ORIGINAL ARTICLE

Predictive value of exhaled nitric oxide and aerobic capacity for sepsis complications after liver transplantation

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SUMMARY

Our objective was to investigate the predictive value of fractional nitric oxide (NO) concentration in exhaled breath (FeNO) and aerobic capacity (peak VO_2) for postoperative sepsis in liver transplantation candidates. Patients were identified and charts of all consecutive patients were prospectively reviewed. Bacterial sepsis represented the commonest postoperative complications (30%), which was attributed to peritonitis, pneumonia, and catheter-related infections. Preoperative FeNO and peak VO_2 values were lower in patients with postoperative sepsis. Patients with sepsis required higher needs for mechanical ventilation and ICU length of stay. Inverse correlation was found between logarithmically FeNO-transformed data and systolic pulmonary artery pressure ($r = -0.348$; $P = 0.018$). Multivariate analyses using bootstrap sampling method indicated that odds of sepsis were associated with lower values of peak exercise VO_2 [OR = 0.790 (0.592; 0.925)] and reduced log(FeNo) [OR = 0.027 (0.001; 0.451)], but not with higher MELD scores [OR = 1.141 (0.970; 1.486)]. By evaluating the cutoff for the ROC curves in each bootstrap resampling, median and 95% confidence interval were calculated for peak VO_2: 17 [16.2; 22] ml/kg/min and FeNO: 17.2 [13.0; 33.9] ppb. We conclude that low peak exercise VO_2 and reduced FeNO may help identify patients who are at risk to develop perioperative sepsis.

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Key words
aerobic capacity (VO_{2max}), exhaled nitric oxide, liver transplantation, sepsis

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Introduction

Orthotopic liver transplantation (LT) has become the treatment of choice for patients with end-stage liver cirrhosis and hepatocellular carcinoma [1,2]. Conditions associated with complications after LT include recipients' poor clinical status, extensive surgery field, and lengthy operative times [3]. Despite improved LT

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perioperative management, sepsis remains a major cause of morbidity and mortality during postoperative care [4–6]. Currently, risk of perioperative adverse events in LT candidates is identified by medical history survey and preoperative evaluation of biological and functional conditions [4–6]. Information regarding identification of specific risk factors for postoperative sepsis is limited [7,8].

Evaluation of aerobic capacity, as objectively measured by peak exercise oxygen uptake (peak VO_2), has emerged as a noninvasive tool providing valuable prognostic information in patients underdoing major surgery. In this context, we and other groups [9–12] have proposed that impaired aerobic capacity is associated with poor outcome in LT candidates. Measurement of fractional nitric oxide (NO) concentration in exhaled breath (FeNO) is proposed as a valuable marker of lung diseases accompanied by airway inflammation and bronchial hyper-responsiveness. In line, elevated FeNO levels have been associated with acute pulmonary complications after abdominal surgery [13]. In a different context, normalization of low FeNO levels by vasodilator treatment has been associated with better survival in patients with pulmonary arterial pressure [14]. Despite the critical role of NO signaling in cardiopulmonary dysfunction in patients with end-stage liver cirrhosis [1], prognostic value of FeNO has not been previously studied in LT candidates.

The aim of this study was to test whether reduced NO lung production and low levels of aerobic capacity (peak VO_2) would prognosticate postoperative sepsis after LT in intensive care unit. Hence, we recorded underlying clinical and functional characteristics of LT candidates and we further investigated the significance of reduced FeNO levels and peak VO_2 in predicting early onset postoperative sepsis after LT. Overall, we demonstrate that reduced FeNO levels and low peak VO_2 may improve identification of LT candidates at increased risk of developing postoperative sepsis after LT.

Patients and methods

This study was conducted in accordance with the Declaration of Helsinki and approved by the appropriate the local ethics authority. A local institutional review board approved the protocol (CHRU Lille Pole Imagerie Explorations Fonctionnelles Ethics Committee – approval number UF 5040; 2010-20). Written informed consents for the procedure and for their medical data to be used in a study were obtained from all subjects, a legal surrogate, or the parents or legal guardians for minor subjects, or that the requirement for written informed consent was waived by the ethics committee. Patients were prospectively included if the local committee, directed by surgeons, anesthesiologist and hepatologists, considered them potentially suitable for LT. All consecutive adult cirrhotic patients transplanted were included. We excluded patients transplanted with national priority in an emergency setting (acute liver failure/fulminant hepatitis without underlying cirrhosis) and those who underwent multi-organ transplantation or liver retransplantation. Patients were not included in this series if they had primary cardiac diseases or moderate-to-severe portopulmonary hypertension. LT candidates were nonactive smokers without a history of atopy and free from bronchial asthma, allergies, pulmonary tuberculosis, or recent respiratory tract infection. From the database of our institution, we prospectively identified and reviewed charts of all consecutive patients who underwent LT at the Lille's University Hospital.

