Preconditioning with Hyperbaric Oxygen in Pancreatico-duodenectomy: a Randomized Double-blind Pilot Study

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Abstract. *In a prospective randomized double-blind study, we evaluated the post-operative biological and clinical effects of a single preoperative hyperbaric-treatment the day before surgery for pancreatic ductal adenocarcinoma. Patients and Methods: Twenty one patients were randomized and divided into two groups: group-A (10 patients, 48%) were exposed to a HyperBaric Oxygen (HBO) session the day before intervention [Pre-Intervention Day (PID)], group-B (11 patients, 52%) breathed air for 40 min in a hyperbaric chamber pressurized to 1.15 ATA (placebo group). For all patients blood samples were obtained before HBO treatment or the placebo procedure (T0); at the end of HBO session or placebo procedure (T1); on the first post-operative day (POD)(T2) and on seventh POD(T3) day, measuring interleukin (IL)-1, IL-6, IL-8, IL-10, IL-12 and TNF-α, recording postoperative pancreatic fistula (POPF), biliary-fistula, fever, intra-abdominal abscess, bleeding, pulmonary complications, delayed gastric emptying and requirement for post-operative antibiotics. The results of the present pilot study suggest that a single preoperative hyperbaric oxygen treatment on the day before surgery may reduce the complication rate in pancreatic resection.*

Hyperbaric oxygen (HBO) therapy involves the intermittent inhalation of 100% oxygen in chambers pressurized between 1.5 and 3.0 atmosphere absolute (ATA) (3). HBO increases both dissolved oxygen and the partial pressure of oxygen in plasma (4, 22). HBO is commonly used in the treatment of decompression sickness, carbon monoxide intoxication, arterial gas embolism, necrotizing soft tissue infections, chronic skin ulcers, severe multiple trauma with ischemia and ischemic diabetic foot ulcers (3-14). A possible mechanism of HBO mediating beneficial effects has been described as attenuation of the production of pro-inflammatory cytokines in response to an inflammatory stimulus such as surgery (15, 38) and modulation of the immune response (3, 5). Previous data by Yang and colleagues on animals demonstrated that HBO inhibits TNF-α production during intestinal ischemia-reperfusion (30) and it has a beneficial effect, mediated by decreased production of both TNF-α and IL-1β, on indomethacin-induced enteropathy (31). The positive role of HBO in human surgery has been demonstrated in cardiovascular (16, 17), orthopedic surgery (18), and after liver transplantation, as reported by Franchello *et al*. which documented a reduction of ischemic areas and an increase of intrahepatic arterial vascularisation by collateral vessels after 20 HBO sessions in a patient affected by Hepatic Artery Thrombosis (HAT) after liver transplantation (19). Pancreatico-duodenectomy (PD) is associated with a significant complication rate ranging between 30%-60% (20), often with prolonged hospital stay and economic resource utilization (21).

The main objective of the present pilot study on patients receiving PD was to identify differences between the concentration of inflammatory cytokines in two groups of patients exposed to HBO or sham prior to PD procedure. A secondary objective was assessment and comparison of the complication rate and duration of hospital stay between the two groups.

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Patients and Methods

Ethics approval was obtained from local regional ethics committee of the University of Verona, Italy (n. 2176/2012). The study was a prospective, randomized double-blind study lasting 6 months. Patients were randomized at the Hyperbaric Institute SpA of Villafranca (Verona, Italy) by gaining exposure to HBO treatment, using a computer-generated allocation schedule and were divided into two groups. Twenty-four hours before the planned PD, patients of group "A" were submitted to a HBO session, while Patients of group "B" breathed air in a multiplace hyperbaric chamber pressurized to 1.15 ATA (sham).

All patients received medical evaluation to exclude absolute contraindications to hyperbaric therapy, including electrocardiography, thoracic X-Ray and the count of white cells, red cells, haemoglobin and hematocrit. Patients aged >80 years or <18 years, or affected by cavitary or current tuberculosis, bronchiectasis, lung abscess, previous spontaneous pneumothorax or traumatic current not already drained, heart failure, pulmonary edema, acute purulent sinusitis, severe glaucoma, hypercapnic respiratory insufficiency with pCO_2 >60 mmHg, previous rupture of inner ear's round or oval membrane, sinus bradycardia, inflammation of the upper respiratory tract, parenchymal or mechanical bronchoconstrictions or epilepsy, were excluded from the study.

