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# Effect of Calibration for Tissue Differentiation Between Healthy and Neoplasm Lung Using Minimally Invasive Electrical Impedance Spectroscopy

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AQ:2 This work involved human subjects or animals in its research. Approval of all ethical and experimental procedures and protocols was granted by the Ethics Committee on Clinical Investigation of the Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, under Application No. CEIC-73/2010.

AQ:3 **ABSTRACT** This study proposes a calibration method and analyses the effect of this calibration in lung measures, using minimally invasive electrical impedance spectroscopy with the 3-electrode method, for tissue differentiation between healthy and neoplasm lung tissue. Tissue measurements were performed in 99 patients [54 healthy tissue and 15 neoplastic tissue samples obtained] with an indicated bronchoscopy. Statistically significant difference ($P < 0.001$) were found between healthy lung tissue and neoplasm lung tissue in bioimpedance parameters. The calibration of the bioimpedance measures with respect to a measure performed in bronchi reduces the inter-patient dispersion, increasing the sensitivity, decreasing the specificity and increasing the area below the ROC curve for three out of four impedance-derived estimators. Results also show that there are no significant differences between healthy lung tissue among smoker, non-smoker and ex-smoker samples, which was initially stated as a possible cause of EIS measurement dispersion in lungs.

**INDEX TERMS** Bronchi, bronchoscopy, calibration, electrical impedance spectroscopy (EIS), lung.

## I. INTRODUCTION

Respiratory disorders have a big impact in the population worldwide. According to the European Respiratory Society, chronic obstructive pulmonary disease (COPD) is the third global cause of death in more developed countries. Moreover, lung cancer is the leading cause of cancer death in the world. Both are smoking-related conditions [1].

In lung cancer, late detection in advance stages is common and is related to poor prognosis [2]. Diagnostic of lung peripheral and central nodules is increasing because of number of patients with indeterminate nodules are discovered in CT screening and verified with other diagnostic options such as minimally invasive bronchoscopic procedures to establish final histological type. However, the diagnostic yield using

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virtual bronchoscopy (VB), radial endobronchial ultrasound (r-EBUS), electromagnetic navigation (EMN) and ultrathin bronchoscopes remains suboptimal [3], [4], and their high economic cost makes them unavailable in most centers.

We aim to use Electrical Impedance Spectroscopy (EIS) to complement the actual methods of diagnosis of lung diseases as it could allow the differentiation between healthy lung tissue and neoplasm lung tissue and help in the choice of the specific sample location.

EIS technique is one of the existing methods of impedance analysis. Impedance is defined as the opposition to the flow of an alternating electrical current which is dependent on the frequency of this current [5]. When the impedance is measured in biological tissue is named as bioimpedance (Z). It measures the passive electrical properties of the tissue after the introduction of a low amplitude alternating current to the organism [5], [6]. The bioimpedance is a complex number with a real part (the resistance, R) and an imaginary part (the reactance, Xc), both parts are dependent of the geometry of the measured region, the location of electrodes and the tissue electrical passive properties [5]. The physiological fluids have low resistance and dominates the measured resistance, while cell membranes act as capacitors, having high impedance at low frequencies and low impedance at high frequencies and contributes mainly to the reactive part. Due to these behaviors, the electrical current introduced in the biological tissue divides into resistive and capacitive pathways and it changes with the frequency [6]. An alternative representation of the Bioimpedance, as a complex number, is the use of the modulus (Z) and the phase angle (PA). The PA represents the relative time lag between the injected current and the generated voltage [7]. Bioimpedance data can be obtained using single or multiple frequencies. When the bioimpedance data is obtained using a broad band of frequencies is known as bioimpedance spectroscopy [6]. The advantage of the EIS method, to measure and analyze bioimpedance data, is based on the fact that current at low frequency (lower than 10 kHz) flows through the extracellular medium while current at high frequencies (over 100 kHz) flows through both, intracellular and extracellular medium, giving more information about the structure of the tissue.

There are previous studies about lung bioimpedance measurements. Toso *et al.* [8], through an impedance plethysmograph emitting 50 kHz alternating current, reported different impedance vector distribution in patients with lung cancer as compared with healthy patients. A reduced Xc and a smaller PA were found while R was preserved in patients with lung cancer. Nierman *et al.* [9] performed transthoracic bioelectrical impedance analysis to quantify extravascular lung water in animal models. Orschulik *et al.* [10] used non-invasive bioimpedance spectroscopy for the diagnosis of acute respiratory distress syndrome in an animal model.

Some previous studies have been carried out by our research group. Sanchez *et al.* [11] performed minimally invasive lung bioimpedance measurements to study the characteristics of lung bioimpedance (calibration and linearity) and the differences between inflated and deflated lung. Later Coll *et al.* [12] and Riu *et al.* [13] present studies demonstrating the potential for tissue differentiation through minimally invasive electrical impedance spectroscopy in lung using the 4-electrode method.

This manuscript (2$^{nd}$ phase) is the continuation of the previous study (1$^{st}$ phase) entitled ''Minimally invasive lung tissue differentiation using electrical impedance spectroscopy: a comparison of the 3- and 4- electrode methods'' performed by Company-Se *et al.* [14]. It compared the capacity of tissue differentiation of the minimally invasive electrical impedance spectroscopy in lungs using the 4-electrode method and the 3-electrode method. The results showed that both methods were adequate for tissue differentiation but 3-electrode method was more feasible for its clinical use because of its lower complexity, both in the catheter configuration (single electrode) and in the measurement system architecture. This previous study proposed for future works to increase the sample size for the differentiation between healthy lung tissue and neoplasm lung tissue using the 3-electrode method.

In this 2$^{nd}$ phase the measures performed in healthy lung tissue and in neoplasm lung tissue showed high inter-patient variability. This variability could hinder the tissue differentiation in lungs. There are several causes for this variability: 1) The measured absolute values of the R and Xc spectra are influenced by the tissue properties (the variable under measurement) but also by the geometry of the measurement (body shape of the patient and electrode positions). Geometrical factors such as body mass index (BMI) has been reported as one significant factor for changes in lung metrics [15]; 2) The breathing produces also impedance changes due to the considerable air volume change from inspiration to expiration and the influence of the non-conductive air contents in the lung tissue. This phenomenon could increase the inter-patient variability as depending on the patient, the breathing cycle will be different; 3) In the 3-electrode method, the electrode impedance of the catheter tip is measured and could increase the intra- and inter-patient variability due to poor contact of the catheter tip against the lung tissue and the liquids accumulation in the airways; 4) Another potential cause for inter-patient variability is cigarette consumption. It could contribute to the increase of the inter-patient dispersion. Smoking-induced epithelial abnormalities can serve both as targets for abnormal inflammatory responses and as initiators of deregulated inflammation. Cytokines, chemokines, and growth factors released by alveolar macrophages, lymphocytes, neutrophils, endothelial cells, and fibroblasts may act to promote epithelial dysfunction and malignant progression [16], [17].

