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Second hand smoke and oscillometry
Omnia cum pretio (Everything with a price)

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Key words:
- Oscillometry
- Second hand smoke
- Tobacco smoke

Oscillometry (also known as the forced oscillation technique) measures the mechanical properties of the respiratory system (upper and intrathoracic airways, lung tissue and chest wall) during quiet tidal breathing, by the application of an oscillating pressure signal (input or forcing signal), most commonly at the mouth. Physiologically oscillometry is fundamentally a different measurement to traditional lung function measurements, i.e. spirometry and lung volumes, single breath diffusion (DLCO), specific airways conductance (sGAW) and usually compliments them.1-3

Although thresholds for positive bronchodilatation have been established (-40% Rrs,+50% Xrs,-80% AX), z-scores are recommended for future definition of a significant response,1 along with an effect size that is a measure that tells you how important a difference is. The method has potential sources of error (patient breathing, bacterial filters, artificial airways) and verification (reactive test load has not been established).4

Nevertheless, oscillometry can be used in various clinical settings which include clinical lung function laboratories, field testing, home monitoring and intensive care. Oscillometry measurements have mostly been applied in airways diseases and paediatric lung diseases, where oscillometry may have the most widespread clinical application. The short testing times and ease of administration for subjects are potential advantages in this setting.

In this issue of the journal, Kairi et al,5 show that acute exposure of healthy non-smokers to second-hand smoke (SHS) leads to alterations of resting breathing mechanics suggestive of a likely broncho-constrictive response to the irritative inhalant, successfully captured by impulse oscillometry (IOS). The authors used the IOS system by Jaeger which in relation to forced oscillation pseudorandom technique causes harmonic distortion and waveforms that induce large volume fluctuations in relation to the underlying breathing volume, especially when the respiratory system behaves in a substantially nonlinear fashion, i.e. in the presence of severe airflow obstruction or cyclic lung recruitment and derecruitment.6 The authors for the first time report on early lung pathology detected by IOS caused by SHS (early not mild COPD), and since pre-post measurements were performed with the same apparatus, with the same technique on the same subject, bias is minimized (coherence function not reported).

Coherence is another important parameter and is used to determine
the validity and quality of the test results. It reflects the reproducibility of the impedance measurements. It is a value between 0 and 1 and, ideally, should be >0.8 at 5 Hz and >0.9 at 20 Hz for the measurement to be considered valid. However, it is important to note that these values are for adults, and there are no standard values reported in children. Coherence can be decreased because of improper technique, irregular breathing, glottis closure, and swallowing.

Tobacco smoke contains thousands of xenobiotics harmful to human health. Their irritant, toxic and carcinogenic potential has been well documented. Passive smoking or exposure to SHS in public places, including workplace, poses major medical problems. In a Greek study, a 1-h exposure to SHS levels at bar/restaurant significantly increased the white blood cells (WBC) for at least 4 h following the exposure time. This effect of SHS on WBC has dose-response characteristics and should be considered to prescribing complete blood count.

In another study, several xenobiotic metabolizing genes especially the EPHX1 (epoxyl hydrolase) low activity diploityp with additional effect modifiers CYP1A1 and GSTT1 (glutathione S tranferase T1) may modify the impact of second-hand tobacco smoke and ambient air pollutants, polycyclic aromatic hydrocarbons and PM2.5, on acute bronchitis in preschool children. Also, short-term cigarette smoke exposure predisposes the lung to secondary injury (second hit).

Following the 2006 legislation (banning smoking in all public places in Scotland), asthma admissions decreased in both younger children (-0.36% [-0.67 to -0.05], p=0.021) and older children (-0.68% [-1.00 to -0.36], p<0.0001), and in children from the most deprived (-0.49% [-0.87 to -0.11], p=0.011) and intermediate deprived (-0.70% [-1.17 to -0.23], p=0.0043) area quintiles, but not in those from the least deprived area quintile.

The recent outbreak of the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19), has led to a worldwide pandemic. Both acute and second-hand smoke were found to increase ACE2 expression in the bronchus with Inhaled corticosteroids decreasing ACE2 expression in the lower airways. No significant effect of genetics on ACE2 expression was observed, but a strong association of DNA- methylation with ACE2 and TMPRSS2- (serine protease) mRNA expression was identified in the bronchus, emphasizing the impact of SHS.