The HBO session was a single hyperbaric exposure at 2.5 ATA for a duration of 116 min: 12-15 min of compression time, 3 periods of 24 min each on 100% O_2 at maximum pressure, interrupted by two air breathing periods of 5 min each. Oxygen was delivered to patients *via* a tight-fitting mask. Decompression to 1 ATA occurred over 18 min, with 3 min at 1.3 ATA. Oxygen was administered starting at 1.6 ATA and during decompression from 2.5 to 1.3 ATA. During the entire exposure time an experienced hyperbaric-trained physician was inside the hyperbaric chamber as required by the practice quality provisions of the Hyperbaric Institute.

In all patients, small venous blood samples were obtained before (T0) and at the end (T1) of HBO session or placebo procedure; at the first post-operative day (POD) (T2) and in the seventh POD (T3). Venous blood samples were collected in heparinized tubes and the plasma obtained stored at –80˚C. The BDTM CBA Human Inflammatory Cytokines kit number 551811 (Becton, Dickinson and Company BD Biosciences San Jose, CA, USA) was used to measure interleukin (IL)-1, IL-6, IL-8, IL-10, IL-12p70 and TNF-α. The FCAP ArrayTM software (Becton, Dickinson and Company BD Biosciences San Jose, CA 95131) was used to generate results in graphical and tabular format.

The following postoperative complications were recorded: post-operative pancreatic fistula (POPF), biliary fistula, fever, intra-abdominal fluid collections, defined as the presence of fluid collection of 5 cm in diameter with or without clinical significance at CT scan or ultrasound presence, bleeding, pulmonary complications, including pneumonia, bronchospasm, respiratory failure and prolonged mechanical ventilation delayed gastric emptying, defined as the need of nasogastric tube decompression for 10 days and the use of postoperative antibiotics. For the evaluation of the complications we followed carefully the definition proposed by Bassi *et al*. (23) and we classified the POPF using the definition proposed by ISGPF (24). Pulmonary complications were diagnosed from presence of three of the following: fever, purulent sputum, tachycardia, tachypnea, inspiratory crackles, bronchial breathing, abnormal chest x-ray, arterial hypoxemia, positive stain and culture, as described by Bassi *et al*. (62).

For statistical analysis, the Chi-squared test after Yates and Fisher tests, when necessary, were used. All statistical tests were two-sided. A *p*-value less than 0.05 indicated statistical significance. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software version 19.0 (SPSS, Chicago, IL).

Results

From 01-02-2012 to 31-07-2012 in our Department 127 patients underwent pancreatic resection (81 [63.8%] pylorus preserving pancreatico-duodenectomy [PPPD], 9 [7.1%] Whipple procedures, 28 [22%] distal pancreatic resections, 7 [5.5%] intermedial pancreatic resections and 2 [1.6%] total pancreatic resections). Out of these patients, 32 were recruited to the present study (25.2%) using "geographic criteria". In fact, all patients recruited in the study lived near the Hyperbaric Institute (maximum 150 km) in order to carry-out the tests and the bureaucracy needed for the HBO therapy and preconditioning. Out of these patients, 5 (15.6%) presented a "borderline" neoplasia when the tumor involved the celiac axis or the superior mesenteric artery (unresectable primary tumor) at CT scan (Stage III sec. AJCC) and they were treated with thermoablation, while other 6 (18.8%) presented liver metastasis (Stage IV sec. AJCC). The remaining 21 (65.6%) patients presented with resectable pancreatic cancer. From these last patients, 11 were randomized to the placebo group (52.4%) and the other 10 (47.6%) to the HBO group. All patients recruited in this study had a resectable lesion and underwent PPPD. The flow chart of the randomization is described in Figure 1.

The results of serum concentrations of IL-1, IL-6, IL-8, IL-10, TNF-α, IL-12p70 at T0, T1, T2 and T3 are listed below (Table I). The maximum concentration of cytokines was at T2. Level of IL-6 was significantly lower in "A" group, while IL-10 level was significantly higher after HBO exposure.

Table I shows the correlation between the age distribution in placebo group and HBO group, the tumor histology, the incidence of POPF and biliary fistula, the incidence of fever, postoperative use of antiobiotics, pulmonary complications, delayed gastric empty (DGE) and bleeding by group. In the group of 21 patients that underwent surgical procedure, 14 (66.7%) underwent PPPD for pancreatic ductal adenocarcinoma and 7 (33.3%) for benign neoplasms (3 main-duct intraductal papillary mucinous neoplasms [IPMNs], 2 mucinous cystic neoplasms [MCNs], 1 symptomatic serous cystadenoma [SCA] and 1 chronic pancreatitis).