While the ventilation-induced impedance modulation effect can be reduced using averaging, the other potential causes of variability need a calibration method capable to reduce this variability in order to perform tissue differentiation with success. For example, electrical impedance measures using the 3-electrode method in cardiology uses a floating measure within the heart (catheter completely

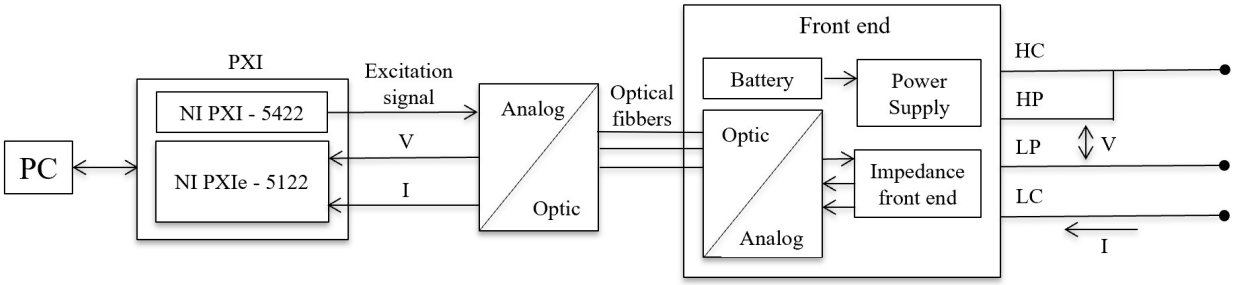



**FIGURE 1.** Schematic representation of the bioimpedance acquisition system.

surrounded by blood) to calibrate the geometrical factors in the bioimpedance measures obtained in contact with the myocardial walls [18], and also, partially, the electrode impedance effect. In lungs, a floating measure completely surrounded by air to calibrate is not viable due to the non-conductive property of the air and it is not feasible to locate the catheter in a place where the tip electrode will be surrounded by a well-known tissue and which will be affected by geometrical factors similar to the ones that will affect the tissue impedance measurements for each patient. For this reason, we proposed to acquire a bioimpedance measure in principal bronchus and use it to calibrate the lung tissue bioimpedance measures.

The aim of this study, by using minimally invasive electrical impedance spectroscopy with the 3-electrode method, is to propose a calibration method and to analyze the effect of this calibration in measures performed in the bronchi for tissue differentiation in different groups: healthy lung tissue (no radiological abnormalities in CT Thorax) and neoplasm lung tissue. Also, the possible differences in the impedance measurements in healthy tissue in smokers, non-smokers and ex-smokers will be verified to check if this factor would affect the ability to differentiate between healthy lung tissue and lung neoplasm.

## II. MATERIALS AND METHODS

### A. PARTICIPANTS

Minimally invasive EIS measures were taken in 99 patients (Age: 65 ± 16 yr; Weight: 76.8 ± 15.6 kg; BMI: 27.7 ± 5.5 $kgm^{-2}$) with a bronchoscopy indicated during the period between November 2021 and February 2022 at the ''Hospital de la Santa Creu i Sant Pau''. All of them underwent bioimpedance measurement. However, 30 of them had other characteristics than healthy lung tissue or neoplasm lung tissue such as emphysema or fibrosis. For this reason, out of the 99 patients measured by bioimpedance, only 69 were considered for analysis (healthy: 54 and neoplastic: 15).

The number of bioimpedance samples obtained in healthy lung tissue were 54 [(non-smokers: n = 22, Age: 59 ± 19 yr; Weight: 70.8 ± 16.6 kg; BMI: 26.7 ± 5.8 $kgm^{-2}$); smokers: n = 9, Age: 66 ± 7 yr; Weight: 83.5 ± 11.9 kg; BMI: 31.0 ± 4.3 $kgm^{-2}$); (ex-smokers: n = 23, Age: 71 ± 12 yr; Weight: 79.3 ± 13.8 kg; BMI: 27.5 ± 4.8 $kgm^{-2}$; years without smoking = 22 ± 11 yr)] while the number of samples obtained from neoplasm lung tissue were 15 (Age: 70 ± 9 yr; Weight: 75.3 ± 11.2 kg; BMI: 26.3 ± 4.1 $kgm^{-2}$).

Ethics approval was obtained from the Hospital de la Santa Creu i Sant Pau (CEIC-73/2020) according to principles of the Declaration of Helsinki for experiments with human beings. All patients proved signed informed consent.

### B. MEASUREMENT SYSTEM

The acquisition of bioimpedance measures were performed using a tetrapolar catheter (Medtronic 5F RF Marinr), 115 cm long with a diameter of 1.65 mm (5 F) and two skin electrodes (Ambu BlueSensor VLC ref: VLC-00-s/10 and 3M Company ref: 9160F) placed on the right side of the patients at the level of the ribs. Only the catheter tip electrode will be used in the measurements.

The measurement system is made up of 3 devices (Fig. 1): 1) an optically insulated battery-powered patient interface insulated front end (that includes the impedance front end); 2) a rugged PC platform based on a PXI system from National Instruments; and 3) an analog-optical interface front-end to connect the PXI with the insulated front end. An arbitrary waveform generator generates a multisine excitation signal that is composed of 26 frequencies between 1 kHz and 1 MHz. To ensure a current lower than the maximum allowable patient auxiliary current stablished in the IEC 60601-1:2005 (<1mA rms measured with the circuit proposed in the IEC 60601-1:2005) the front end includes an AC-coupled current source that attenuates the low-frequency components accordingly with the current limit pattern specified by this standard. The system was verified including the 26 frequency components (1 kHz – 1MHz) simultaneously.

The voltage (V(t)) and current (I(t)) are simultaneously acquired. Then, with the optical-analog interface connected to the PXI, the excitation is converted into an optical signal. The optical signal is then converted back into an electrical signal inside the front end. The voltage and current signals, optically transmitted from the front end to the optical-electrical interface, are acquired with the digitizer card. The acquisition

