

Exhaled Breath Condensate Acidification Occurs During Surgery for Abdominal Cancer

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Abstract. *Background/Aim:* Acidification of exhaled breath condensate (EBC), reflecting airway inflammation and oxidative stress, has been reported in lung cancer patients undergoing lobectomy. We undertook this study to examine EBC pH changes during surgery for abdominal cancer. *Patients and Methods:* EBC pH was measured from 20 patients undergoing abdominal cancer resection before and during surgery. Repeated-measures of ANOVA and random-effects linear models were applied to compare mean EBC pH values in samples collected at different times. Cox and linear regression models were used to determine the association of EBC pH with occurrence of acute bronchospasm intraoperatively and the duration of hospitalization. *Results:* Significant acidification of EBC was observed during surgery ($p=0.007$) associated with 0.77% (95% confidence interval=-0.14-1.68, p -value=0.097) increase in the risk for developing acute bronchospasm, after adjustment for potential confounders. *Conclusion:* EBC acidification occurs in patients undergoing abdominal cancer resection and is associated with the occurrence of acute bronchospasm intraoperatively.

Exhaled breath condensate (EBC) is a source for biomarkers of airway inflammation and oxidative stress that could be potentially used as a diagnostic tool, prognosticator of

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outcome or to guide therapeutic interventions (1-6). Collection of EBC is a simple, non-invasive and safe procedure that has been used in actively participating individuals breathing on their own, as well as in mechanically-ventilated patients, for studying the composition of airway lining fluid and to detect lung inflammation (7-9). However, issues of standardization and several technical aspects limit its application in everyday clinical practice (10). In the past decade, researchers have focused on the measurement of EBC pH as it is inexpensive, can be repeatedly performed without adversely affecting a patient and, most importantly, correlates well with other indices of airway inflammation and varies substantially in diseased states (11-16). Additionally, a tendency for lower EBC pH values has been observed after intubation and during mechanical ventilation in ICU patients related to the presence of either subclinical or an overt lung inflammation (7, 18). Interestingly, continuous EBC pH monitoring in ventilated patients showed an acidification of EBC pH during clinical deterioration followed by its normalization during recovery (19). The results of the latter study are very promising and suggest a diagnostic as well as a prognostic utility of EBC pH measurements.

Finally, in their study Moloney *et al.* reported significant acidification of EBC in patients with lung cancer after lobectomy but not in cardiac patients after coronary bypass graft surgery with cardiopulmonary bypass (8). The authors attributed this discrepancy to the fact that the insult of cardiac surgery is less than that of lobectomy.

There is a lack of data regarding patients undergoing non-thoracic oncological surgery and who are under anesthesia and short-term mechanical ventilation, procedures related to increased risk for acute lung injury (20, 21). We undertook this study to determine whether EBC pH changes occur in

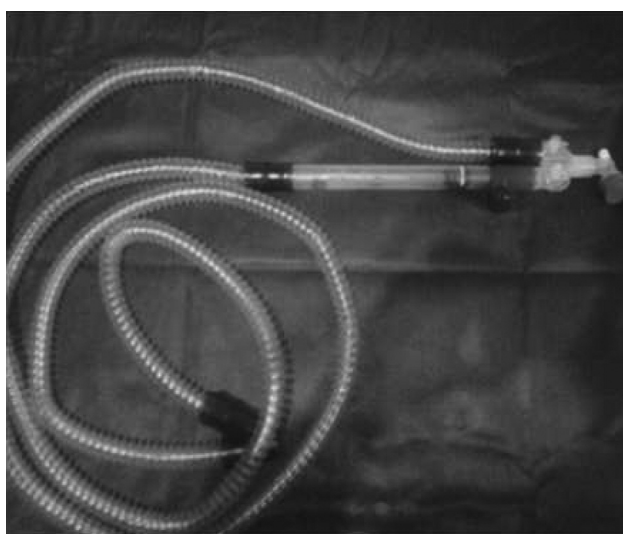


Figure 1. Exhaled breath condensate collection system. The R-Tube has been incorporated into a ventilator circuit.

the operating room in patients undergoing abdominal cancer resection compared to their preoperative values taking into account several factors that may influence EBC acidity. Furthermore, we investigate the potential prognostic utility of EBC pH in these patients by assessing its association with the development of acute respiratory events intra-operatively as well as with the duration of hospitalization.