Clinical and cardiopulmonary functional evaluation

Clinical information about comorbidities, medical history, pre-, and post-transplant status of recipients was recorded. Individual patient record included medical conditions, biological parameters, MELD score at LT [model for end-stage liver disease (calculated from serum bilirubin, INR, serum creatinine levels)], simplified acute physiology score at ICU admittance, arterial blood gas analyses, and arterial pressure. Transthoracic echocardiography was systematically performed in the preoperative period according to the American Society of Echocardiography guidelines. Pulmonary artery systolic pressure (PAPs) was first evaluated. If PAPs was elevated, right-side heart catheterization was performed. Patients with pre-existing severe precapillary pulmonary hypertension (mean pulmonary artery pressure >35 mmHg) associated with severe right ventricular dysfunction or high level of pulmonary vascular resistance were excluded from this study. All patients abstained from food and coffee for 4 h prior to body plethysmography lung function tests, which included total lung capacity (TLC), forced vital capacity (FVC), forced expiratory volume in first second (FEV1), FEV1/FVC, and transfer factor for carbon monoxide/alveolar volume ratio (K_{CO}), and were performed with a Lilly type flow transducer (Masterscreen Body; CareFusion; Hochberg, Germany). K_{CO} data were expressed as percent of predicted values (% predicted). Breath-by-breath

expired gases were collected and analyzed online data (Vyntus CPX; CareFusion; Hochberg, Germany) were measured at rest, warm-up, and incremental exercise testing during cycling (VIAsprint™ 150P Bike; CareFusion) to calculate minute ventilation (V_E), oxygen uptake (VO_2), and carbon dioxide output (VCO_2). During exercise test, cardiac function evaluated noninvasively by means of twelve-lead electrocardiography, peak oxygen pulse (VO_2/heart rate), and mean arterial pressure.

Exhaled NO measurement

All patients abstained from food and coffee for 4 h prior to studies were asked to not participate in strenuous activity for 1 h prior to fraction of exhaled NO (FeNO) measurements. FeNO levels were measured with a chemiluminescence NO analyzer (NO analyzer CLD 88 Series Eco Medics AG; Duernten, Switzerland). Measurements were conducted according to the ATS recommendations [15]. Subjects were seated and nose clips were not used. After deep inspiration of NO-free air, NO concentrations were measured during controlled expiration at the first stable FeNO plateau for at least three seconds (FeNO variations <10% or 1 ppb). Reproducible exhalations were performed to obtain at least two NO plateau values within 10% deviation. FeNO was recorded as mean of two FeNO measurements at 50 ml/s expiratory flow. Reproducibility of exhaled NO was evaluated in adult controls with an intraclass correlation coefficients for FeNO measured at 50 ml/s expiratory flow >0.98.

Surgical procedure and intensive care management

Donor variables, including the donor risk index, donors' age, and length of time on the waiting list, were collected at the time of transplantation. Variables, including blood loss, duration of cold ischemia, and operative times, were collected as well. Liver grafts were obtained from deceased donors and preserved using University of Wisconsin solution according to our transplant center protocol. LT was performed under standardized anesthetic protocol using the side-to-side cavo-caval technique. Following initial clinical supervision in the postanesthesia recovery room, patients were transferred to the specialized ICU for end-stage cirrhotic patients. Weaning from the ventilator and extubation were performed within the first 6 h post-LT. After weaning, patients were transferred to a specialized surgical care unit until hospital discharge. All patients received similar postoperative care with a routine immunosuppressive regimen including corticosteroids, mycophenolate mofetil, and tacrolimus or cyclosporine. In cases of renal dysfunction prior to LT, two infusions of basiliximab were performed allowing to delay tacrolimus/cyclosporine introduction to day 5. No fresh frozen plasma was used. All patients received piperacillin for 48 h following local prophylactic protocol.