In a COPD study, early intervention triggered by worsening of oscillometric indices was not associated with any differences in hospitalisation, or symptoms. However, there was a significant reduction in repeat hospitalisations, leading to significantly reduced healthcare costs. Self-reported chronic bronchitis or emphysema or COPD was associated with higher pulmonary resistance and lower pulmonary reactance measured by IOS, both among subjects with and without COPD according to GOLD criteria. IOS may have the potential to detect pathology associated with COPD earlier than spirometry.

Impulse oscillometry parameters demonstrated greater sensitivity compared with spirometry for monitoring reversibility of airway obstruction and the effect of maintenance therapy. Impulse oscillometry may facilitate early treatment dose optimization and personalized medicine for chronic obstructive pulmonary disease patients.

Small airway wall area percentage (Aw% 7-9), an EB-OCT (endobronchial optical coherence tomography) parameter, correlated significantly with Fes (resonant frequency) and R5-R20 (difference in resistance between 5 and 10 Hz) in early COPD, but not spirometry.

The cut-offs for small airway disease are difference in resistance at 5 Hz and resistance at 20 Hz greater than 0.07 kPa/(L/s), reactance at 5 Hz less than -0.12 kPa/(L/s), Fes greater than 14.14 Hz, and area under reactance curve between 5 Hz and resonant frequency greater than 0.44 kPa/L.

The reported values although did not reach these thresholds, were statistically significant and show that even short exposure acute effects of SHS are detrimental on lung health, particularly if exposure is intermittent and repetitive.

CONFLICT OF INTEREST
None

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δράσεις στον τύπο της αναπνοής, στην κεντρική αναπνευστική ωσή και στη μηχανική της ήρεμης αναπνοής σε υγιείς νεαρούς ενήλικες Πνεύμων 2018; 31(3):151-8.
The role of diabetes mellitus and obesity in COVID 19 patients

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Key words:
- COVID 19
- Diabetes Mellitus
- Obesity

ABSTRACT
Several risk factors (old age, hypertension, cardiovascular disease, immunodeficiency) have been related with coronavirus disease 2019 (COVID 19). Among them, Diabetes Mellitus (DM) and obesity are recognized to increase the susceptibility and severity of the infection. A higher inflammatory response observed in these patients, the immune system dysfunction and increased expression of angiotensin converting enzyme 2 (ACE2) which is a the target for SARS-CoV-2, contribute to the worse outcome of diabetic and obese patients. Adequate blood glucose control improves the prognosis, while critically ill COVID 19 patients with high metabolic risk should be monitored for new onset DM.


INTRODUCTION

According to the World Health Organization, 820,000 deaths approximately are attributed to COVID-19 (coronavirus disease 2019) until August 2020. The disease is caused by SARS-CoV-2 virus and its clinical presentation ranges from mild respiratory symptoms to severe pneumonia with respiratory failure, Acute Respiratory Distress Syndrome (ARDS) and septic shock¹. Elderly patients, mainly males, patients with comorbidities (hypertension, cardiovascular disease, diabetes mellitus [DM], chronic respiratory disease), patients with cancer and immunosuppression are at increased risk for severe COVID 19. However, emerging data show that young people without comorbidities are also susceptible and obesity is a significant risk factor for severe disease.

