wound healing. Although hypoxia stimulates mRNA synthesis, the translation of mRNA to proteins or post-translational modification is limited by hypoxia. Cell replication, collagen synthesis and the expression of cytokines is reduced in case of chronic hypoxia [13–15]. Collagen synthesis is also decreased by acute hypoxia [16]. Siddiqui *et al.* showed an increase in fibroblast proliferation in cellular fibroblast cultures after an acute hypoxia. However, after a hypoxia lasting for 6 weeks there was a prominent decrease in fibroblast proliferation and α-1-procollagen synthesis [13]. The delayed healing of ischemic wounds compared with the nonischemic wounds reveals a crucial role of oxygen in wound healing.

Many pathways in wound healing are oxygen dependent. Numerous cells recruited around the wound need energy for protein synthesis and proliferation.

Collagen, by far the most important component of the connective tissue, is essential in wound repair. Collagen deposition to wound surface is correlated with the oxygen tension within the wound [16]. Low oxygen tension, secondary to tissue hypoxia, will decrease the synthesis of collagen from fibroblasts [13]. Furthermore, collagen needs a post-translational modification for maturation and transportation outside the cell. Proline residues within collagen are hydroxylated by an enzyme called prolyl hydroxylase, which is oxygen dependent. This enzyme exhibits 50% maximum enzymatic activity when the oxygen tension is 20 mmHg, and 90% maximum enzymatic activity in the case of 150 mmHg oxygen tension [17].

There is a very considerable difference in oxygen pressure between the capillaries in the wound tissue and the repair cells in the wound. Oxygen has to travel a distance of up to 150 µm from the capillaries to reach the wound tissue [18]. This distance can be achieved only the oxygen dissolved in the plasma and not by the one bounded to hemoglobin. So increasing this soluble oxygen in plasma will yield to a better oxygenation for the wound. Hyperoxemia caused by HBOT will lead to an increased

Figure 1. An algorithm of the use of hyperbaric oxygen therapy in nonhealing wounds of patients with critical limb ischemia.



HBOT: Hyperbaric oxygen therapy.

amount of dissolved oxygen in plasma [19,20]. The effective diffusion distance is 64 µm with partial oxygen pressure (pO_2) of 100 mmHg and 246 µm with a pO_2 of 2000 mmHg [18]. After 90 min of HBOT, oxygen pressure in the wound rises to as much as 600 mmHg and this persists, while decreasing, for 1 h after hyperoxia treatment [4]. Following repeated HBO treatments, neovascularization-related tissue oxygenation is permanently adjusted [5,6].

An additional requirement to oxygen is for cellular replication. Hehenberger *et al.* showed that the proliferation rate of the fibroblasts increases with HBOT. The highest proliferation rate was achieved at a pO_2 of 1875 mmHg in normal fibroblasts (43 ± 10% increase) and at a pO_2 of 1440 mmHg in diabetic's fibroblasts (63 ± 11% increase) [21]. HBOT increases fibroblast proliferation and this effect persists even after the treatment [22]. The increase in fibroblast proliferation lasts for 72 h after a 120 min of HBOT. However, doubling the daily therapy does not have an additive effect, nor does the increase in atmospheric pressure from 2.4 atm to 4 atm [23]. HBOT also increases the hyaluronic acid and proteoglycan synthesis of fibroblasts taken from normal and wound tissue [24]. In addition, HBOT increases endothelial cell proliferation. This effect begins within 15 min of therapy [23]. Dimitrijevich *et al.* exposed normal human dermal fibroblasts, keratinocytes, dermal equivalents and skin equivalents to HBOT at 1–3 ATA for up to 10 days. They observed that HBOT increases proliferation of the fibroblasts and the differentiation and epidermopoesis of the keratinocytes [25].