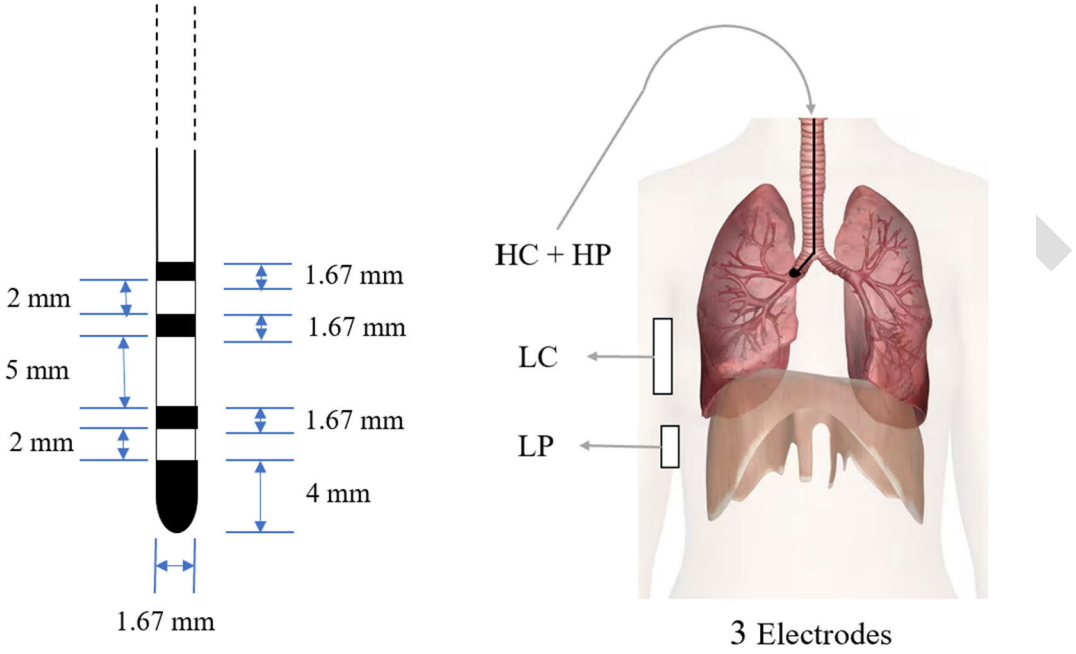



**FIGURE 2.** Right figure: Schematic representation of the 3-electrode method. In the 3-electrode method the LC and LP electrodes are placed on the skin using skin surface electrodes. Left figure: dimensions of the catheter. Only the tip electrode of the catheter is used to perform the measurements.

system takes simultaneous samples of voltage and current at 20 MSamples/s. From the acquired signals, 60 impedance spectra per second are obtained.

Bioimpedance measures were obtained using the 3-electrode method. To inject the current (HC) and detect the potential (HP) the electrode located at the tip of the catheter is used. The two skin electrodes are used as low current (LC) and loc potential (LP) electrodes (Fig. 2).

### C. MEASUREMENT PROTOCOL

Bronchoscopy, a procedure used to inspect the airways, was performed to obtain the bioimpedance measures. As part of the diagnostic process, radiological imaging technique (CT or PET/CT) were performed in each patient before bronchoscopy procedure. To obtain the bioimpedance measures, the catheter was inserted through a port of the bronchoscope. During the bronchoscopy, patients are placed in a supine position with the upper airways anaesthetized with topical 2% lidocaine. Moreover, intravenous sedation is provided with midazolam, fentanyl and propofol. During the process, measures in bronchial tissue, healthy lung tissue and neoplasm lung tissue, if applicable, were taken. The acquisition of the measures had a duration of 12 seconds.

### D. EIS MEASUREMENTS

To obtain the EIS measurements the system applies a multisine current signal and acquires the voltage and current signals. The Fast Fourier Transform (FFT) is used to obtain the ratio between the voltage and current coefficients of the FFT corresponding to each injected frequency.

The acquisition takes 12 s at 60 spectra per second. The 3-electrode measurements were calibrated with a measurement over a known resistor (600 Ohms) connected to the catheter tip and to the external electrode connectors.

### E. CALIBRATION USING BRONCHUS

To remove the geometrical factors of the patients a multiplicative factor calibration of the bioimpedance of the lung measures is proposed. The proposed method aims to calibrate the lung measures with respect to a measure performed in the bronchial tissue (principal bronchus) for each respective patient. A measurement in the bronchi is of no interest in clinical practice, therefore, impedance measurement in bronchial tissue offers the advantage of calibration while not losing relevant clinical information. Moreover, because of its low cell content, bronchial tissue should have a flat impedance spectrum, thus being suitable as calibration reference [14]. The obtained impedance modulus (|Z|) of the lung is divided by the mean value (mean value of impedance at each frequency, during a time interval) of |Z| of the bronchial tissue and then multiplied by a factor of 100 Ω, which is the expected impedance magnitude value obtained in the bronchi [12] (1). The PA calibrated of the lung measure is obtained by subtracting the original value of the PA of the lung measure minus the mean value of the PA obtained in the bronchi tissue

sample (2).

$$|Z(f,t)|_{calibrated} = 100 * |Z(f,t)|_{lung} / mean(|Z(f,t)|_{bronchi}) \quad (1)$$

$$PA_{calibrated}(f,t) = PA_{lung}(f,t) - mean(PA_{bronchi}(f,t)) \quad (2)$$

### F. DATA ANALYSIS

For tissue differentiation analysis among non-smoker, smoker and ex-smoker samples in healthy lung tissue samples as well as for tissue differentiation analysis between healthy lung tissue and neoplasm lung tissue the averaged spectra of the bioimpedance measurements, obtained using the 3-electrode method, throughout the acquisition time was used.

The frequency range chosen to visualize and analyze the data was 15 kHz – 307 kHz. The values from frequencies higher and lower than this range were discarded due to electrode effects at low frequency and capacitive coupling errors at high frequency. For tissue differentiation analysis the frequency of 15 kHz for |Z| and R and the frequency of 307 kHz for PA and Xc were chosen. These frequencies were chosen based on the higher distance between the means of the groups used to perform the tissue differentiation.

The normality of the distribution of the variables was determined by the Kolmogorv-Smirnov (healthy lung tissue samples) test and Shapiro-Wilk test (neoplasm lung tissue samples). The variables normally distributed are shown as the mean ± standard deviation (SD) and 95% confidence interval (CI) for the mean (lower bound and upper bound). Non-normally distributed variables are shown as statistic median (interquartile range, IQR) and minimum - maximum. One-way analysis of variance (ANOVA) was used to determine statistically significant differences in the |Z|, PA, R and Xc values among smokers, non-smokers and ex-smoker samples in healthy lung tissue. Repeated measures t-test was used to determine statistically significant differences in the |Z|, PA, R and Xc values between non-calibrated data and calibrated data among smokers, non-smokers and ex-smoker healthy lung samples. One-way analysis of variance (ANOVA, parametric data) and Mann–Whitney U test (non-parametric data) was used to determine statistically significant differences in the |Z|, PA, R and Xc values between healthy lung tissue and neoplasm lung tissue. In addition, the area under the Receiver Operating Characteristic (ROC) curve was used to measure the discriminative capacity of the non-calibrated and calibrated measure of |Z|, PA, R and Xc according to tissue classification (1: healthy lung tissue; 2: neoplasm lung tissue) by biopsy. Following the ROC analysis area under curve (AUC) above 0.9 is considered a very good model and AUC above 0.97 it is considered as excellent. A value less than 0.5 indicates the model is no better than random prediction.

The statistical software IBM® SPSS® version 28.0 (IBM Corp, Armonk, NY, United States) was used for data analysis. The level of statistical significance was set at $P < 0.05$.

## III. RESULTS

### A. MULTI-FREQUENCY RESPONSE FOR MINIMALLY INVASIVE HEALTHY LUNG TISSUE MEASUREMENTS

Fig. 3 shows the mean (continuous line) and SD (dashed lines) values of |Z|, PA, R and Xc plotted along the frequency range (15 kHz – 307 kHz) used for the measures obtained in healthy lung tissue divided in smoker patients (red), non-smokers patients (green) and ex-smoker patients (blue) for non-calibrated (left) bioimpedance measures and calibrated bioimpedance measures (right) showing an inter-sample reduction of the dispersion and increasing data homogeneity.