The incidence of DGE (19%) was not statistically different between the 2 groups: 3 in HBO group and 1 in placebo group. There were 5 instances (23.8%) of postoperative bleeding (3 in HBO group and 2 in placebo group, PNS). One patient (placebo group) required laparotomy 2 h after the initial surgery for common hepatic artery repair. In the randomized patients, there were 6 POPF

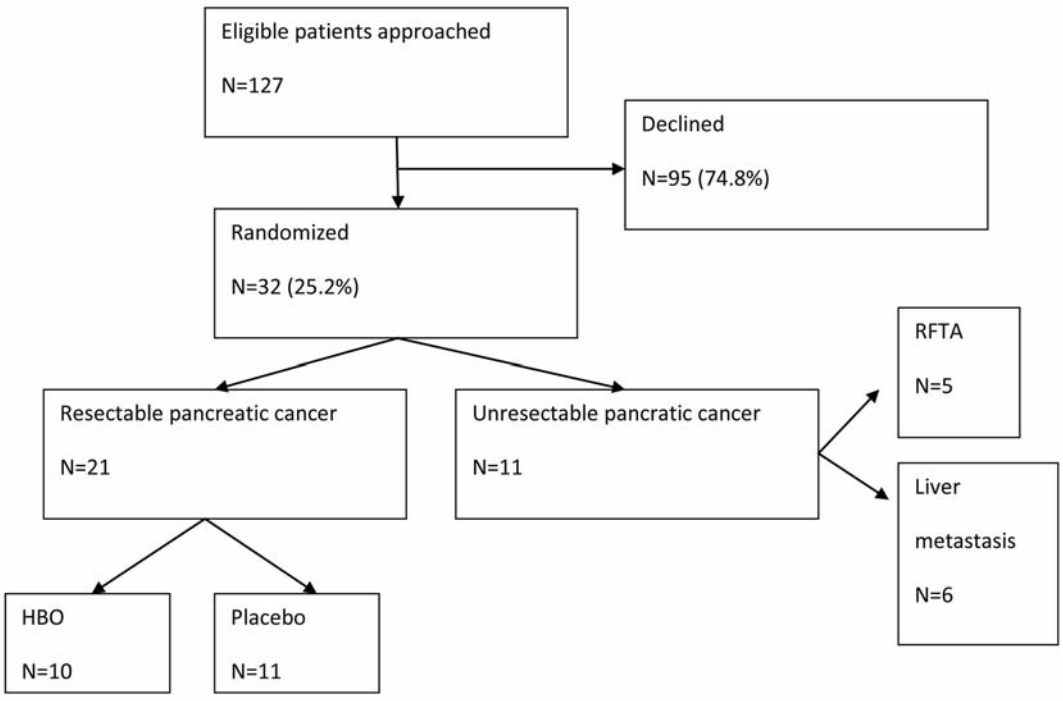

Figure 1. *Flow-chart for randomization*.

(28.6%) (2 type A, 2 type B and 2 type C sec. ISGPF), 3 in the HBO group, 3 in the placebo group. There was one biliary fistula (4.8%) in a patient of the placebo group. Significant differences in favor of HBO were found in pulmonary complications (none in HBO group *versus* 6 in placebo group; $p=0.023$).

In the patients that underwent PPPD, 6 pulmonary infections were present, significantly less frequent in the HBO group *vs*. placebo group ($p=0.023$), but were not correlated with gender, histology, POPF, biliary fistula, fever, use of antibiotics or age (Table II). There were 3 pulmonary infections in males (50%) and 3 in females (50%). In 2 cases (33.3%) pulmonary infections occurred in patients >65 years. In 2 cases (33.3%), POPF and pulmonary infection were present in the same patient and in 1 case (16.7%) the pulmonary infection was present with biliary fistula. Use of antibiotics was not significantly different between groups (4 in HBO group and 6 in placebo group, $p=0.67$) and the incidence of fever was the same in the two groups (4 in HBO group and 4 in sham group, $p=1$).

Table III shows the correlation between gender, POPF, biliary fistula, HBO, fever, DGE and the concentrations of cytokines. IL-1 and TNF-α were correlated with fever ($p=0.006$ and $p=0.04$ respectively). IL-6 was correlated with biliary fistula and its concentration was affected by HBO exposure, as was IL-10 ($p=0.035$). TNF-α was also correlated with POPF ($p=0.026$). On the other hand, IL-8 and IL-12 were not influenced by HBO exposure ($p=0.065$ and $p=0.69$ respectively).