Patients and Methods

Twenty patients (45% male, mean age=62.25 years, range=31-83 years) undergoing surgery for abdominal cancer were enrolled. Information on chronic diseases, smoking habits and somatometry were extracted from the medical files. Written informed consent was obtained from all patients and the protocol was approved by the independent Research Ethics Committees of the involved collaborating centers.

EBC collection. The RTube EBC collection system (Respiratory Research, Inc, Charlottesville, VA, USA) was used. The cooling devices were kept at -80°C between measurements and taken out of the freezer immediately before each collection. The first sample of EBC was collected in the evening before surgery. All patients provided a 20-min collection of EBC of about 1.0-1.5 ml of condensate, in the ward while wearing nose clips at normal tidal breathing at least 2 h after their last ingestion of any food or drink. The second sample was collected intraoperatively 1 h after the induction of anesthesia and stabilization and securing of the airway with a cuffed endotracheal tube. The third sample was collected from the intubated patient after the final occlusion of the surgical incision, before arousal. As we observed that in intubated patients 20 min was not long enough to collect an adequate volume of condensate we decided to increase the intraoperative collection period. Therefore both intraoperative samples of EBC were

Table I. Demographic and clinical characteristics of the study population (N=20).

Characteristic	Value
Gender, n (%)	
Male	9 (45)
Female	11 (55)
Age, mean \pm SD (years)	62.3 \pm 12.8
BMI, mean \pm SD (kg/m ²)	28.3 \pm 6.00
Smoking status, n (%)	
Never	10 (50)
Smoker	10 (50)
Chronic diseases, n (%)	
COPD or chronic bronchitis	3 (15)
GERD	3 (15)
ASA score, n (%)	
2	18 (90)
3	2 (10)
Type of abdominal surgery, n (%)	
Major abdominal-gynecological	4 (20)
Major urological	2 (10)
Major digestive	14 (70)
Duration of operation, mean \pm SD (min):	212 \pm 60.2
Outcome of interest	
Bronchospasm	5 (25%)
Duration of hospitalization, mean \pm SD (days):	6.85 \pm 2.7

BMI: Body mass index; COPD, chronic obstructive pulmonary disease; GERD, gastroesophageal reflux disease; ASA, American Society of Anesthesiologists score.

collected for a 30-min period by inserting the RTube early into the exhalation limb of the ventilator tubing directly after the Y-shaped connecting piece (Figure 1). The same humidification, in the form of a heat and moisture exchanger, was used for all patients and was not disconnected during EBC collection. The fraction of inspired oxygen was 0.4-0.5 and the tidal volume (TV) was standardized either at 8 or 10 ml/kg ideal body weight (IBW), depending on the anesthesiologist, without positive end-expiratory pressure (PEEP). Immediately after collection, all EBC samples were frozen in aliquots and stored at -80°C until analyzed.

pH measurement. Samples were processed for pH measurement every 10 patients (batch of 30 aliquots). A stable pH was achieved in all cases after de-aeration of the EBC specimens by bubbling argon gas (350 ml/min for 10 min) and pH was measured by means of a pH meter (Jenway-350; Jenway; Gransmore Green, UK) with a -2.000 to 16.000 pH range and a mean resolution/accuracy of the order of $0.001/0.01/0.1$ pH (resolution) and ± 0.003 pH (accuracy). This pH measurement system was calibrated at pH 4, pH 7 and pH 10 against appropriate technical buffers provided by the manufacturer of the instrument.

Clinical data. In the operating room, electrocardiographic, hemodynamic (heart rate, arterial blood pressure), respiratory parameters (respiratory rate, TV, peak inspiratory airway pressure, oxygen saturation, end tidal CO₂, arterial blood gases) and temperature were continuously monitored and recorded. Data regarding the need to increase inspired oxygen concentration and

Table II. Volume of exhaled breath condensate (EBC) and EBC pH values of all participants (N=20) in the three sampling periods; values are given as the mean±SD.

	Preoperative [†]	First intraoperative [‡]	Second intraoperative [§]
Duration of collection (min)	20	30	30
Volume (µl)	953±251	858±253	855±216
EBC pH	7.450±0.466*	7.171±0.273*	7.182±0.352*

[†]Spontaneously breathing; [‡]1 h after the induction of anesthesia (under mechanical ventilation); [§]after the final occlusion of the surgical incision and before arousal (under mechanical ventilation). *ANOVA *p*-value=0.007.

use of adjuvants such as bronchodilators, vasodilators, vasopressors or antiarrhythmic drugs were collected. In addition, we recorded episodes of bronchospasm occurring between the two intraoperative EBC collections. The American Society of Anesthesiologists score was determined for each patient preoperatively.