DM AND OBESITY: PREVALENCE, INCIDENCE AND MORTALITY

DM is related to severe COVID 19 with a prevalence that varies and ranges from 7-20% in Chinese studies, to 33-35% in studies from Italy while Bhatraju et al, in USA, in a small study with 24 patients reported a prevalence of 58%².³. In a meta-analysis of 21 studies from China with
47.344 patients, 7.7% of COVID 19 patients had DM, while in a retrospective study with 1591 critically ill patients in Lombardy-Italy, the incidence of DM was 17%4,5. In New York City in a study with 5700 patients the most common comorbidities were hypertension (56.6%), obesity (41.7%) and DM (33.8 %)6. An even 50% rise of mortality is reported in diabetics with COVID 19 while in the past DM was related to increased complications and mortality in Severe Acute Respiratory Syndrome (SARS-CoV-1) epidemic in 2002-2003 in China, in influenza A (H1N1) pandemic in 2009 and in Middle East Respiratory Syndrome Coronavirus (MERS-CoV) outbreaks in 2012 in Middle East7. In the USA, according to the Centers for Disease Control and Prevention (CDC) the prevalence of DM in patients hospitalized in the ICUs was 32% while the Chinese Center for Disease Control and Prevention reported a case fatality rate of 7.3% in diabetic patients8,9. Zeng-Hong Wu et al in a meta-analysis, which included 9 studies, reported that DM was significantly related to COVID 19 mortality with odds ratio of 1.7 (p = 0.006) and in another study with 1099 patients 16% of patients who had severe disease and only 6% of those with mild disease were diabetic10-11. Ian Huang et al, in a meta-analysis of 30 studies concluded that DM was related to a worse outcome (Risk Ratio [RR] 2.38), increased mortality (RR 2.12), severe disease (RR 2.45), ARDS (RR 4.64) and disease progression (RR 3.31). Old age (p = 0.003) and hypertension (p <0.001) influenced the association of DM to worse prognosis, which was stronger in young people and patients without hypertension according to a meta-regression analysis12.

Obesity seems to be an independent risk factor for severe COVID 19, especially in the younger patients and usually coexists with DM increasing further the risk of these patients13. Severe obesity (BMI ≥35 kg/m²) was independently related to mortality in 200 COVID 19 patients in New York City, while in Chinese studies obese patients had a 3.4 higher probability of having severe disease (OR 3.40, p <0.007) in comparison to patients with normal body weight14-16. In a Greek multi-centre observational study in 8 ICUs where 90 patients were included, 34.4% of patients were obese and 18.9% were diabetics. The mean BMI was 28 kg/m² and younger patients (<55 years) had a significantly higher BMI (30.8 kg/m²). The incidence of DM and mortality were similar among different age groups. In this cohort, 30.8% of patients who died and 16.7% of those who remained hospitalized in the ICUs were diabetics while none of the patients who were discharged had DM (p = 0.074). Additionally, obesity was more common in patients who died (46.2%), rather than in survivors (26.7%) (p=0.077)17.

**PATHOPHYSIOLOGY**

The pathophysiologic mechanisms that contribute to increased incidence of severe COVID 19 and worse prognosis for obese and diabetic patients are not fully understood. DM predisposes to infections due to immune system disorder and especially alterations of innate immunity. The higher frequency of DM in the older patients and the common coexistence with cardiovascular disease and other comorbidities may interpret the worse prognosis of these patients18. Also, angiotensin converting enzyme 2 (ACE2) may be implicated in the increased severity of COVID 19 in diabetics. The virus SARS CoV-2 (COVID-19), like SARS CoV-1, enters host cells after binding to ACE2. ACE2 receptors are expressed in epithelial cells of lung, upper respiratory system, intestine, kidneys, heart, vessels and pancreas. ACE2 decreases the levels of angiotensin II and interleukin 6 (IL-6) and increases angiotensin 1-7 which has anti-inflammatory effect and causes vasodilation12. After its connection with SARS CoV-2, ACE 2 is decreased, losing its protective role3. DM and hypertension are characterized by activation of the renin-angiotensin system in different tissues, while the increased expression of ACE2 that is observed in diabetic patients may predispose to COVID 1919. Also, diabetic patients are often treated with angiotensin-converting enzyme inhibitors (ACEI) and angiotensin receptor blockers (ARB) which increase the levels of ACE2 facilitating possibly the entry of SARS-CoV-2 in alveolar cells12. Additionally, increased levels of proinflammatory cytokines and especially of interleukin 1 (IL-1), interleukin 6 (IL-6) and tumor necrosis factor – α (TNF-α) in diabetic patients contribute to cytokine “storm” that characterizes severe COVID 19 and leads to rapid deterioration12. Patients with uncontrolled DM and COVID 19 have higher values of inflammation markers (polymorphonuclear neutrophils, CRP, d-dimers, ferritin, Erythrocyte Sedimentation Rate [ESR], IL-6 and fibrinogen) compared with patients with well controlled DM that exhibit a milder inflammatory response18,20. Respectively, obesity is related to chronic, low-grade, subclinical inflammation due to cytokines and hormones released from adipose tissue, while often is accompanied by decreased physical activity leading to insulin resistance and immunological disorder7,18. Finally, the decreased vital capacity and compliance of
the use of ACEI/ARB in patients with COVID 19, DM and hypertension the findings are conflicting. The European Society of Cardiology, the European Society of Hypertension and the American Heart Association do not recommend the discontinuation of ACEI/ARB in patients with DM and COVID 19, as currently there are no sufficient data that ACEI/ARB increase the risk of severe disease or the susceptibility to the infection. On the contrary in experimental models, ARB exhibit a possible protective effect on the lung and losartan is currently being evaluated for the treatment of COVID 19\textsuperscript{3,18}.