Postoperative complications

Electronic charts and transplant database include information regarding bleeding, acute rejection, causes of death, cardiovascular, pulmonary, renal and neurological events, hepatic vascular thrombosis, biliary complications, total length of hospital stay in the specialized surgical unit, duration of mechanical ventilation, need for postoperative renal replacement therapy, and infectious complications. In order to limit post-transplant infectious episodes coming from transplanted liver, documented bacterial infection in the donor prior procurement led to LT contraindication. Careful review of the donor's medical and social history and assessment of donor pre-existing or latent infections were routinely performed. Survey of donor-derived infectious disease transmissions was currently monitored at the time of liver procurement, recovering, packaging, and transport by the French Agency of Biomedicine.

Bacterial infections, that is, surgical site and intra-abdominal infections, pneumonia, bacteremia, and catheter-related infections, were recorded. Criteria used for identifying and classifying healthcare-associated infections were those outlined by the US National Healthcare Safety Network. The criteria for sepsis were defined according to European Society of Intensive Care Medicine (ESICM) guidelines. Sepsis was defined as systemic inflammatory response caused by known or suspected infectious. Severe sepsis associated sepsis with acute organ dysfunction including hypotension (systolic blood pressure less than 90 mmHg or a 40 mmHg reduction from baseline pressure). Septic shock associated sepsis with persistent or refractory hypotension or tissue hypoperfusion despite adequate fluid resuscitation and subsequently needs administration of vasopressor agents. Medical staff of the surgical unit, which recorded all pre- and postoperative data, was blinded for preoperative cardiopulmonary functional evaluation including FeNO and peak VO_2.

Statistical analysis

Results are presented as means ± SD standard deviation or median and interquartile range where indicated.

Descriptive statistics with tests of normality (Shapiro–Wilk test) for quantitative variables were used. Two independent groups of data were compared by either unpaired Student's *t*-tests or nonparametric tests depending on whether the data had normal distribution or were skewed. Chi-square (χ^2) tests were employed to compare intergroup distribution of qualitative variables. As FeNO values were not normally distributed, data were log-transformed prior to analysis. Correlation between PAPs and FeNO was performed by calculating Pearson's product–moment coefficient using logarithmically FeNO-transformed data. Logistic regression analysis was performed to analyze association between outcome, perioperative variables, and occurrence of sepsis after LT. Potential predictors for outcome with a P value <0.10 in bivariate analysis were added to the multivariate stepwise logistic regression model using backward variable selection to identify those factors that independently predicted sepsis after liver transplantation. As recommended, we used bootstrap method to determine the strength of the evidence that a given variable truly is an independent predictor of the outcome [16]. So, this process was repeated using the 5000 bootstrap samples. It was then determined the proportion of regression models in which each of the candidate variables was retained. Robustness analysis was performed by calculation of OR and 95% CI using resampling bootstrap based on 5000 iterations. To estimate the cutoff level of FeNO and peak VO_2 for prediction of postoperative sepsis, ROC curve analyses were implemented. The optimal cutoff for the ROC curves was defined as corresponding to the maximum value of Youden's index (sensitivity + specificity − 1). We repeated this process using the 5000 bootstrap samples to evaluate a 95% confidence interval for the cutoff for the ROC curves. Level of statistical significance was set at a P value <0.05. All statistical analyses were performed with SAS 9.4 software (SAS Institute Inc., Cary, NC, USA).

Results

Study Population

Between January 2010 and May 2015, a total of 214 patients underwent LT in our institution. Of these, 84 patients were excluded from the study because of multiorgan transplantation, urgent transplantation for acute liver failure, or urgent retransplantation for primary liver nonfunction. In addition, 52 patients were excluded from the study because they were unable to perform exercise testing and or FeNO evaluation for disability or lack of understanding. Thus, 78 patients receiving grafts from deceased donor donors were enrolled in the present study. The most common underlying diagnosis was alcoholic cirrhosis (87%). LT indication was end-stage liver cirrhosis in 53 patients (68%) and hepatocellular carcinoma in 25 patients (32%). Mean age of the study population was 55 ± 8 years. Clinical information, medical history, and pretransplant status are shown in Tables 1 and 2. Waiting time for LT and time between pulmonary function evaluation and LT was 9.6 ± 5.4 and 9.2 ± 4.2 months, respectively. Donor information and perioperative characteristics of patients undergoing LT are shown in Table 3.