Quality assurance/quality control. Standard operating procedures were applied for the collection of EBC and for EBC pH measurements (see above).

Analysis. Descriptive statistics such as proportions, means, and SDs were used to summarize the characteristics of study population. A repeated-measures analysis of variance (repeated ANOVA) was applied to compare the mean EBC pH values at the different sample times. When appropriate, *post hoc* comparisons were carried out using Bonferroni correction for multiple comparisons. Significance was set at a *p*-value of less than 0.05. We used random-effects linear models, incorporating a random intercept, to compare the EBC pH measurements at the different sample times taking into account various possible confounders such as age (years), sex, body mass index (BMI; kg/m²), smoking habit, history of gastroesophageal reflux disease (GERD) or chronic obstructive pulmonary disease (COPD), arterial blood pH measured at the same time with the collection of the first intra-operative EBC sample, duration of operation (min) and TV (ml/kg). We used Cox proportional-hazards models for the occurrence of bronchospasm during surgery. We used the time between the first and second measurement of EBC pH during surgery as the timescale and the EBC pH measurement 1 h after induction of anesthesia (first intraoperative measurement) as the exposure under study, which was analyzed as a time-varying variable. Censoring was carried out on the first occasion bronchospasm occurred or at the time of second measurement of EBC pH before arousal (second intra-operative measurement), whichever came first. We also developed linear regression models to relate the duration of hospitalization (days) to EBC pH. We used three different models, one for every EBC pH measurement. We checked the robustness of the results of the models developed by gradually including potential confounders such as age, sex, BMI, smoking habits, history of gastritis and COPD and duration of surgery. The statistical analysis was performed using STATA version 13 (StataCorp., College Station, TX, USA).

Results

In Table I, participants' demographic and clinical characteristics are presented. Acute bronchospasm, between the two EBC

Table III. Post-hoc comparisons between the three exhaled breath condensate (EBC) pH measurements using Bonferroni correction for multiple comparisons.

EBC pH	Mean difference (Bonferroni 95% CI)	<i>p</i> -Value
First intraoperative [†]		
Preoperative	-0.279 (-0.513--0.045)	0.015
Second intraoperative [‡]		
Preoperative	-0.267 (-0.501--0.034)	0.020
First intraoperative [†]	0.011 (-0.222-0.245)	>>0.05

CI: Confidence interval. [†]At 1 h after the induction of anesthesia (under mechanical ventilation); [‡]after the final occlusion of the surgical incision and before arousal (under mechanical ventilation).

collections occurred in five patients and none of them was associated with cardiac dysfunction or fluid overload. In Table II, the volume of condensate in exhaled air obtained in the three samples (mean values for each collection) as well as the EBC pH values are shown. The mean volume of EBC of the study participants while normally breathing over a period of 20 min was 953 (±251) µl, whilst for the same patients intra-operatively and while mechanically ventilated, the mean volume of the first collection was 858 (± 253) µl and of the second 855 (±216) µl over a period of 30 min. Significant acidification of the EBC (*p*=0.007) was observed between the preoperative (spontaneous breathing) and intraoperative samples (mechanically ventilated) (Table II). The mean preoperative EBC pH was significantly higher by 0.279 (95% confidence interval (CI)=0.045-0.513, *p*=0.015) and 0.267 (95% CI=0.034-0.501, *p*=0.020) units from the first intraoperative sample (collected 1 h after the induction of anesthesia) and the second intraoperative sample (before arousal), whilst the difference of the means between the two intra-operative EBC pH measurements did not reach the nominal level of significance (Table III). Interestingly, the aforementioned significant differences remained robust using mixed models adjusting alternatively for age, sex, BMI, smoking, history of GERD or COPD, arterial blood pH corresponding to the first

Table IV. Mean change of the pH of preoperative exhaled breath condensate (EBC) compared to the first and second intraoperative measurements using mixed models adjusting for potential confounders.