In conclusion, both DM and obesity are related to severe COVID 19 (ARDS, ICU hospitalization, mechanical ventilation) and increased mortality. Possible responsible pathophysiologic mechanisms include the severe inflammatory response in diabetics and obese patients, the over expression of ACE2 and the immune system dysfunction. The optimal glycemic control and the monitoring for new onset DM are essential in critically ill COVID 19 patients.

CONFLICT OF INTEREST
None.
REFERENCES


Immediate effects of second-hand smoke on the mechanics of tidal breathing

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Key words:
- Respiratory Resistance,
- Respiratory mechanics,
- Impulse Oscillometry,
- Passive smoking,
- SHS

Abbreviations:
ATS/ERS: American Thoracic Society/European Respiratory Society
AX: Reactance Area
BMI: Body Mass Index
COPD: Chronic obstructive pulmonary disease
EBC: Exhaled Breath Condensate
FeNO: Exhaled Nitrogen Oxide
fdr: Frequency Dependence of Resistance
FRC: Functional Residual Capacity
fre: Resonant Frequency
IOS: Impulse Oscillometry
IQR: Inter-quartile range
RS: Resistance at 5 Hz
R10: Resistance at 10 Hz
R20: Resistance at 20 Hz
SD: Standard deviation
SHS: Second-Hand Smoke
10/250: exposure for 10 min. in a 250 μg/m3 concentration of PM2.5
20/250: exposure for 20 min. in a 250 μg/m3 concentration of PM2.5
10/500: exposure for 10 min. in a 500 μg/m3 concentration of PM2.5
20/500: exposure for 20 min. in a 500 μg/m3 concentration of PM2.5
X5: Distal Capacity Reactance at 5 Hz
X20: Reactance at 20 Hz
Z5: Total Impedance at 5 Hz

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ABSTRACT
BACKGROUND: Limited studies have examined the implications of Second-Hand Smoke (SHS) on lung function; majority used traditional diagnostic lung function tests requiring forced respiratory manoeuvres. Aim of our study was to assess the immediate effects of exposure to SHS on the respiratory mechanics during tidal breathing.

METHODS: 20 healthy non-smokers 18-45-years-old participated in four exposure sessions; 10 minutes in 250 μg/m3 PM2.5 (10/250), 20 minutes in 250 μg/m3 PM2.5 (20/250), 10 minutes in 500 μg/m3 PM2.5 (10/500) and 20 minutes in 500 μg/m3 PM2.5 (20/500). A pre and an immediately post exposure IOS measurement were obtained. Differences in Impulse Oscillometry (IOS) parameters pre and post exposure for each session were assessed with paired t-tests or Wilcoxon tests. Differences between exposure sessions were assessed with mixed linear models. Analysis was performed in Stata 14.

RESULTS: Statistically significant differences were observed in IOS parameters in all exposure sessions, with most changes observed in 10/500 and least in 20/500 session. Analysis between sessions showed significantly different results between 20/250 compared to 10/250 session in many IOS parameters, while 10/500 differed statistically significantly to 10/250 only in R10 inspiratory.

CONCLUSIONS: Present study is the first to show that acute exposure of healthy non-smokers to SHS leads to alterations of resting breathing mechanics, successfully captured by IOS. Alterations were expressed by increased Resistance of peripheral and central airways, findings suggestive of a likely broncho-constrictive response to the irritative inhalant. A mild, linear effect of exposure duration was found, while no clear effect was observed for the level of exposure.