The basal mean level of cytokines in patients with unresectable pancreatic cancer was statistically different from patients with resectable pancreatic cancer, especially for IL-6 and IL-8 ($p=0.0001$ and $p=0.046$ respectively).

Discussion

In the present study, we evaluated post-operative biological and clinical effects of preoperative HBO in patients undergoing pancreaticoduodenectomy. There were no complications from the hyperbaric exposure in all patients.

Pre-oxygenation studies. As already known, modulation of the inflammatory response resulting from exposure to oxygen in a hyperbaric chamber modifies the synthesis of growth factors (VEGF) (1), reduces the concentration of pro-inflammatory cytokines, and modulates tissue myeloperoxidase and lipoperoxides (2).

During the reperfusion of ischemic tissue, oxygenated blood increases number and activities of oxidants generated in tissues. Reperfusion increases the hazardous effects of early ischemic injury by release of cytokines and reactive oxygen species (ROS) such as hydroxyl radical (OH^-),

Table I. *Wilcoxon matched pairs test between HBO and placebo conditions for IL-1, IL-6, IL-8, IL-10, IL-12, TNF measure at T0, T1, T2, T3.*

	IL-1 (HBO)	IL-1 (placebo)	*p*-Value	IL-6 (HBO)	IL-6 (placebo)	*p*-Value	IL-8 (HBO)	IL-8 (placebo)	*p*-Value
T0	0.41	0.47	0.196	5.06	5.97	0.009	15.95	15.77	0.71
T1	0.51	0.74		5.81	24.06		22.6	25.14	
T2	1.4	1.35		141.23	179.54		38.05	37.54	
T3	1.04	1.69		21.76	24.5		16.88	16.62	
	IL-10 (HBO)	IL-10 (placebo)	*p*-Value	IL-12 (HBO)	IL-12 (placebo)	*p*-Value	TNF (HBO)	TNF (placebo)	*p*-Value
T0	2.01	4.04	0.034	0.46	0.41	0.8	0.38	0.4	0.18
T1	2.41	4.43		0.65	2.41		1	1.3	
T2	5.6	7.76		0.59	0.11		0.73	0.61	
T3	3.34	4.14		0.67	0.6		1.04	1.1	

superoxide radical (O_2^-), and hydrogen peroxide (H_2O_2) and by the activation of complement system. This phenomenon has been broadly named "ischemia-reperfusion (I/R) injury". Neural tissues as well as most visceral tissues can be all involved in I/R, and animal models of different organs' tolerance and vulnerability have been widely studied (39).

A critical role in ischemia-reperfusion injury is played by TNF-α and IL-1β, as shown by Yang and Bosco (30-31) the increased of TNF-α serum levels, occur in the early phase of inflammation, whereas elevation of IL-1β levels occur in the later phase, suggesting that this cytokine could sustain the inflammation process. The same authors showed how an ischemia-reperfusion injury could strike multiple organs: increased TNF-α serum levels, sequel intestinal ischemia-reperfusion injury, is responsible of acute lung injury (31). In fact, TNF-α goes into the systemic circulation and is received by resident population of macrophages in liver and lungs, promoting the inflammation process also in other organs (33).

There exist many studies investigating I/R injury in different tissues, such as kidney, liver, lung, testis, brain, heart muscle, and skeletal muscle in the literature (34). I/R injury in skeletal muscles (32) and also in bone may also occur with vascular problems, including atherosclerotic occlusive disease, arterial thrombosis, or arterial embolism, after organ transplantation, cardiovascular surgery, and vascular trauma (35). In addition, limb ischemia-reperfusion occurs often in surgical procedures, when using a tourniquet to provide a bloodless surgical field (32, 35). Although hyperbaric oxygen (HBO) causes oxidative stress (36, 37) similar to ischemic-preconditioning (PC), HBO-PC generates a non-lethal level of ROS, which induces ischemic tolerance and has protective effect after severe ischemic attacks (37, 38). Certain studies have shown that HBO-PC reduces I/R damage by its antioxidant effect, hyperoxygenation, and vasoconstriction. Furthermore, HBO is believed to exert its antioxidant effect by reducing leucocyte recruitment and activation, edema, cellular necrosis, and increasing the efficacy of antioxidant enzymes (32, 36, 37).