Revascularization of the newly formed granulation tissue is an essential process in wound healing. New vessels provide nutrients for the active cells to promote granulation tissue formation and facilitate the clearance of debris. Although hypoxia is a well-known stimulus for angiogenesis, chronic hypoxia inhibits angiogenesis [26]. HBOT has been shown to increase angiogenesis and wound perfusion in animal models [4,26]. In addition, increased transcutaneous partial oxygen pressure ($TcpO_2$) has been reported in clinical studies after a course of HBOT [5,8]. Postulated mechanisms of hyperoxia-induced angiogenesis include increased VEGF production and collagen deposition [4]. VEGF levels increase by 40% after 5 days of HBOT and decrease to control levels 3 days after the last HBOT [4].

Leukocytes, by the stimulation of chemoattractans, are the first to migrate to the damaged tissue area. Should an infection not occur, they will remain within the wound for 3 days, leaving their place to macrophages. Leukocytes together with macrophages and the complement system form the nonspecific defense system against microorganisms. This is by far the most effective defense mechanism in infected wounds. Leukocytes use two systems for the killing of these microorganisms: the nonoxygen dependent- and oxygen-dependent pathways. The nonoxygen-dependent system consists of enzymes within lysosomes. The phagosomes that engulf microorganisms unite with the antibacterial enzyme-laden lysosome. Although very effective, this system is not enough for an efficient killing of microorganisms. Infection is much more prominent in hypoxic wounds because of an impairment of the oxygen-dependent defense system [27]. The bacterial killing via oxygen is effectuated by an enzyme called phagosomal oxidase located at the membrane of the phagosomes. This enzyme uses oxygen as a substrate to generate high amounts of oxidants to kill microorganisms. The activity of the phagosomal oxidase enzyme is correlated with oxygen tension. In case of a pO_2 between 45 and 80 mmHg, the activity of the enzyme is halved; it is significantly decreased in case of a pO_2 under 30 mmHg, while it is 90% of its maximum enzymatic speed at a pO_2 of 300 mmHg [28]. Since the activity of phagosomal oxidase is reduced at low-oxygen tensions, the bacterial killing capacity of leukocytes is impaired in hypoxic wounds, leading to wound infection [28]. HBOT restores the bacterial killing capacity of leukocytes in hypoxic wounds by increasing tissue oxygen tension [29].

Furthermore, HBOT is bactericidal to anaerobic bacteria because it increases the formation of oxygen-free radicals, which are lethal to anaerobic metabolisms [30]. HBOT displays bacteriostatic effectiveness against aerobic and facultative anaerobic bacteria by impairing the protein synthesis and functions of nucleic acids and membrane-transport mechanisms [31,32]. HBOT inhibits α-toxin (oxygen-stable) production and detoxifies the θ-toxin (oxygen-labile) of *Clostridium perfiringens* [33–35].

HBOT also acts synergistically with a number of antibiotics. It potentializes sulfonamide activity in *Pseudomonas* infections [36]. At the same time, HBO therapy prolongs the

Figure 2. Multiplace chamber (inside view).

postantibiotic efficacy of aminoglucosides [37] and increases the efficacy of nitrofurantoin by increasing the production of reactive main products [38]. Experimental studies have shown that HBOT combined with gentamicin and cefazolin acts synergistically, exhibiting a potential additive effect [39,40].

Platelet-derived growth factor (PDGF) is a glycoprotein, which is carried in α-granules of platelets and included in the wound repair process. Recombinant human PDGF is used in the treatment of diabetic foot ulcers. Zhao *et al.* studied the combination of HBOT with growth factors in a rabbit ear ischemic wound model [41]. They found that PDGF and transforming growth factor-β 1 (TGF-β 1) are more effective than HBOT in accelerating impaired wound healing. However, the combination of HBOT with either PDGF or TGF-β 1 act synergistically and completely reverse the wound healing deficit produced by ischemia [41]. In a similar animal model, Bonomo *et al.* demonstrated that HBOT increased the PDGF β-receptor mRNA expression when combined with PDGF treatment [42].