INTRODUCTION
Second-Hand Smoke (SHS) is defined as the mixture of fine particles and gases emitted by the burning cigarette (sidestream) and through the
smoker’s expiration (mainstream). It is composed of thousands of compounds known for their irritative, toxicant and carcinogenic properties1,2.

The adverse health effects associated with exposure to second hand smoke were first published in 19813,4, showing that spouses of smokers were at increased risk for lung cancer; since then, scientific evidence and concerning epidemiological data have led to the development of protective legislation and educational campaigns.

Most previous studies on SHS exposure have been performed in animals, cell cultures, or in humans in laboratory settings, using traditionally burning cigarettes or smoking machines to simulate the SHS5. Majority of studies have examined epidemiological data, symptoms6,7, association with cardiovascular and respiratory disorders5, effects on pregnancy and foetus, as well as physical and cognitive development of children and adolescents8. To quantify the effects, other studies have simulated exposure to specific conditions such as inside the cars9, bars and restaurants10. Limited studies have examined the implications on lung function; some have examined the chronic occupational effect on exercise testing11, while others examined exhaled nitrogen oxide (FeNO) and biomarkers in the exhaled breath condensate (EBC)12. The majority of those examining respiratory mechanics have enabled the traditional diagnostic lung function tests that require forced respiratory manoeuvres, such as spirometry and body plethysmography, however with conflicting results8,13. Only Schivinski et al. have used both forced and resting breathing techniques, such as spirometry and Impulse Oscillometry (IOS), to study the respiratory mechanics in children and adolescents who were chronically exposed to SHS at home, in comparison with those non exposed14.

To date, the gold standard test for diagnosis and lung function evaluation is considered spirometry and the flow-volume loop, which however mainly reflect the abnormalities (obstruction) of the conducting (large and medium size) airways; when the earlier FEV1 reduction is captured by spirometry, a substantial area of small airways has already been affected by the disease process, thus the “silent lung zone”15. Furthermore, the forced spirometric manoeuvres greatly depend on the subjects’ collaboration, a disadvantage by default; in contrast, IOS allows the evaluation of the respiratory mechanics by superimposing multiple frequencies over resting (tidal) breathing, a great advantage being the easy, effort independent technique in addition to the continuous measurement and the possibility for intra-breath analysis of the inspiratory and expiratory component respectively15. While of low specificity, not useful for diagnostic purposes, the method yields however a high sensitivity16 making it an ideal test to detect mild disorders, evaluate the response to treatment and bronchoprovocation challenge, as well as for detecting the impact of exposure to various hazardous inhalants including cigarette smoke17.

To the authors knowledge, there is currently a literature gap in studies that have examined the effect of exposure to SHS on respiratory mechanics during tidal breathing as well as the effect of duration and level of exposure. Therefore, the present study aimed to assess the immediate effects of exposure to SHS on the respiratory mechanics of healthy non-smokers, in a controlled environment, during tidal breathing and to examine the effect of duration and level of exposure, in addition to the intra-breath analysis of this effect.

METHODS

A total of 20 individuals, males and females were voluntarily recruited from Athens area. Eligibility to participate in the study was based on the following criteria: Non-smokers, aged 18–45 years, healthy (insignificant medical history, normal physical examination), BMI <30 kg/m2, spirometry within normal limits according to the American Thoracic Society/European Respiratory Society (ATS/ERS) Task Force 2005, no current or recent illness or acute infection (< 4 weeks prior to enrolment), no recent surgery (<2 months prior to enrolment), no medication intake including contraceptives, no pregnancy or lactation.

Study design

A four-session experimental study was designed to measure the effect of exposure to SHS on healthy non-smokers, who were individually exposed one at a time. The sessions took place in four separate days and included a pre and an immediately post exposure IOS measurement:

- Session (10/250): exposure for 10 minutes in a 250 μg/m3 concentration of PM2.5
- Session (20/250): exposure for 20 minutes in a 250 μg/m3 concentration of PM2.5
- Session (10/500): exposure for 10 minutes in a 500 μg/m3 concentration of PM2.5
- Session (20/500): exposure for 20 minutes in a 500 μg/m3 concentration of PM2.5.
Ethics approval & informed consent

Participants were informed of the study’s aim and their right to access and withdraw at any time. Their informed consent was given in writing prior to the study. Ethics approval was issued by the Ethics Committee of the National and Kapodistrian University of Athens School of Medicine (protocol number 5109/17.02.2012).