Despite improvements in surgical techniques and in anesthetic management, ischemia-reperfusion injury remains an inevitable event of cardiac surgery, resulting in significant postoperative complications and multiple-organ dysfunction. To date, brain injury after cardio-pulmonary bypass (CPB) for cardiac surgery has been well-documented. Sequelae can be as mild as postoperative cognitive dysfunction and postoperative delirium and as severe as stroke (40). The etiology of cerebral injuries is probably multi-factorial, from an interaction among cerebral microemboli, global cerebral hypoperfusion, inflammation, cerebral temperature modulation and genetic susceptibility (41). Ischemia-reperfusion injury during CPB also leads to myocardial stunning, necrosis, or apoptosis that manifest clinically either acutely as low cardiac output or chronically as heart failure (42).

Preconditioning is the application of an intervention to activate endogenous protective mechanisms to reduce the morphological and functional sequelae of a subsequent ischemia insult. The phenomenon of ischemic preconditioning was first described in a canine myocardium ischemia-reperfusion injury model (43) and subsequently was shown in the brain (44). Since then, intense research in the field of pharmacology ensued to identify agents such as volatile anesthetic agents and ischemic preconditioning (45-47) that could duplicate the protective effects of preconditioning for cardiac surgery.

Despite the increasing number of basic science publications on this issue, studies describing HBO preconditioning in the clinical practice of general surgery remain scarce. To date, only a few studies have investigated the preconditioning effects of HBO in the human brain and myocardium (48). In 2004 Sharifi *et al*. described the use of HBO to inhibit restenosis after PTCI in acute myocardial

Table II. *Correlation between age, histology, POPF, biliary fistula, fever, postoperative antibiotics use, pulmonary complications, DGE, bleeding and HBO exposition.*

	HBO (N=10)	Placebo (N=11)	*p*-Value
Age (HBO - placebo)			
M (5-4)	62.8±11.1	62.6±12.6	n.s.
F (5-7)	69±3.3	72.2±4.2	
Histology			
Benign	3	4	n.s.
Cancer	7	7	
POPF			
No	7	8	n.s.
Yes	3	3	
Biliary fisula			
No	10	10	n.s.
Yes	0	1	
Fever			
No	6	7	n.s.
Yes	4	4	
Antibiotics			
No	6	5	n.s.
Yes	4	6	
Pulmonary complications			
No	10	5	0.023
Yes	0	6	
DGE			
No	7	10	n.s.
Yes	3	1	
Bleeding			
No	7	9	n.s.
Yes	3	2	

ns: Not significant.

Table III. *Correlation between gender, POPF, biliary fistula, HBO, fever, DGE and the concentrations of cytokines.*

	Gender	POPF	Biliary fistula	HBO	Fever	DGE
IL-1	n.s.	n.s.	n.s.	n.s.	0.006	n.s.
IL-6	n.s.	n.s.	0.04	0.009	n.s.	n.s.
IL-8	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
IL-10	n.s.	n.s.	n.s.	0.03	n.s.	n.s.
IL-12	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
TNF-α	n.s.	0.019	n.s.	n.s.	0.04	n.s.

ns: Not significant.

infarction (49). In 2005, Alex *et al*. observed that repetitive pre-treatment with three sessions of HBO at 2.4 ATA before on-pump coronary artery bypass graft (CABG) surgery reduced neuropsychometric dysfunction and modulated favorably the inflammatory response after CPB (16). Yogaratnam *et al*. reported that preconditioning with a single session of HBO at 2.5 ATA before on-pump CABG surgery improved left ventricular stroke work post-CABG surgery while reducing intraoperative blood loss, intensive care unit (ICU) length of stay, and postoperative complications (17).

Recently, Li *et al*. aimed to determine whether HBO preconditioning could decrease the release of cerebral and myocardial biochemical markers. Endpoints of this study included serum troponin I, inotrope usage, ventilator hours, length of ICU stay, postoperative length of hospital stay, hemodynamic parameters, and serum CAT activity (37).

In conclusion, in this randomized pilot study for 32 patients selected using geographical criteria affected by pancreatic cancer, several novel findings came to light, as for the first time we evaluated the biological and clinical effects of HBO in patients undergoing pancreatic surgery.