Recent studies have revealed that nitric oxide (NO) is a key mediator of normal wound healing. NO may play a vital role in the several steps of wound healing including angiogenesis, inflammation, cell proliferation, matrix deposition and remodeling [43]. It has been shown that wound NO production is reduced in diabetic patients with chronic foot ulcers. It has been suggested that beneficial effects of HBOT on wound healing might be mediated by the combined effects of hyperoxia and the increased local (wound) production of NO [44]. In a recent study, Boykin and Baylis found that wound nitrate and nitrite levels, the stable oxidation products of NO, are increased after HBOT in patients who have favorably responded to HBOT [45].

HBOT also decreases neutrophil infiltration by inhibiting neutrophil β-2 integrin (CD11/CD18) function [46], as well as the expression of intercellular adhesion molecule-1 on endothelial cells [47] after ischemia-reperfusion injury.

Stem cells are being increasingly studied in the treatment of CLI [48]. After intramuscular injection of bone-marrow-derived stem cells, a significant increase in $TcpO_2$ and pain-free walking time has been reported in patients with CLI [49]. An important study form Thom *et al.* demonstrated that HBOT mobilizes stem/progenitor cells from bone marrow by increasing the bone marrow NO level [50,51]. The same group showed that HBOT augments mobilization of stem/progenitor cells, recruitment to ischemic wounds and hastens ischemic wound healing [51].

Clinical studies

Several studies investigated the effectiveness of HBOT on nonhealing wounds. Most of these studies include patients with diabetes-related lower extremity wounds.

In a nonrandomized, nonblinded, controlled study, Baroni *et al.* assessed the effectiveness of HBOT in 28 diabetic patients with foot lesions [52]. Patients were divided into HBOT (n = 18) and control group (n = 10). Both groups were treated with strict metabolic control and daily wound debridement. In the HBOT group, 16 (88%) patients were relieved and two (11%) had an amputation. While in the control group only one in ten patients (10%) were relieved, with four patients (40%) amputated and five with no relief. They concluded that HBOT as an adjunctive therapy to the standard therapy procedures reduces the amputation rate in diabetic patients.

Urayama *et al.* studied the effectiveness of HBOT on a series of 50 patients with chronic obstructive arterial diseases [53]. Patients complained of intermittent claudication, ischemia and ulcerations. They received 3–40 HBOT sessions. Of the six patients (83%), five suffering from rest pain were relieved. A total of 16 out of 30 patients (53%) with foot ulcerations were relieved, and the $TcpO_2$ at the skin adjacent to the wound increased during HBOT.

Faglia *et al.* studied the effect of HBOT on diabetic foot ulcers in an unblinded, randomized and controlled study [5]. A total of 35 patients were included in the HBOT group and 33 in the control group. Patients from both groups received a standard treatment protocol consisting of glycemic regulation, antibiotherapy, wound debridement, vascular surgery, graft procedure or pressure-relief measures. In the HBOT group, patients received 38.8 ± 8 HBOT sessions. Three patients (8.6%) in the HBOT group and 11 patients (33.3%) in the control group underwent major amputation. The difference between the groups was statistically significant ($p = 0.016$). They suggested HBOT as an effective adjunctive therapy procedure and pointed out that HBOT decreases the rate of major amputation in diabetic patients with severe prevalently ischemic foot ulcers.

In a recent study, Abidia *et al.* studied HBOT for ischemic lower-extremity chronic ulcers in a placebo-controlled study [55]. Patients were randomly assigned either to receive 100% oxygen (treatment group) or air (control group) at 2.4 atm of absolute pressure for 90 min daily (total of 30 treatments). Complete healing was achieved in five out of eight ulcers in the HBOT group compared with one out of eight ulcers in the control group. They postulated that HBOT increased the healing rate of chronic ischemic wounds in diabetic patients, and that HBOT was a significant adjunctive procedure in those cases that had no chance of vascular reconstructive surgery.