### B. TISSUE DIFFERENTIATION AMONG NON-SMOKERS, SMOKERS AND EX-SMOKERS PATIENTS IN CALIBRATED AND NON-CALIBRATED DATA

Table 1 lists the descriptive parameters, specified as the mean ± SD, 95% confidence interval for mean (lower bound and upper bound) of |Z|, PA, R and Xc and the results of the one-way ANOVA including the Fisher coefficient (F) for the minimally-invasive bioimpedance measures performed in healthy lung tissue (non-smokers: n = 22; smokers: n = 9; ex-smokers: n = 23) for the measures calibrated and non-calibrated. No statistically significant differences ($P > 0.05$) related to the smoking condition are found among the three groups analyzed for both calibrated and non-calibrated
data.

### C. MULTI-FREQUENCY RESPONSE FOR MINIMALLY INVASIVE HEALTHY LUNG TISSUE AND NEOPLASM LUNG TISSUE MEASUREMENTS

Fig. 4 shows the mean (continuous line) and SD (dashed lines) values of |Z|, PA, R and Xc plotted along the frequency range (15 kHz – 307 kHz) used for the measures obtained in healthy lung tissue (green) and neoplasm lung tissue (black) before (left) and after (right) calibration respectively. Results show an increase in the separation between tissues in |Z|, R and Xc, especially the first two.

### D. TISSUE DIFFERENTIATION BETWEEN HEALTHY LUNG TISSUE AND NEOPLASM LUNG TISSUE

Table 2 lists the descriptive parameters, specified as the mean ± SD, 95% confidence interval for mean (lower bound and upper bound) for normally distributed variables and specified as statistic median (interquartile range, IQR) and minimum – maximum for non-normally distributed variables of |Z|, PA, R and Xc and the results of the one-way ANOVA including the Fisher coefficient (F) and the Mann–Whitney U test results including the U statistic (U) for the minimally invasive bioimpedance measures performed in healthy lung tissue (n = 54) and in neoplasm lung tissue (n = 15) for the measures calibrated and non-calibrated. Statistically significant differences ($P < 0.001$) are found between

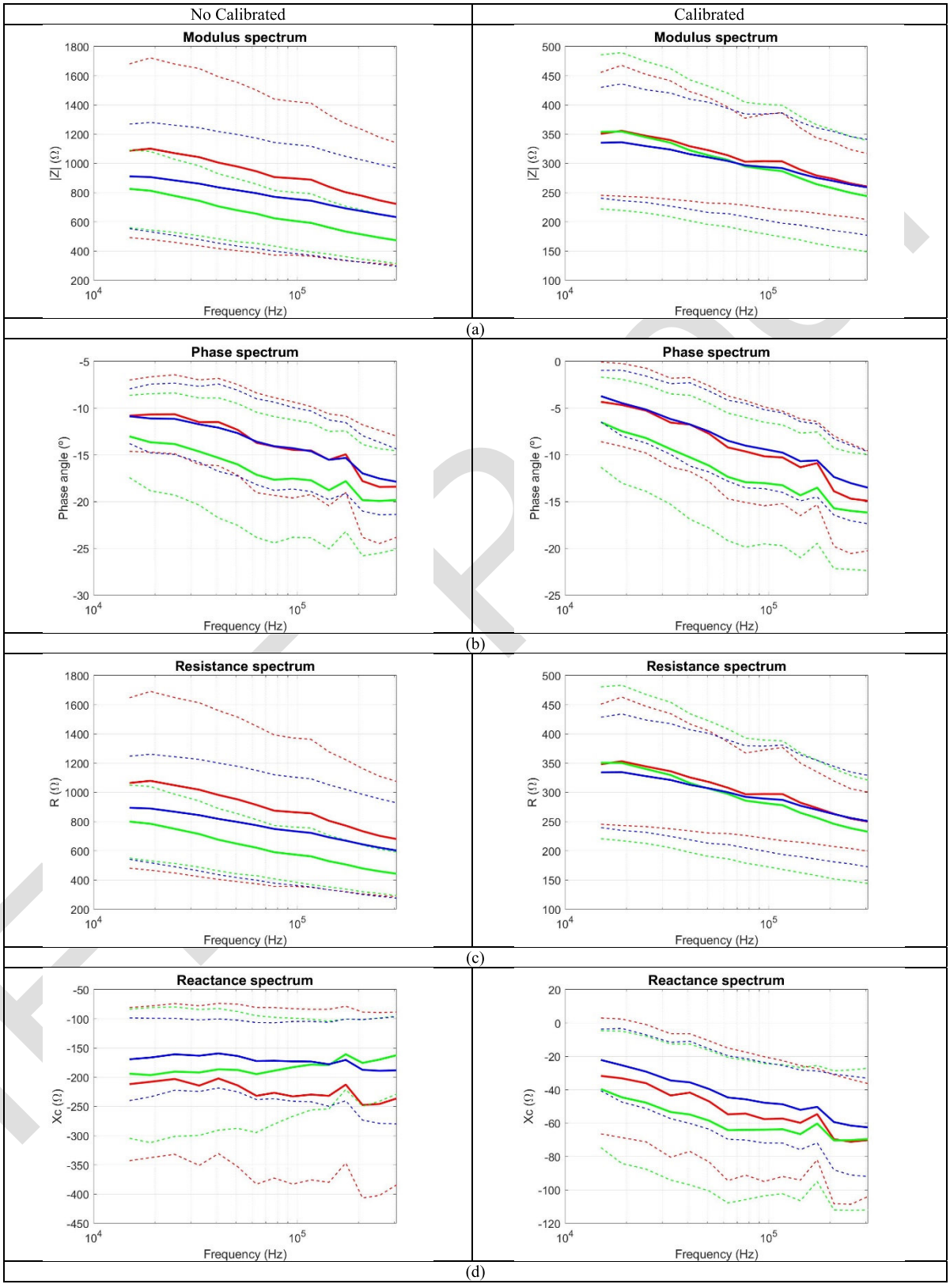



**FIGURE 3.** Results of the non-calibrated (left) and calibrated (right) mean (continuous line) and SD (dashed lines) parameters extracted from the bioimpedance signal along the different frequencies analyzed (15 kHz – 307 kHz). In order, (a) Modulus, (b) Phase angle, (c) Resistance and (d) Reactance of the bioimpedance of all the different measures taken in healthy lung tissue classified according to cigarette consumption. Green: non-smoker; blue: ex-smokers; red: smokers.