Biological effects in our patients. The preoperative hyperbaric therapy modulates the levels of IL-6 (p=0.009) and IL-10 (p=0.03). It also seems that there is a statistically significant relationship between IL-6 and biliary fistula (p=0.009). IL-1 (p=0.006) and TNF-α (p=0.04) are correlated with the hyperpyrexia and, in addition, TNF-α should be in relation with the POPF (p=0.019). The relationship of IL-1 with hyperbaric therapy has been demonstrated only in orthopedic surgery (18) and in perianal Crohn's disease treatment by Weisz *et al*. (26).

In the present study we found a relationship between HBO exposure and the modulation of the level of IL-6 and IL-10 with a relationship between IL-6 and biliary fistula, IL-1 and fever and a doubtful correlation between TNF- α and POPF. Preoperative HBO action significantly decreased serum concentration of IL-6 (p=0.009). In addition, we observed a correlation between the occurrence of biliary fistula and serum level of IL-6 (p=0.04).

Patients with unresectable tumors with metastasis (Stage IV sec. AJCC) or with infiltration of adjacent structures (stage III sec. AJCC) had a higher level of interleukins than resectable patients. This difference is very marked as regards the IL-6 (p=0.0001, resectable *vs*. unresectable) and conceivably could play a role in determining tumor staging. This relationship was already suggested by Okisu *et al*. (27) that also observed that the interaction with androgens receptors' and the cytokine concentration were in relation with mortality. Talar-Wojnarowska *et al*. demonstrated that elevated levels of IL-6 could be related to a high metastatic potential through the stimulation of growth factors, including VEGF (28). Pini *et al*. confirmed it in mice pretreated with anti-IL-6 antibodies that it participates in delayed recovery from acute inflammation and may favor development of a pro-tumorigenic environment through prolonged activation of STAT-3, induction of MMP-7 and sustained production of chemokines (50). Boreddy *et al*. demonstrated that IL-6 drastically increased the expression of HIF-1α (hypoxia inducible factor) and VEGF expression that play a crucial role in pancreatic cancer progression (51).

On the other hand, in a recent article, Grote *et al*. demonstrated that IL-6 does not seem to have a role with respect to risk of pancreatic cancer (52).

IL-10 is an anti-inflammatory cytokine with protective activity, its post-operative serum concentration results are higher in patients who underwent HBO (p=0.03), such as literature showed (29). In 2011, Ribatti *et al*. (53) demonstrated that through the secretion of immunosuppressive cytokines such as IL-10, mast cells down-regulate the immune response to tumors. Mast cells, in fact, produce and secrete potent angiogenic molecules, and have been implicated in angiogenesis in various malignancies, including laryngeal squamous cell carcinomas, lung cancers and malignant melanomas (54).

The levels of IL-8, IL-10 and TNF-α were not modulated by HBO.

Clinical effects in our patients. We found a relationship between the different clinical variables and cytokines such as the correlation between fever and IL-1 and TNF-α and the pancreatic fistula with only TNF-α. Patients who underwent a hyperbaric therapy session expressed a reduction in postoperative pulmonary infections (p=0.023 *vs*. placebo) but this effect was not associated with a statistically significant decrease in the length of hospital stay (p=0.063), fever (p=0.146) or use of antibiotics (p=0.063). The occurrence of POPF can't be explained only by an altered inflammatory state (23-25) so the modulation of the inflammatory response has only a marginal role (p=1). In fact, the risk of POPF formation has been shown to be directly proportional to the consistency of the residual organ by virtually all authors (55-63) and is further supported by the fact that anastomosis performed on chronic pancreatitis patients with fibrotic glands has a significantly reduced clinical incidence of complications related to anastomosis (64). In conclusion, the preoperative exposure to hyperbaric environment is safe and can be applied to all patients after careful clinical evaluation and identification of absolute contraindications as per chemotherapic protocol (65).

IL-6 expression in these cases is usually increased and particularly related with the biliary fistula, but shows itself to be sharply influenced under hyperbaric oxygen exposure conditions. This may suggest a potential HBO in decreasing the incidence of postoperative biliary fistulas and pneumonia. Moreover this cytokine may be useful as a prognostic marker or indicator of disease progression. Maybe it is possible that high basal levels of IL-6 may be related to the presence of a tumor in advanced stage and therefore the patient is candidabile to neoadjuvant treatment.

In conclusion, in this pilot randomized study we found that a single preoperative HBO session the day before pancreatic surgery should modulate the inflammatory response, especially for IL-6 and IL-10 with a decrease in postoperative pneumonia. Further studies are required to evaluate the response to HBO in a larger number of patients and in other surgical procedures, especially major surgeries leading to postoperative ICU admission.

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