Postoperative outcomes

Length of stay in specialized surgical care unit was 22 (17.2–28) (median 25–75% percentile). Mean simplified acute physiology score II at ICU admission was 35 ± 8. Postoperative renal replacement therapy was performed in ten patients (13%). Five patients developed postoperative heart failure (6.4%). Postoperative atrial fibrillation was recorded in ten patients (13%). Surgical site bleeding requiring additional surgical procedures occurred in four patients (5%). Two patients died due to intracerebral hemorrhage during the post-LT ICU period (2.5%). None of transplanted livers were infected at the time of liver procurement, packaging, and transport. Absence of latent infection of the transplanted liver was ascertained by National Agency of Biomedicine survey.

Sepsis was identified for 23 of the 78 LT procedures (29.5%), among them three patients fulfilled septic shock criteria. Overall, six patients died from sepsis during the post-LT ICU period (26%). Postoperative sepsis developed within the first 2 weeks after LT. Causes of sepsis were pneumonia (12 patients), intra-abdominal infections (6 patients), catheter-related infections (2 patients), and isolated bacteremia (2 patients). Infectious agents (*Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*) were identified for 13 of the 23 septic patients. Age, gender, BMI, comorbid conditions, indication of LT, donor data, and intraoperative characteristics were similar in patients with or without post-LT sepsis. Compared with nonseptic patients, those who developed sepsis had higher rate of reintubation/mechanical ventilation and ICU length of stay (median; 25–75% percentile: 28; 23–52 vs. 20; 17–23 days, $P < 0.001$).

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Table 1. Clinical and laboratory test characteristics of patients undergoing liver transplantation.

Characteristics of patients	All	No sepsis	Sepsis	*P*
Patients (number)	(*N* = 78)	(*N* = 55)	(*N* = 23)	
Age (years), mean ± SD	55 ± 8	56 ± 8	51 ± 8	0.04
Gender (male/female), *n*	72/6	51/4	21/2	0.83
BMI (kg/m^2), mean ± SD	26.5 ± 5.0	26.2 ± 4.6	27.1 ± 5.6	0.46
Diabetes, *n* (%)	21 (26.9)	15 (27.3)	6 (26.1)	0.91
Hypertension, *n* (%)	19 (24.4)	13 (23.6)	6 (26.1)	0.81
COPD, *n* (%)	23 (29.5)	14 (25.5)	9 (39.1)	0.23
Use of beta-blockers, *n* (%)	32 (41.0)	23 (41.8)	11 (47.8)	0.62
Alcoholic liver disease, *n* (%)	68 (87.1)	49 (89.1)	19 (82.6)	0.43
Viral hepatitis, *n* (%)	8 (10.3)	6 (10.9)	2 (8.7)	0.77
Indication for LT				
Hepatocarcinoma, *n* (%)	25 (32.1)	17 (30.9)	8 (34.8)	0.74
Cirrhosis, *n* (%)	53 (67.9)	37 (67.3)	16 (69.6)	0.84
MELD, mean ± SD	13.1 ± 6.3	11.6 ± 4.6	16.8 ± 5.8	<0.001
Child-Pugh, median (25–75%)	12 (10–14)	11 (9–14)	12 (11–14)	0.46
Creatinine (mg/l), mean ± SD	8.8 ± 2.5	8.8 ± 2.1	8.7 ± 3.2	0.84
Bilirubin (mg/l), mean ± SD	45 ± 68	31 ± 52	75 ± 87	0.01
Platelet (×10^9/l), mean ± SD	101 ± 84	105 ± 85	100 ± 95	0.82
Hemoglobin (g/dl), mean ± SD	11.9 ± 2.6	12.6 ± 1.9	10.4 ± 3.4	<0.01

Data are mean ± standard deviation (SD) except Child-Pugh score (median 25–75% percentile).

BMI, body mass index; COPD, chronic obstructive pulmonary disease; LT, liver transplantation; MELD, model for end-stage liver disease score.

Table 2. Functional characteristics of patients undergoing liver transplantation.