Model	β -Coefficient (95% CI)	p-Value	Model	β -Coefficient (95% CI)	p-Value
Main model [§]			Main model+ blood pH		
Preoperative EBC pH		0.002*	Preoperative EBC pH		0.002*
First intra-operative [†]	-0.279 (-0.457--0.100)	0.002	First intra-operative [†]	-0.279 (-0.457--0.100)	0.002
Second intra-operative [‡]	-0.267 (-0.446--0.089)	0.003	Second intra-operative [‡]	-0.267 (-0.446--0.089)	0.003
Main model+ age (years)			Blood pH	3.027 (0.882-5.172)	0.006
Preoperative EBC pH		0.002*	Main model+ duration of operation (min)		
First intra-operative [†]	-0.279 (-0.457--0.100)	0.002	Preoperative EBC pH		0.002*
Second intra-operative [‡]	-0.267 (-0.446--0.089)	0.003	First intra-operative [†]	-0.279 (-0.457--0.100)	0.002
Age	-0.003 (-0.013-0.006)	0.512	Second intra-operative [‡]	-0.267 (-0.446--0.089)	0.003
Main model+gender			Duration of operation	-0.002 (-0.004--0.001)	0.005
Preoperative EBC pH		0.002*	Main model+		
First intra-operative [†]	-0.279 (-0.457--0.100)	0.002	Tidal volume (ml/kg)		
Second intra-operative [‡]	-0.267 (-0.446--0.089)	0.003	Preoperative EBC pH		0.002*
Gender (Male)	-0.012 (-0.256-0.232)	0.924	First intra-operative [†]	-0.279 (-0.457--0.100)	0.002
Main model+ BMI (kg/m ²)			Second intra-operative [‡]	-0.267 (-0.446--0.089)	0.003
Preoperative EBC pH		0.002*	Tidal volume	0.107 (-0.006-0.220)	0.063
First intra-operative [†]	-0.279 (-0.457--0.100)	0.002	Main model+ COPD history		
Second intra-operative [‡]	-0.267 (-0.446--0.089)	0.003	+ blood pH + duration of operation (min)		
BMI kg/m ²	-0.009 (-0.030-0.011)	0.381	Preoperative EBC pH		0.002*
Main model+smoking habit			First intra-operative [†]	-0.279 (-0.457--0.100)	0.002
Preoperative EBC pH		0.002*	Second intra-operative [‡]	-0.267 (-0.446--0.089)	0.003
First intra-operative [†]	-0.279 (-0.457--0.100)	0.002	COPD (Yes)	-0.163 (-0.518,0.193)	0.370
Second intra-operative [‡]	-0.267 (-0.446--0.089)	0.003	Blood pH	1.845 (-0.354-4.045)	0.100
Smoking (Yes)	-0.095 (-0.334-0.144)	0.435	Duration of operation	-0.002 (-0.003-0.0002)	0.091
Main model+ GERD history					
Preoperative EBC pH		0.002*			
First intra-operative [†]	-0.279 (-0.457--0.100)	0.002			
Second intra-operative [‡]	-0.267 (-0.446--0.089)	0.003			
GERD (Yes)	0.281 (-0.035-0.598)	0.081			
Main model+ COPD history					
Preoperative EBC pH		0.002*			
First intra-operative [†]	-0.279 (-0.457--0.100)	0.002			
Second intra-operative [‡]	-0.267 (-0.446--0.089)	0.003			
COPD (Yes)	-0.413 (-0.775--0.051)	0.025			

BMI: Body mass index; COPD, chronic obstructive pulmonary disease; GERD, gastroesophageal reflux disease. [§]The main model included EBC pH as dependent variable and an indicator for the different samples as the independent one; [†]1 h after the induction of anesthesia (under mechanical ventilation); [‡]after the final occlusion of the surgical incision and before the arousal (under mechanical ventilation). *Significance of difference between the samples of EBC pH overall.

intraoperative measurement of EBC pH, duration of operation and TV. When we introduced all the parameters identified to be significantly associated with EBC pH, namely duration of operation ($p=0.005$), arterial blood pH corresponding to the first intraoperative EBC pH ($p=0.006$) and history of COPD ($p=0.025$) to a multivariable mixed model, the relationship between the three EBC pH measurements remained unchanged (Table IV).