**TABLE 1.** Descriptions of bioimpedance measurements performed in healthy lung tissue for non-smokers, smokers and ex-smokers. The variables normally distributed are shown as mean ± SD, 95% confidence interval for mean (lower bound and upper bound). In addition, the Fisher (F) coefficient for variance analysis and the statistical significance (P) are also shown.

| No calibrated data | | | | | |
|---|---|---|---|---|---|
| | Mean ± SD<br>95% CI<br>(lower bound – upper bound) | | | | |
| | Non-smokers<br>(n = 22) | Smokers<br>(n = 9) | Ex-smokers<br>(n = 23) | F | P |
| \|Z\| (Ω) | 670.99 ± 308.96<br>(433.51 - 908.48) | 1086.97 ± 594.40<br>(630.07 - 1543.86) | 1016.66 ± 492.09<br>(638.40 - 1394.92) | 1.523 | 0.228 |
| PA (°) | -17.47 ± 6.77<br>(-22.68 – (-12.27)) | -18.40 ± 5.41<br>(-22.56 – (-14.24)) | -17.56 ± 4.08<br>(-20.70 – (-14.42)) | 1.000 | 0.375 |
| R (Ω) | 649.35 ± 289.72<br>(426.65 - 872.05) | 1064.34 ± 583.33<br>(615.95 - 1512.72) | 997.28 ± 487.75<br>(622.36 - 1372.20) | 1.646 | 0.203 |
| Xc (Ω) | -116.62 ± 64.24<br>(-166.00 – (-67.24)) | -236.36 ± 147.90<br>(-350.05 – (-122.68)) | -203.36 ± 115.60<br>(-292.22 – (-114.50)) | 1.929 | 0.156 |
| Calibrated data | | | | | |
| | Mean ± SD<br>95% CI<br>(lower bound – upper bound) | | | | |
| | Non-smokers<br>(n = 22) | Smokers<br>(n = 9) | Ex-smokers<br>(n = 23) | F | P |
| \|Z\| (Ω) | 298.72 ± 107.35<br>(216.21 - 381.24) | 350.61 ± 105.20<br>(269.74 - 431.47) | 304.16 ± 86.61<br>(237.58 - 370.74) | 0.156 | 0.856 |
| PA (°) | -14.88 ± 8.01<br>(-21.04 – (-8.72)) | -14.91 ± 5.34<br>(-19.01 – (-10.81)) | -12.53 ± 5.02<br>(-16.38 – (-8.67)) | 1.422 | 0.881 |
| R (Ω) | 296.07 ± 105.24<br>(215.18 - 376.96) | 348.24 ± 102.79<br>(269.23 - 427.25) | 302.71 ± 85.53<br>(236.96 - 368.46) | 0.127 | 0.881 |
| Xc (Ω) | -50.69 ± 30.68<br>(-74.27 – (-27.11)) | -70.10 ± 33.83<br>(-96.11 – (-44.10)) | -50.20 ± 22.79<br>(-67.72 – (-32.69)) | 0.255 | 0.776 |

healthy and neoplasm lung tissue for both calibrated and non-calibrated data.

### *E. EFFECTS OF CALIBRATION IN DATA VARIABILITY IN HEALTHY LUNG TISSUE AND IN NEOPLASM LUNG TISSUE*

Fig. 5 shows the effect of calibration in bioimpedance data variability for healthy lung tissue and neoplasm lung tissue respectively for |Z| and R at 15 kHz and for PA and Xc at 307 kHz. Results show a decrease in data dispersion within the same tissue group, especially in |Z| and R parameters, after the calibration of the bioimpedance data. Fig. 6 shows the receiver operating characteristic (ROC) curves for |Z|, PA, R and Xc before and after calibration for healthy lung tissue and neoplasm lung tissue groups. Results show an increase of the area under curve (AUC) after the calibration of the bioimpedance data in |Z|, R and Xc (AUC $>$ 0.96) and a decrease of the AUC in PA (AUC $<$ 0.95).

## IV. DISCUSSION

This project evaluates the need of the calibration of the minimally invasive EIS bioimpedance measures performed in lung tissue using a measure performed in bronchial tissue. Moreover, it evaluates the influence of cigarette smoking in healthy lung tissue bioimpedance measures as a possible cause of dispersion. Finally, it differentiates between healthy and neoplasm lung tissue and assesses the possible improvement of this differentiation using the calibration.

Lungs are organs that belong to the respiratory system whose principal function is to produce gas exchange. Structures from the respiratory system include trachea, bronchi and terminal bronchioles. Each of these structures has its own anatomical and histological characteristics [19]. Therefore, differences in bioimpedance measurements can be expected based not only on the type of tissue but also on its state.

This work reports the use of minimally invasive EIS in lungs through a bronchoscopy process using the 3-electrode method to differentiate among smoker, non-smokers and ex-smoker healthy lung tissue samples, in order to analyze its potential role in the measurements variability and to differentiate between healthy lung tissue and neoplasm lung tissue. Both tissue differentiations are used to evaluate the inter-patient variability in the mentioned groups and to evaluate the utility of calibration using a bioimpedance measure performed in a principal bronchus. This strategy of taking a measure to calibrate the other measures has been previously used in heart applications [18].

The inflammatory response due to cigarette consumption is not differentiable through bioimpedance EIS measures neither with the non-calibrated measurements nor with the calibrated measures. Therefore, the initial hypothesis that the smoking condition could be a cause of dispersion in the EIS-derived estimators can be discarded. According to **Fig. 3** the |Z| and R show a decrease in their values when calibrating with respect to a bronchi measurement while PA and Xc increase their values (nearer to 0 than the non-calibrated