Patients	All	No sepsis	Sepsis	*P*
Echocardiography	(*N* = 59)	(*N* = 39)	(*N* = 20)	
LVEF (%)	64.4 ± 4.8	64.3 ± 4.5	64.5 ± 5.4	0.87
PAPs (mmHg)	28 (23–33)	25 (22–31)	30 (25–39)	<0.01
Lung function tests	(*N* = 78)	(*N* = 55)	(*N* = 23)	
FEV1 (l)	3.3 ± 0.7	3.3 ± 0.7	3.3 ± 0.7	0.97
FVC (l)	4.4 ± 0.8	4.4 ± 0.8	4.5 ± 0.9	0.63
FEV1/FVC	0.76 ± 0.83	0.76 ± 0.88	0.75 ± 0.70	0.74
TLC (l)	6.70 ± 1.12	6.67 ± 1.02	6.77 ± 1.37	0.72
K_{CO} (% predicted)	80 ± 17	82 ± 16	73 ± 17	0.04
FeNO (ppb)	19 (16–29)	21 (17–39)	17.0 (11–24)	0.02
Peak aerobic capacity	(*N* = 78)	(*N* = 55)	(*N* = 23)	
Workload (W)	100 ± 22	105 ± 20	87 ± 22	<0.01
VO_2 (ml/kg/min)	20 ± 5	21 ± 4	17 ± 4	<0.001
VO_2 (% predicted)	67 ± 16	72 ± 13	55 ± 16	<0.001
Heart rate (% max)	74 ± 14	75 ± 14	70 ± 13	0.11
MAP (mmHg)	115 ± 18	116 ± 20	112 ± 13	0.37

Data are mean ± standard deviation (SD) except PAPs and FeNO (median 25–75% percentile).

LVEF, left ventricle ejection fraction; PAPs, pulmonary artery systolic pressure; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; K_{CO}, transfer coefficient for carbon monoxide; FeNO, fraction of exhaled nitric oxide; ppb, parts per billion; VO_2, oxygen uptake at peak exercise; MAP, mean arterial pressure at peak exercise.

Table 3 compares the pretransplantation clinical characteristics and laboratory values of patients whether or not they developed sepsis after LT. Significant inverse correlation was found between log(FeNo) and PAPs ($r = -0.348$; $P = 0.018$). By bivariate analysis, age, MELD, hemoglobin before LT, K_{CO}, log (FeNO), and

Table 3. Donor information and perioperative characteristics of patients undergoing liver transplantation.

Patients	All $N = 78$	No sepsis $N = 55$	Sepsis $N = 23$	P
Waiting time for LT (months), mean ± SD	9.6 ± 5.4	10.2 ± 4.5	8.9 ± 5.6	0.28
Hospitalization prior to LT, *n* (%)	16 (20.5)	12 (21.8)	4 (30.4)	0.66
Donor conditions				
Age (years), mean ± SD	53 ± 6	53 ± 5	52 ± 7	0.48
Neurological cause of death, *n* (%)	12 (15.4)	8 (14.5)	4 (17.4)	0.75
Cold ischemia (min), mean ± SD	560 ± 125	570 ± 120	550 ± 130	0.51
Bypass duration (min), mean ± SD	125 ± 60	120 ± 75	135 ± 55	0.39
Operative time (min), mean ± SD	470 ± 100	460 ± 110	470 ± 90	0.70
Intraoperative fluids (l), mean ± SD	4.7 ± 2.4	5.1 ± 3.0	4.4 ± 2.5	0.33
RBC transfusion, mean ± SD	4.0 ± 4.0	3.5 ± 3.6	4.4 ± 3.8	0.33
Basiliximab use, *n* (%)	16 (19%)	11 (20%)	5 (22%)	0.86
SAPS II	38 ± 12	38 ± 10	39 ± 16	0.74

Data are mean ± standard deviation (SD).

LT, liver transplantation; RBC, packed red blood cells; SAPS, simplified acute physiology score.

Table 4. Clinical and functional parameters related to sepsis at bivariate and multivariate regression analysis.

	Regression coefficient for model with one predictor		Regression coefficient for model with six predictors	
Predictors	β (SE)	P (β)	β (SE)	P (β)
Age	−0.064 (0.031)	0.039	−0.058 (0.052)	0.262
MELD	0.179 (0.053)	<0.001	0.082 (0.087)	0.344
Hemoglobin before LT	0.036 (0.118)	0.004	0.069 (0.197)	0.725
K_{CO}	0.031 (0.015)	0.042	−0.023 (0.024)	0.328
log (FeNO)	−2.637 (1.185)	0.026	−3.45 (1.178)	0.052
Peak VO_2	−0.257 (0.079)	0.001	−0.252 (0.118)	0.032