Kalani *et al.* investigated the long-term effects of HBOT in a prospective controlled study [56]. A total of 38 diabetic patients with chronic foot ulcers were divided into two HBOT (n = 17) and control groups (n = 21). Patients in the HBOT group received 40–60 sessions of HBOT, while patients in the control group were treated conventionally. A 3-year follow-up demonstrated complete healing in 76% of patients and amputation in 12% of patients in the HBOT group, while complete healing was seen in 48% and amputation in 33% of the control group. The authors concluded that HBOT accelerates the rate of healing, reduces the need for amputation and increases the number of wounds that are completely healed on long-term follow-up.

Patient selection for HBOT

Before adding HBOT as an adjunct to the standard treatments for nonhealing wounds, the chance for an arterial reconstruction should be evaluated in patients with critical limb ischemia. Suitable patients should undergo surgical by-pass or endovascular procedures. However, it should be noted that even after successful revascularization, tissue hypoxia can persist and wounds can take months to heal. HBOT should be initiated in cases with no chance for surgery or in cases with no relief after surgical by-pass or endovascular procedures.

Another point is that cutaneous oxygenation continues to improve for up to 1 month after revascularization. When the surgical approach can be delayed, the best timing to perform a more aggressive debridement or minor amputations is 3–4 weeks after successful revascularization [57].

Measurement of $TcpO_2$ is a simple, noninvasive reliable, diagnostic procedure assessing the oxygenation and the perfusion of the soft tissue surrounding the wound in critically ischemic leg lesions. $TcpO_2$ is measured while the patient is breathing room air. This measurement helps the physician in assessing the healing potential of a wound, in determining the exact level of amputation and in evaluating the rate of success of a skin graft [58,59]. Smart *et al.* reviewed the studies evaluating the role of $TcpO_2$ in problem wounds [60]. If the $TcpO_2$ around the wound is higher than 40 mmHg, this will be enough for a good healing. It has been suggested that a $TcpO_2$

Figure 3. Multiplace chamber (outside view).

of 50 mmHg or less is more suitable for patients with diabetes [60]. Under 20 mmHg is said to have a bad prognosis [9]. HBOT is not considered in patients with a $TcpO_2$ of more than 40 mmHg in the absence of infection [61].

$TcpO_2$ measurements can also be obtained while the patient is breathing 100% oxygen at 1 ATA or at 2.5 ATA inside the hyperbaric chamber. These measurements guide the physician to predict which patient will benefit from HBOT and to evaluate the response to HBOT [58,60,61]. A critical value of $TcpO_2$ to select patients for HBOT has not been determined. Grolman *et al.* suggested that patients with an increase of $TcpO_2$ of more than 10 mmHg with breathing 100% oxygen at 1 ATA may benefit from HBOT [62]. They did not evaluate in-chamber $TcpO_2$ in their study. Smith *et al.* measured $TcpO_2$ during breathing air, oxygen and HBOT at 2.4 ATA in patients who will receive HBOT. They concluded that if in-chamber $TcpO_2$ is higher than 800 mmHg, the patient is likely to respond to HBOT [63]. In-chamber TcpO2 has proved the most accurate single factor in predicting the outcome of HBOT [64,65]. Fife *et al.* suggested that if the $TcpO_2$ at 2.5 ATA with breathing oxygen is higher than 200 mmHg, HBOT is said to be beneficial, but if lower than 100 mmHg it is very rare for a patient to benefit from HBOT [64,65]. Patients with an in-chamber $TcpO_2$ between 100 and 200 mmHg should be evaluated case-by-case and HBOT can be employed when the patient has no chance for vascular surgery and amputation is the only alternative [60]. We have presented an algorithm of the use of HBOT in nonhealing wounds of patients with CLI in **Figure 1**.

Treatment protocol

There are two types of hyperbaric chamber: monoplace and multiplace (walk-in). In the monoplace chamber, a single patient is treated and internal pressure is raised with oxygen. Multiplace chambers accommodate two or more patients at the same time and permit medical staff to accompany patients to care for and assist them during treatment (**Figures 2 & 3**). In multiplace chambers, pressure is raised with compressed air and patients breathe oxygen through masks.