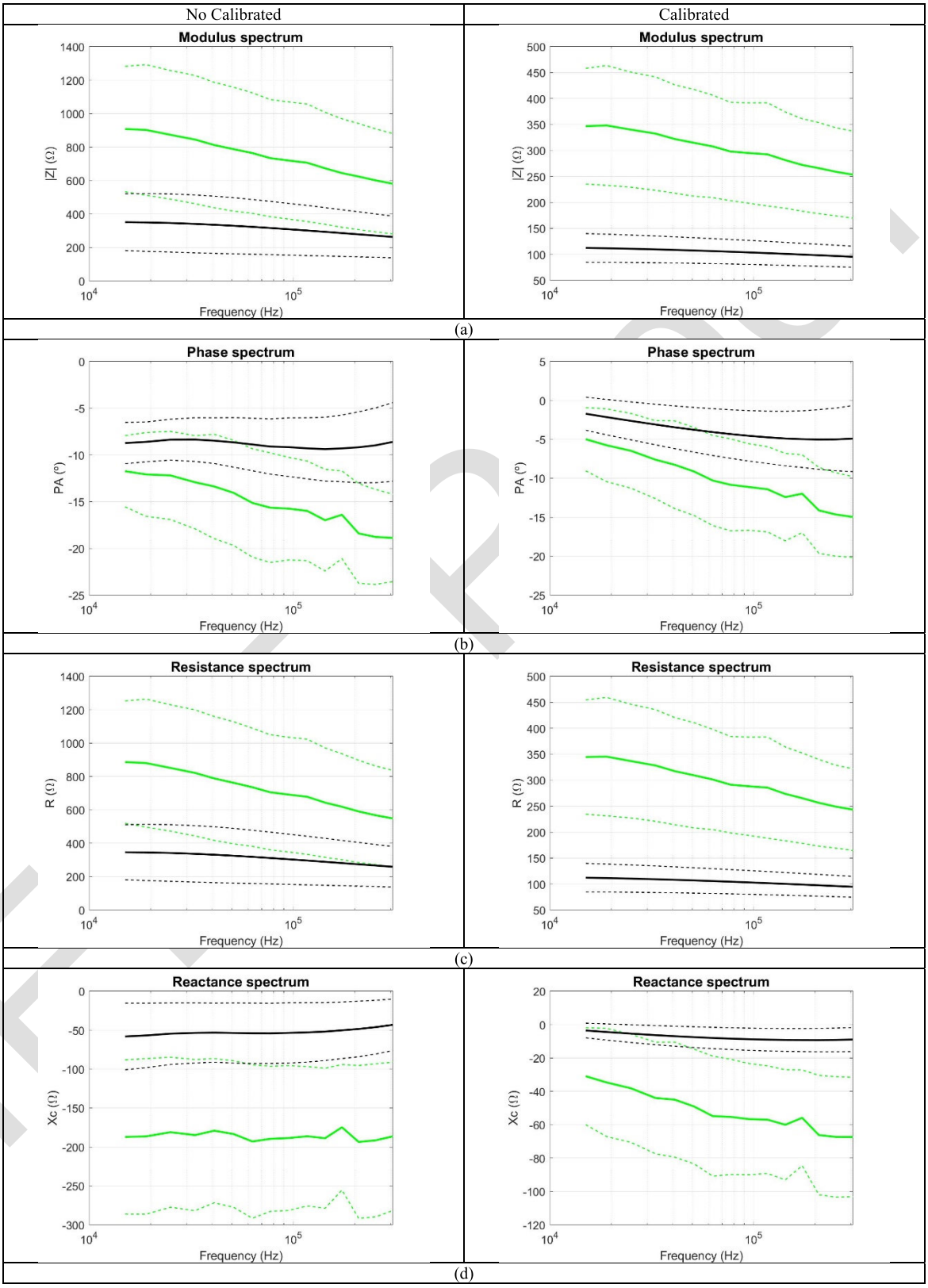



**FIGURE 4.** The mean (continuous line) and SD (dashed lines) values from the bioimpedance signal along the different frequencies analyzed before and after calibration. The (a) modulus, (b) phase angle, (c) resistance and (d) capacitive reactance. Green: healthy lung tissue; black: neoplasm lung tissue.

**TABLE 2.** Descriptions of minimally-invasive bioimpedance measurements for healthy lung tissue and neo-plasm lung tissue. The variables normally distributed are shown as mean ± SD, 95% confidence interval for mean (lower bound and upper bound) while that non-normally distributed data are shown as statistic median (interquartile range, IQR) and minimum-maximum. In addition, the statistic of the Mann Whitney U test (U), the Fisher (F) coefficient for variance analysis and the statistical significance (P) are also shown.

| | No calibrated data | | | | Calibrated data | | | |
|---|---|---|---|---|---|---|---|---|
| | Healthy (n = 54) | Neoplasm (n = 15) | U | P | Healthy (n = 54) | Neoplasm (n = 15) | F | P |
| \|Z\| (Ω) | 751.97 (759.66) (305.85 - 1823.16) | 284.95 (308.09) (134.68 - 668.53) | 44 | <0.001 | 283.26 ± 80.68 (238.58 - 327.94) | 112.38 ± 27.56 (97.12 - 127.65) | 64.735 | <0.001 |
| PA (°) | -15.98 ± 5.60 (-19.08 – (-12.88)) | -8.61 ± 4.21 (-10.94 – (-6.28)) | 36 | <0.001 | -11.61 ± 5.21 (-14.49 – (-8.72)) | -4.90 ± 4.24 (-7.25 – (-2.55)) | 47.597 | <0.001 |
| R (Ω) | 741.72 (731.03) (303.32 - 1799.63) | 283.41 (302.44) (133.39 - 650.79) | 44 | <0.001 | 281.77 ± 79.71 (237.63 - 325.91) | 112.26 ± 27.51 (97.02 - 127.49) | 65.099 | <0.001 |
| Xc (Ω) | -146.04 (139.73) (-405.34 – (-21.95) | -43.17 ± 33.05 (-61.48 – (-24.87)) | 39 | <0.001 | -44.46 ± 22.51 (-56.92 – (-31.99)) | -8.97 ± 7.18 (-12.94 – (-4.99)) | 39.142 | <0.001 |

data). The non-calibrated |Z| and R as well as the PA show slightly although non-significative higher values in those samples in which cigarette consumption is present. However, Xc present lower values in smoker samples. When we calibrate the bioimpedance measurements we show an intra-sample variability reduction. This variability reduction specially affects |Z| and R due to the geometrical dependence of R and the high correlation between |Z| and R [5], [6].

Emphasizing the importance of the analysis of R and Xc according to the theory of Lukaski *et al.* [6], [7], Piccoli *et al.* [20], and Lukaski *et al.* [21] we selected the frequencies (15 kHz and 307 kHz) to check the hypothetical differentiation among non-smokers, smokers and ex-smokers healthy lung tissue samples following the calculation of the maximum distance between means of the three groups. From the bioimpedance parameters, R describes the behavior of the medium through which the current flows while Xc describes the capacitive component of the cell membranes. The values of |Z| and PA are dependent of R and Xc [6], [7].

The significance of the test was determined with the p-value which is the probability of obtaining test results at least as extreme as the result observed, assuming that the null hypothesis is correct. Therefore, considering the level of significance set, results will be statistically significant if a $P < 0.05$ is obtained in the test. Regarding tissue differentiation among non-smokers, smokers and ex-smokers healthy lung tissue samples, one-way ANOVA reported non-significant results ($P > 0.05$) for all variables (|Z|, PA, R and Xc) for both, the non-calibrated and the calibrated measures. No post-hoc test has been done as no significant results have been found. The Fisher coefficient parameter (F) represents the relationship between the inter-group variance and the intra-group variance. Therefore, a higher F coefficient indicates a higher inter-group variance than intra-group variance [22]. According to the results obtained in **Table 1**, the F coefficient obtained in the non-calibrated data is higher in |Z|, R and Xc than in the calibrated data. In contrast, F coefficient in PA obtained in the calibrated data is higher than in the non-calibrated data. Therefore, the statistical results obtained show that the effect of cigarette consumption should not be considered to perform tissue differentiation through bioimpedance analysis. Moreover, results show an intra-sample dispersion reduction with the effect of calibration, especially in |Z| and R, which depend on the geometrical factors.

Regarding tissue differentiation between healthy lung tissue and neoplasm lung tissue we have taken all the healthy lung tissue samples without considering the tabaco habits as it has been demonstrated that this factor is not significant ($P > 0.05$). Lung cancer is a highly complex neoplasm and comprise several histological types. The groups most frequently are the non-small cell lung cancer (NSCLC) such as adenocarcinoma and squamous carcinoma, followed by small cell lung cancer (SCLC) [23]. Lung cancer are the results of the accumulation of genetic and epigenetic changes, including abnormalities of the inactivation of tumour-suppression genes and the activation of oncogenes [24]. For the tissue differentiation between healthy lung tissue and neoplasm lung tissue all cancer types have been included in the same group so we assume that the remaining dispersion in neoplasm lung tissue might be due to the differences within lung cancer types. We have selected the frequencies (15 kHz and 307 kHz) that offered us a better discriminatory response between healthy lung tissue and neoplasm by taking the frequency with the maximum difference between the mean of the healthy lung tissue and the mean of the neoplasm lung tissue. We have also visualized the mean impedance spectrum and SD of the healthy lung tissue samples and the neoplasm lung tissue samples between the frequency range analyzed (15 kHz – 307 kHz) with the data non-calibrated and calibrated to show the effects of the calibration. According to the results obtained in **Fig. 4** the calibration of the bioimpedance measures with respect to a measure performed in bronchi reduces the intra-group variability and, in consequence, increases the inter-patient distance in both, the healthy lung tissue and the neoplasm lung tissue, especially in |Z| and R, which are the two parameters that are dependent on geometrical factors (**Fig. 5**). Results obtained show a higher |Z| and R and a lower PA and Xc in healthy lung tissue than in neoplasm lung tissue. Moreover, |Z| and R show higher difference between the lower frequencies and the higher frequencies in healthy lung tissue than in neoplasm lung tissue.