β, coefficient of logistic regression; SE, standard error; MELD, model for end-stage liver disease score; LT, liver transplantation; K_{CO}, transfer coefficient for carbon monoxide; FeNO, fraction of exhaled nitric oxide; peak VO_2, oxygen uptake at peak exercise.

peak oxygen uptake (peak VO_2) were significantly associated with occurrence of postoperative sepsis. Reliable and accurate measurement of pulmonary artery systolic pressure (PAPs) was only available in 59 patients (Table 2). Although statistically significant at bivariate analysis, PAPs was not considered in the multivariate model. This selection had limited impact on the statistical analysis as colinearity due to correlation between log(FeNo) and PAPs was avoided in the multivariate model. Table 4 displays logistic regression model for one and six possible predictor variables. In the 5000 bootstrap samples, multivariable logistic regression with automatic backward stepwise elimination selected two predictors in 52.8% times and three predictors 21% times among six predictors [age, MELD, hemoglobin before LT, K_{CO}, log (FeNO), and peak oxygen uptake (peak VO_2)]. The number of times that each variable was selected using each of the six variable selection techniques is reported in Table 5. Peak VO_2 and log (FeNO) were identified as independent predictors of occurrence of sepsis in less than half of the 5000 bootstrap samples using backward stepwise elimination. MELD was identified as independent predictor of occurrence of sepsis in 38.4% of the bootstrap samples using backward selection. Therefore, logistic regression analysis was performed using these three identified predictors, that is, peak exercise VO_2, log(FeNo), and MELD. Odds ratios are shown in Table 6. The bootstrap method confirmed that odds of sepsis were associated with lower values of peak exercise VO_2 [OR = 0.790 (0.592; 0.925)] and reduced log(FeNo) [OR = 0.027 (0.001; 0.451)] but not with higher MELD scores [OR = 1.141 (0.970; 1.486)]. Finally, based on the results of ROC curve analyses, we estimated the

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Table 5. Number of times each variable was selected in 5,000 bootstrap sampling method.

	n	%
Predictors		
Age	1535	30.7
MELD	1921	38.4
Hemoglobin before LT	490	9.8
K_{CO}	514	10.3
log (FeNO)	2692	53.8
Peak VO_2	3583	71.7

Number of times each variable was selected by backward variable selection methods to logistic regression between sepsis and clinical and functional predictors in the 5000 bootstrap samples.

MELD, model for end-stage liver disease score; LT, liver transplantation; K_{CO}, transfer coefficient for carbon monoxide; FeNO, fraction of exhaled nitric oxide; peak VO_2, oxygen uptake at peak exercise.

optimal cutoff point of FeNO and peak VO_2 for postoperative sepsis diagnosis (Fig. 1). Areas under the curve (AUCs) for the ROC of FeNO and peak VO_2 for postoperative sepsis were 0.665 [0.532; 0.798] and 0.735 [0.609; 0.861], respectively. AUC for combined FeNO and peak VO_2 model was 0.803 [0.690; 0.916] with an optimal cutoff for FeNO and VO_2 at about 17 ppb and 17 ml/kg/min, respectively. By re-evaluating the cutoff for the ROC curves in each bootstrap resampling, median and 95% confidence interval were calculated for peak VO_2: 17 [16.2; 22] ml/kg/min and FeNO: 17.2 [13.0; 33.9] ppb.

Discussion

Postoperative sepsis is a major concern in patients undergoing liver transplantation [4,17,18]. In our study, bacterial sepsis represented the commonest postoperative complications and occured within the first 2 weeks of hospital stay. Sepsis was attributed to nonopportunistic bacterial intra-abdominal infection, pneumonia, and catheter-related infections. Patients with sepsis had higher needs for mechanical ventilation and in hospital length of stay. Reduced levels of fractional nitric oxide (NO) concentration in exhaled breath (FeNO) and poor aerobic capacity (peak VO_2) in LT candidates were independent risk factors of postoperative sepsis.