The therapy protocols differ among HBOT centers. HBOT is usually administered at 2–3 ATA, and the duration of treatment varies from 60 to 300 min. The frequency and duration of HBOT and the pressure at which treatments are administered are determined by the disease

and patient's condition. In general, patients are exposed to 100% of oxygen at 2.4 or 2.5 ATA for a total of 100 min at depth, with 5 min air breaks interspersed between 30 min oxygen periods for nonhealing wounds. However, some centers treat nonhealing wounds for 90 min at 2.4 ATA with no air breaks. However, this could be modified regarding the status of the wound. If the infection is severe, the HBOT could be started twice a day and continued once a day after a relief in the infection. After the restoration of a good capillary bed, reconstructive procedures such as skin grafts, flaps and other therapeutic surgical alternatives should be considered in order to increase the healing rate of the wound.

HBOT should be instituted as an adjunctive therapy to the standard therapy modality. Antibiotherapy, metabolic control of the patient, daily wound care and debridement should not be overlooked during HBOT. The total number of treatments is determined according to the patient's response to HBOT. For chronic wounds, 30–50 sessions is usually enough. If no progress is observed after 15 or 20 sessions, HBOT should be stopped.

HBOT is a reliable treatment method. Most of the side effects observed during treatment are slight and reversible, but they may occasionally be very severe [66]. The most frequently observed side effect is middle ear barotraumas [66]. The most undesirable side effect of HBOT is oxygen toxicity. The intermittent inhalation of oxygen with air breaks and use of 2.0 ATA reduces the incidence of oxygen toxicity. Reversible myopia arises due to toxic effects on the lens and resolves within weeks after the completion of treatment [67]. The symptoms of CNS oxygen toxicity include twitching, auditory hallucinations, disorientation and seizures. The incidence of seizures is reported as 1/40,000 treatments [68]. They are self-limiting and do not cause permanent damage. Pulmonary toxicity-induced coughing, tightening of the chest and temporary impairment in pulmonary functions may be observed [69].

Expert commentary & outlook

HBOT is widely used in the treatment of nonhealing wounds. Basic research explored the possibility that intermittent hyperoxia caused by HBOT in ischemic wounds regulates local tissue oxygenation and cellular energy metabolisms, increases the killing capacity of leukocytes, collagen synthesis and maturation, angiogenesis and fibroblast proliferation. However, the effectiveness of HBOT in the management of chronic wounds is still debated because of the lack of the high-quality clinical studies. A recent systematic review suggests a potential role for HBOT as an adjunct in the management of chronic wounds in diabetic patients [70].

Treatment of chronic wounds in patients with CLI requires a multidisciplinary approach. HBOT should be used as an adjunctive therapy to the standard therapy modality. HBOT is not a miraculous treatment modality. It is a good adjunctive therapy that increases the healing rate of wounds in selected patients. Patients that are observed not to benefit from HBOT should not continue the therapy, while patients that would benefit from HBOT in regards of lowering the level of amputation or even preventing amputation should not be deprived of this therapy.

Executive summary

Effects of hyperbaric oxygen therapy on impaired wound healing

- Hyperbaric oxygen therapy (HBOT) in ischemic wounds regulates local tissue oxygenation and cellular energy metabolisms, increases the killing capacity of leukocytes, collagen synthesis and maturation, angiogenesis and fibroblast proliferation, and mobilizes stem cells from bone marrow.

Patient selection for hyperbaric oxygen therapy

- If possible, revascularization should be considered before initiation of HBOT in patients with ischemic wounds.
- HBOT should be initiated in cases with no chance for vascular surgery or in cases with no relief after successful surgical by-pass or endovascular procedures.
- Transcutaneous oxymetry is an important test to predict which patient will benefit from HBOT.

Conclusions

- HBOT is a good adjunctive therapy that increases the healing rate of wounds in selected patients.
- HBOT is not used in the treatment of intermittent claudication in patients with critical limb ischemia.
- HBOT should be stopped in patients with no progress after 15–20 sessions.

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