In Table V, the association between the first intraoperative measurement of EBC pH and the occurrence of bronchospasm during surgery and short mechanical ventilation in the operating room is shown. A 0.1 unit decrease of the first intraoperative EBC pH, that is after the induction of anesthesia and under mechanical ventilation, was found to increase the risk for development of bronchospasm intraoperatively by 0.77% (95% CI=-0.14-1.68, $p=0.097$). This result is of

borderline significance ($0.05 < p < 0.10$) but it remained practically the same after controlling for different sets of potential confounders. A negative association was also revealed when we examined the relation between EBC pH measurements and duration of hospitalization. A 0.1 unit decrease of either the preoperative EBC pH, or the first or second intra-operative ones was found to increase the duration of hospitalization by 0.08 (95% CI=-0.20-0.36), 0.20 (95% CI=-0.28-0.68) or 0.17 (95% CI=-0.20-0.54) days, respectively. However, none of these negative associations reached the nominal level of significance.

Discussion

In this study, we observed an acidification of EBC during surgery and mechanical ventilation in patients undergoing

Table V. Association of the pH of the first intraoperative exhaled breath condensate (EBC) (sample collected 1 h after the induction of anesthesia and under mechanical ventilation) with the occurrence of acute bronchospasm intra-operatively. Results from Cox models.

Model	HR (95% CI)	p-Value
EBC pH	0.926 (0.846-1.014)	0.097
EBC pH + gender (male)	0.919 (0.837-1.008)	0.074
EBC pH + age (years)	0.918 (0.831-1.015)	0.095
EBC pH + BMI (kg/m ²)	0.938 (0.858-1.025)	0.159
EBC pH + smoking (yes)	0.935 (0.864-1.012)	0.096
EBC pH + COPD (yes)	0.939 (0.852-1.034)	0.199
EBC pH + GERD (yes)	0.907 (0.812-1.012)	0.082
EBC pH + gender (male) + age (years)	0.918 (0.836-1.009)	0.077
EBC pH + gender (male) + GERD (yes)	0.909 (0.818-1.011)	0.078
EBC pH + age (years) + GERD (yes)	0.903 (0.804-1.013)	0.083
EBC pH + gender (male) + age (years) + GERD (yes)	0.909 (0.816-1.014)	0.086
EBC pH + gender (male) + age (years) + smoking (yes)	0.968 (0.892-1.050)	0.430
EBC pH + gender (male) + GERD (yes) + smoking (yes)	0.943 (0.871-1.021)	0.151
EBC pH + age (years) + GERD (yes) + smoking (yes)	0.947 (0.877-1.022)	0.158
EBC pH + gender (male) + age (years) + GERD (yes) + smoking (yes)	0.973 (0.899-1.053)	0.499
EBC pH + gender (male) + age (years) + BMI (kg/m ²) + GERD (yes)	0.927 (0.830-1.037)	0.185
EBC pH + gender (male) + age (years) + BMI (kg/m ²) + GERD (yes) + smoking (yes)	0.968 (0.883-1.060)	0.483
EBC pH + gender (male) + age (years) + BMI (kg/m ²) + COPD (yes) + GERD (yes)	0.969 (0.880-1.067)	0.522
EBC pH + gender (male) + age (years) + BMI (kg/m ²) + COPD (yes) + GERD (yes) + smoking (yes)	0.933 (0.825-1.054)	0.265

HR, Hazard ratio; CI, confidence interval; BMI, body mass index; COPD, chronic obstructive pulmonary disease; GERD, gastroesophageal reflux disease.

resection of abdominal cancer that was associated with increased risk for occurrence of acute bronchospasm intra-operatively. Furthermore, the acidification of EBC collected at different times was associated with an increase in the duration of hospitalization that did not, however, reach the nominal level of significance.

Three decades ago, EBC collection and analysis were introduced into the study of inflammatory lung diseases and since then an increasing number of articles argue over its utility (22, 23, 25). Among several molecules contained in EBC (3, 17, 29) and different parameters, research has also focused on the use of the pH of this non-invasively and safely collected material as a biomarker of airway inflammation and oxidative stress (11). Previous studies have demonstrated lower EBC pH values in individuals having asthma (11, 24), COPD (11, 25) or GERD (26), as well as a more pronounced acidification during deterioration of several respiratory clinical conditions, mainly obstructive lung disease (17, 24, 27, 28). Furthermore, a tendency for lower EBC pH values after intubation and during mechanical ventilation has been shown in ICU patients both with (12, 19) and without lung injury (18). In their study, Suzuki *et al.* showed that oxidative stress, using reactive oxygen species produced by neutrophils, was greater in patients with cancer (21). In the present study, we noted in patients with abdominal cancer without known lung injury, that there was a consistent and significant drop of the EBC pH value, compared to the preoperative one, which persisted throughout the