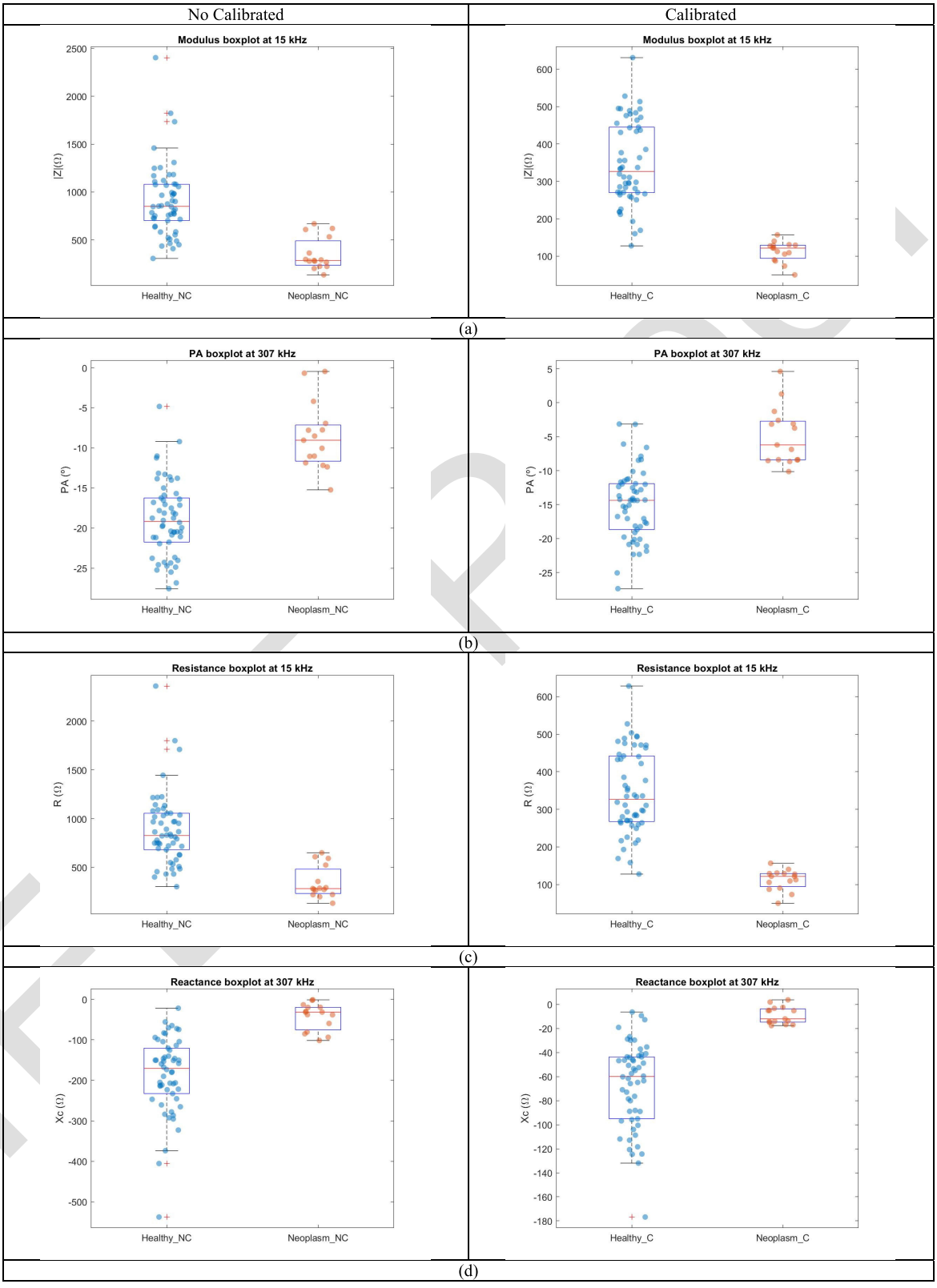
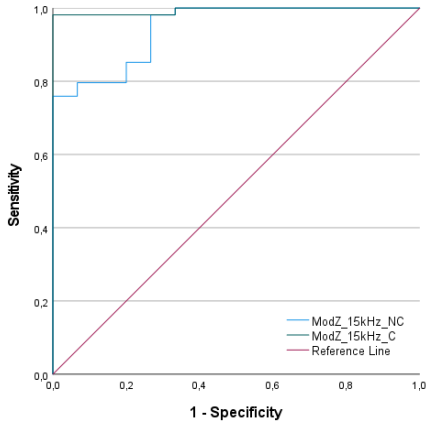
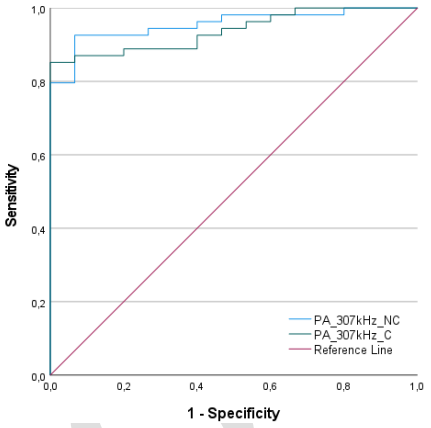



**FIGURE 5.** Boxplot of bioimpedance calibrated (C) and non-calibrated (NC) data of healthy lung tissue and neoplasm lung tissue for (a) |Z| and (c) R at 15 kHz and for (b) PA and (d) Xc at 307 kHz. The central mark of each box indicates the median, and the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively. The whiskers extend to the most extreme data points that are not considered outliers. In addition, the bioimpedance data values for the calibrated (blue) and non-calibrated (orange) measures. Vertical axis are different for the calibrated and non-calibrated data for better data visualization.