Incidence, site, and type of postoperative infections observed in our study were in agreement with previously published data [4–6]. High incidence of post-LT sepsis in the ICU has been attributed to many factors including recipient's underlying extrahepatic diseases, major immunosuppressive protocol, and extensive LT surgical technique [4–6,17,18]. In addition, impaired cardiopulmonary functional status of LT candidates has been consistently associated with poor postoperative outcomes [6,7]. For example, routine pulmonary function tests performed in LT candidates have identified reduced total lung capacity (TLC) as an independent risk factor of postoperative pulmonary complications [4,7]. Furthermore, preoperative cardiovascular evaluation indicates that elevated pulmonary artery systolic pressure (PAPs) is associated with pulmonary complications and increased mortality after LT [4,19,20]. In our series, reduced total lung capacity (TLC) was not associated with increased incidence of septic postoperative complications. Reduced transfer factor for carbon monoxide (TLCO) to alveolar volume (VA) ratio adjusted for hemoglobin concentration (K_{CO}) was significantly associated with postoperative sepsis after LT, whereas multivariate analysis failed to identify K_{CO} as an independent predictor. Our results confirmed that pulmonary artery systolic pressure (PAPs) was associated with increased incidence of septic complications after LT. Interestingly, we identified an inverse correlation between PAPs and log-transformed exhaled NO values. This observation is consistent with previous studies showing that NO levels in exhaled air and NO metabolites in bronchoalveolar lavage fluid are inversely

Table 6. Clinical and functional parameters related to sepsis at multivariate regression analysis.

Multivariate analysis	β	SE	*P* (β)	OR (95% CI)
MELD	0.124	0.063	0.050	1.132 (1.000–1.282)
log (FeNO)	−3.251	1.447	0.025	0.039 (0.002–0.660)
Peak VO_2	−0.214	0.090	0.018	0.807 (0.677–0.963)

β, coefficient of logistic regression; SE, standard error; OR, odd ratio; CI, confidence interval; MELD, model for end-stage liver disease score; LT, liver transplantation; K_{CO}, transfer coefficient for carbon monoxide; FeNO, fraction of exhaled nitric oxide; peak VO_2, oxygen uptake at peak exercise.

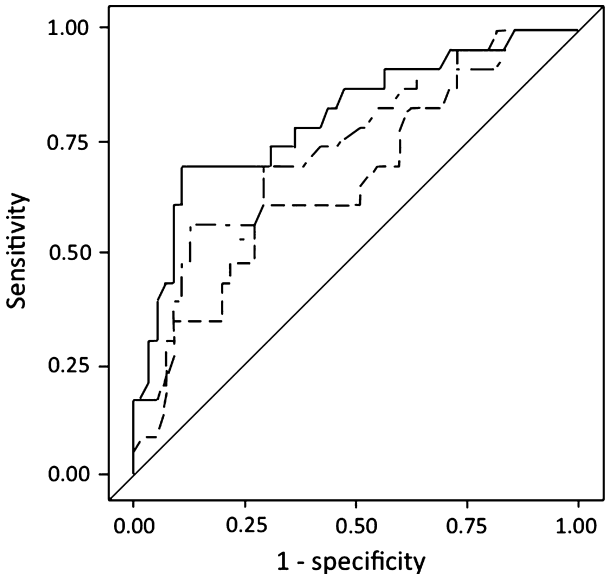

Figure 1 ROC curve for the diagnostic value of FeNO (- - -), peak VO_2 (- –), and combined FeNO–peak VO_2 (——), to predict postoperative sepsis in LT candidates. Area under the curve (AUC) for the ROC of FeNO: 0.665 [0.532; 0.798], peak VO_2 0.735 [0.609; 0.861], and combined FeNO–peak VO_2 model was 0.803 [0.690; 0.916], with an optimal cutoff of FeNO and VO_2 at about 17 ppb and 17 ml/kg/min, respectively.

related to the magnitude of pulmonary hypertension [21].