operation and was robust when we adjusted for several factors reported previously to influence EBC acidity, obstructive lung disease and GERD included. A plausible explanation of our results is that positive-pressure ventilation during surgery for abdominal cancer in patients without pre-existing lung injury induces subclinical pulmonary inflammation and oxidative stress following physical disruption of tissues and cells. Consistent with this is our observation that EBC acidification precedes and is positively related to the development of acute bronchospasm intra-operatively. Thus, it further strengthens the hypothesis that short-term ventilation during surgery may induce subclinical pulmonary injury, implying a diagnostic as well as a prognostic utility of EBC pH measurements. After considering the conflicting results of previous studies and in an effort to clarify the role of short-term mechanical ventilation and mainly the use of different ventilator settings in the initiation of lung injury (21, 29-31), we also introduced TV into our models as a continuous variable. However, we did not note any difference in EBC pH changes. This observation implies no different effect on airway lining fluid acidification when using TV between 8 and 10 ml/kg IBW without PEEP and recruitment maneuvers and is in line with the results of Determann *et al.* (31) who showed that mechanical ventilation with lower TV (6 ml/kg) and PEEP (10 cmH₂O) did not have a different effect on levels of biomarkers of lung epithelial injury compared with conventional mechanical ventilation (TV of 12 ml/kg without PEEP).

There is a lack of data concerning EBC use in non-cardiothoracic surgical patients. Garcia de la Asuncion *et al.* investigated the time course of several markers of oxidative stress simultaneous in EBC and in blood during pulmonary lobectomy. Both EBC and blood showed significant increases in oxidative stress markers. These results may explain the acute lung injury that occurs after pulmonary lobectomy (9). In their study concerning individuals undergoing either coronary artery bypass grafting (CABG) or lobectomy for lung cancer, Moloney *et al.* collected EBC in endotracheally intubated patients at the beginning and at the end of CABG, as well as of lobectomy (8). They detected higher inflammatory markers and lower EBC pH values only in patients after lobectomy and not after CABG, suggesting that the insult associated with cardiac surgery is less than that associated with lobectomy. Using a similar design to that of Moloney *et al.* (8), we also found no significant changes in the EBC pH when we compared the mean values of the samples collected at the beginning and at the end of surgery for abdominal cancer, in which the insult can also be considered less than that associated with lobectomy. However, due to our study design, by also having collected EBC preoperatively from the same patients, while non-intubated and while spontaneously breathing, we were able to demonstrate that the critical time interval for EBC acidification is the one after intubation, induction of anesthesia and initiation of mechanical ventilation, implying an injurious effect on the airways and the lungs.

An advantage of the present study is that based on previous literature, we applied standardized operating procedures to maximize the validity of our results (32). In their study, Lema *et al.* showed that the volume of condensate is directly related to the volume of air inhaled and that it takes at least 15 min to generate a sufficient volume of condensate (33). In the present study, we ensured acceptable EBC volume by determining collection time, a strategy that is considered to be valid (16, 33). However, we noted that in intubated and mechanically-ventilated patients, it takes more time to collect the same volume of condensate compared to non-intubated and normally breathing individuals (34). This may be due to the trend towards lower TVs in mechanical ventilation such that EBC collection volumes may be lower due to decreased minute ventilation. Unfortunately, the procedure we used to collect EBC in our patients did not allow us to measure minute ventilation in the study participants while normally breathing in order to compare it to that while mechanically ventilated. Additionally, in our study, bubbling was used as the degassing method because Lin *et al.* found that bubbling samples reach pH equilibrium faster, pH variation between samples is very small, and volume loss is not a major concern (35).

To the best of our knowledge, this is the first study that compares EBC pH in samples taken at different time points from patients submitted to abdominal cancer resection and

implies a prognostic role of EBC acidification regarding respiratory adverse events occurring intra-operatively.

Our results suggest that EBC pH measurement is a noninvasive technique that could be safely used in the operating room in surgical patients with cancer to monitor the lower airway as an early biomarker of respiratory complications. Our findings are consistent with previous studies in other patient groups and add to the evidence of a diagnostic and prognostic utility of EBC pH measurements. Further research in larger populations is needed to confirm our findings and establish its use as a non-invasive real-time prognostic biomarker. Continuous monitoring of EBC pH at the bedside and in the operating room could guide interventions in order to prevent potential perioperative morbidity or mortality.

Conflicts of Interest

None.

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