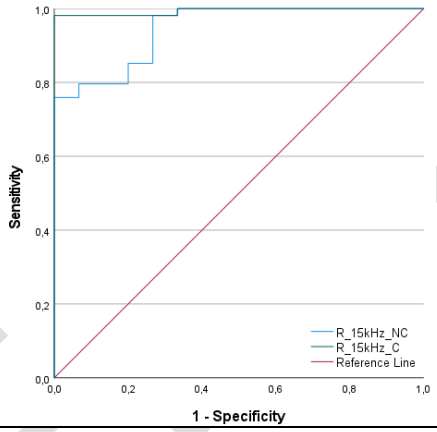

| | |Z|-NC | |Z|-C |
|---|---|---|
| **AUC** | 0.946 | 0.994 |
| **SE** | 0.0028 | 0.007 |
| **Asymptotic Sig.** | < 0.001 | < 0.001 |
| **Sensitivity (%)** | 79.6 | 98.1 |
| **Specificity (%)** | 93.3 | 67.0 |
| **\|Z\|-VALUE (Ω)** | 627.45 | 128.30 |

(a)



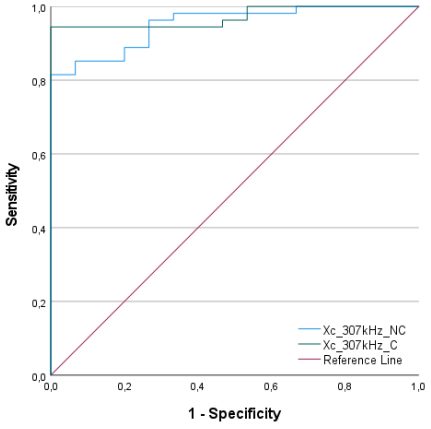

| | PA-NC | PA-C |
|---|---|---|
| **AUC** | 0.956 | 0.938 |
| **SE** | 0.0023 | 0.027 |
| **Asymptotic Sig.** | < 0.001 | < 0.001 |
| **Sensitivity (%)** | 92.6 | 87.0 |
| **Specificity (%)** | 86.7 | 86.7 |
| **PA-VALUE (°)** | 12.27 | 8.58 |

(b)




| | R-NC | R-C |
|---|---|---|
| **AUC** | 0.946 | 0.994 |
| **SE** | 0.0028 | 0.007 |
| **Asymptotic Sig.** | < 0.001 | < 0.001 |
| **Sensitivity (%)** | 79.6 | 98.1 |
| **Specificity (%)** | 93.3 | 67.0 |
| **R-VALUE (Ω)** | 619.72 | 128.22 |

(c)




| | Xc-NC | Xc-C |
|---|---|---|
| **AUC** | 0.952 | 0.972 |
| **SE** | 0.0024 | 0.018 |
| **Asymp.Sig.** | < 0.001 | < 0.001 |
| **Sensitivity (%)** | 85.2 | 94.4 |
| **Specificity (%)** | 93.3 | 53.3 |
| **Xc-VALUE (Ω)** | 93.95 | 13.14 |

(d)

**FIGURE 6.** **Receiver operating characteristic (ROC) curves to assess the predictive ability of the different electrical impedance parameters before and after calibration between healthy and neoplasm lung tissue. In (a) the results of the |Z| before calibration (NC) and after calibration (C) at 15 kHz. In (b) the results of the PA before calibration (NC) and after calibration (C) at 307 kHz. In (c) the results of the R before (NC) and after (C) calibration at 15 kHz. In (d) the results of the Xc before (NC) and after (C) calibration at 307 kHz.**

EIS assumes that current at low frequency flows through the extracellular space while current at high frequencies flows through both, intracellular and extracellular space. Moreover, healthy lung tissue is composed of alveolar epithelial and endothelial cells separated by a thin basement membrane and interstitial space. Interstitial space is a non-conductive medium, than the neoplasm lung tissue. These two main characteristics produce a higher |Z| and R in healthy lung tissue

than in neoplasm lung tissue. Lung cancer produce multiples histological changes of the normal bronchial mucosa. Proliferation of epithelial cells with abundant cytoplasm and vesicular nuclei, intercellular bridging, thickening of alveolar septa and others pathological changes [25]. The morphologic features in neoplasm lung tissue seem to contribute to lose their capacitive behavior which is translated into a PA and Xc flat mean impedance spectrum, as compared to the healthy lung tissue mean impedance spectrum (**Fig. 4**). Regarding tissue differentiation between healthy lung tissue and neoplasm lung tissue, one-way ANOVA for the calibrated data and Mann–Whitney U test for the non-calibrated data (**Table 2**) show statistically significant differences between the two groups (healthy and neoplasm lung tissue, $P < 0.001$). These statistical differences are probably due to the histological differences between both groups by minimally-invasive EIS measurements. Focusing only in the calibrated data results in **Table 2** show higher significance in |Z| and R than in PA and Xc as the F coefficient is higher in the first two parameters.

The study has shown that there is an effect on the measurement when calibrating, reducing the dispersion of the measurements (**Fig. 5**). Calibration doesn't change the outcome of the hypothesis test, showing a statistically significant difference in both cases, but the higher-F coefficient (Fisher coefficient from one-way ANOVA test, used for comparing the factors of the total deviation) than U (statistic from Mann–Whitney U test, used to assess whether two sampled groups are likely to derive from the same population) suggests stronger separation between the groups (**Table 2**), which is highly significant ($P < 0.01$) for both calibrated and non-calibrated measures. On the other hand, according to the ROC curve analysis, (**Fig. 6**) we have observed that the area under the curve (AUC) is equally excellent in all the variables (AUC $> 0.9$) both calibrating and not calibrating, although higher AUC values are observed when calibrating. After calibrating, the AUC is greater than 0.96 for all cases except in PA. The |Z|, R and Xc increase the sensitivity (true positive fraction) and decrease the specificity (false positive fraction) after calibration. Only PA showed a decrease in sensitivity maintaining its specificity (**Fig. 6**). Considering that PA has a trigonometric relationship between R and Xc and that these improve with calibration, the authors recommend performing the calibration of the measurements with respect to the bronchi.

In the previous study performed by Company-Se *et al.* [14] we performed tissue differentiation between healthy lung tissue and bronchi tissue. We proposed continuing with the study by including neoplasm lung tissue for lung tissue differentiation. Results obtained in **Table 2** show that minimally invasive electrical impedance spectroscopy using the 3-electrode method is able to discriminate with both, calibrated data (not considering geometrical factors) and with non-calibrated data. In future studies we aim to include other lung pathologies with other histological characteristics.

## V. CONCLUSION

In conclusion, results of the healthy lung tissue bioimpedance measurements show that there are no significant differences between healthy lung tissue among smoker, non-smoker and ex-smoker measures, which was initially stated as a possible cause of EIS measurement dispersion in lungs. Then, to perform tissue differentiation between healthy lung tissue and neoplasm lung tissue the effect of tobacco habit will not be considered. Also, this effect will not be considered in our future studies.

On the other hand, we found that there is a statistically significant difference in both calibrated and non-calibrated measurements at 15 kHz (|Z| and R) and 307 kHz (Xc and PA) between healthy and neoplasm lung tissue. This shows that minimally invasive electrical impedance spectroscopy measurements using the 3-electrode method are able to discriminate between healthy lung and neoplasm both with and without calibration.

Calibration has, however, been demonstrated to reduce data variability and increase the tissue state separation capability, which will be useful in future studies when including other pathologies with similar pathological mechanisms.

Moreover, significant differences are found between calibrated and non-calibrated paired samples of smoker, non-smoker ex-smoker and neoplasm lung tissue showing that calibration is beneficial to reduce intra-sample variability.

The authors recommend calibrating the measures obtained with respect to the bronchi given that it is demonstrated that it increases the sensitivity of the 3-electrode minimally invasive electrical impedance spectroscopy for tissue differentiation.

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