Intrahepatic and systemic hemodynamic perturbations that characterize end-stage cirrhotic patients have attributed, at least in part, to abnormal nitric oxide signaling [22–25]. In the cirrhotic liver, reduced bioavailability of NO contributes to impaired endothelium-dependent relaxation within the microcirculation and elevated intrahepatic vascular resistance [25]. Decreased intrahepatic NO is mainly attributed to reduction of endothelial NO synthase (eNOS) activity and NO scavenging by increased superoxide vascular production. Of note, NO production is increased in the splanchnic vascular bed, which contributes to increased endothelium-dependent relaxation in the systemic circulation and inappropriate hyperdynamic circulatory state [22–24]. Surprisingly, increased NO levels have been detected in the exhaled air of end-stage cirrhotic patients, suggesting that the lung is a source of NO [26–30]. Indeed, direct production of NO by the lung is required to explain increased NO levels in the exhaled air because overproduction of NO in the splanchnic vascular bed is rapidly scavenged by circulating hemoglobin before reaching the alveoli. In our study, average levels of exhaled NO were within the normal ranges before LT, whether or not patients developed postoperative sepsis. However, exhaled NO levels were significantly lower in patients who developed sepsis after LT compared with those who did not. Reduced NO levels in exhaled air may be attributed to combined effects of lung inflammatory response and local oxidative stress on NO production, deregulation of which may impede anti-infectious lung host defenses [31,33]. In line, low exhaled NO levels have been previously associated with poor outcomes in patients suffering from acute lung injury elicited by cardiopulmonary bypass and lung transplantation [33–35]. Overall, low levels of NO in the exhaled air may indicate impaired NO production associated with poor lung function conditions.

Evaluation of aerobic capacity has emerged as a valuable noninvasive tool to improve clinical evaluation in LT candidates. We [9,10] and other groups [11,12] have shown that aerobic capacity evaluated before surgery can predict 1-year survival after LT. In the present study, we found that odds of early onset sepsis after LT were associated with lower values of peak exercise VO_2. This finding suggests that impaired aerobic capacity may predict patient's inability to face increased metabolic demand related to major surgical stress [9,10]. Limited physiological reserve would predict poor cardiovascular adaptation with inadequate oxygen delivery increase, which in turn aggravates factors leading to postoperative sepsis. Such deleterious factors in cirrhotic patients include decreased bowel motility, bacterial overgrowth, increased intestinal permeability, which all increase the risk of the translocation of intestinal microbiota to the mesenteric lymph nodes [22,36]. Another possible explanation is that reduced aerobic capacity represents a surrogate marker for deregulated inflammatory state with abnormal immune response [37, 38]. In line, connection between physical inactivity and one's risk of developing sepsis is supported by studies that suggest associations between poor aerobic capacity, immune function, and susceptibility to respiratory infections [38–40].

We recognized several limitations of this study. First, our study confined to a population of LT candidates included by a single surgical center. The prognostic value of reduced FeNO and aerobic capacity requires further confirmation in large-scale, multicenter studies. Furthermore, because normal individuals demonstrate a fairly wide range of FeNO and knowing that alveolar NO is increased in cirrhotic patients, magnitude of changes in FeNO between consecutive measurements may be more informative than an absolute value. We failed to measure exhaled NO at multiple flow rates because of frequent passivity and inadequate patient motivation. Hence, we were unable

to provide relevant information regarding sources of NO production in alveolar and bronchial wall compartments. Second, limited postoperative sepsis episodes were reported in our study. When the number of events is low relative to the number of predictors, standard regression could produce overfitted risk models that make inaccurate predictions. Accurate statistical analyses using multivariate models with bootstrap somewhat balance this limitation. Even though another stochastic element may be added in the analysis, variable selection was based on both statistical (VO_2, FeNO) and clinical (MELD) pertinence. This approach was favored to avoid computing mean and 95% CI based on the distribution over the 5000 resamples from different combinations of variables which may be irrelevant and noninformative in the bootstrap procedure. Third, we included LT candidates who were able to perform lung function tests and cycling exercise. Hence, we have excluded sicker patients who were likely more prone to develop postoperative complications after LT. In our series, moderate MELD score calculation in LT candidates further indicated inclusion of less severe patients.

In conclusion, our study confirms that sepsis is a common complication after LT and is a major cause of morbidity and longer hospital stay. Associations between reduced exhaled NO levels with the development of sepsis represent the most interesting and new finding of the present study. We conclude that reduced FeNO levels and poor aerobic capacity may help identify patients who are likely to develop perioperative sepsis. However, these results warrant, however, further investigations in a large cohort of patients to determine the relevance of these preoperative risk factors. Attractive strategies to improve post-LT overall outcome include pre-LT programs of personalized and adapted physical activity, which may reduce postoperative complications, duration of hospitalization, and survival.

Authorship

RN, TDP, HS, EJL, DA, BE, DS and LG: make substantial contributions to conception and design, and/or acquisition of data, and/or analysis interpretation of data and writing up of the paper and give final approval of the version to be submitted and any revised version. DA, BE, DS and LG: participated in drafting the article or revising it critically for important intellectual content and give final approval of the version to be submitted and any revised version.

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