Exposure room & equipment

Participants were exposed one at a time in a 20 m³ room. The room had an interior door to the rest of the office apartment and a window to the exterior, both closed during exposure to keep the levels of pollution stable and as designed per each session.

SHS pollution was created using a custom-made smoking machine. The levels of SHS pollution in terms of PM₂.₅ concentrations were monitored using an AM 510 SIDEPAK calibrated according to the manufacturer’s guidelines. The PM₂.₅ concentration was created using one and two cigarettes for the 250 and 500 session respectively; in case an adjustment to a lower level was needed, the desired concentration was achieved by simple room ventilation.

For standardization purposes the same cigarette brand was used (nicotine: 0.8mg tar: 10mg) throughout all sessions and for all participants.

IOS measurement was performed using a Viasys Jaeger Masterscreen IOS system (Franklin Lake, NJ, USA), according to ATS/ERS guidelines¹⁷.

Participants were asked to take an upright, neutral sitting position, with legs uncrossed, apply a nose clip and lightly support their cheeks by own hands and finally, they were instructed to breathe normally at the Functional Residual Capacity (FRC) level for 90 seconds, avoiding to swallow, cough, or sigh.

IOS parameters measured

Total Impedance at 5 Hz (Z₅), Resistance at 5, 10 and 20 Hz (R₅, R₁₀ and R₂₀), Distal Capacity Reactance at 5 Hz (X₅), Reactance at 20 Hz (X₂₀), Resonant Frequency (fres) and Reactance Area (AX) were measured, in addition to their inspiratory and expiratory components. Additionally, the parameters R₅ and R₂₀ were used to assess whether R₅ > R₂₀, to identify the presence of Frequency Dependence of Resistance.

Statistical analysis

Statistical analysis was performed for each exposure session, looking at differences of the IOS parameters within each session. Additionally, analysis was performed between exposure sessions to assess if the different exposure conditions affected the IOS parameters differently.

Normality of the data was assessed with the Shapiro-Wilk statistic. Descriptive characteristics are presented as mean and standard deviation for the normally distributed variables while median and interquartile range (IQR), defined as the 25th and 75th percentile, are presented for the non-normally distributed variables. Effect sizes have been calculated by implementing Cohen’s d formula.

To look at differences in the IOS parameters within each exposure session, taking into account the measurement before each session (pre) and the measurement after each session (post), paired t-tests for the normally distributed variables and Wilcoxon tests for the non-normally distributed variables were performed.

To test for differences between exposure sessions, mixed linear models were introduced. Based on our study design, repeated measurements were on two levels; on the participant level (the same individuals participated in all exposure sessions) and on the IOS parameter level (two measurements per individual were obtained within each exposure session). To remove one level and make models less complicated, we calculated the difference of the measurements post - pre exposure of the IOS parameters for each individual. This difference for each IOS parameter was introduced as the dependent variable in each mixed model. The different exposure sessions were introduced as a single categorical variable in the models, with the lowest exposure session (10 minutes in 250 μg/m³) as the reference category. Regression coefficients (β), their standard errors and their corresponding p-values are presented.

Statistical significance was set at p <0.05, while all p-values presented are two-tailed. Analysis was performed in Stata 14 (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, Texas: StataCorp LP).

RESULTS

A total of 20 volunteers participated in the study, 9 males and 11 females. Median age of participants was 31 years old. Participants were of a normal weight (mean BMI 21.9) (Table 1).

Exposure of 10 minutes in 250 μg/m³

Statistically significant differences were observed between pre vs post the 10/250 session in X₂₀, X₂₀ ex-
piratory and expiratory. Mean $X_{20}$ was 0.09 kPa/(L/s) pre compared to 0.10 kPa/(L/s) post exposure ($p=0.03$) with a medium effect size of 0.53 and a mean percentage change of 18.5% among participants. Mean $X_{20}$ expiratory was 0.085 kPa/(L/s) pre compared to 0.094 kPa/(L/s) post exposure ($p=0.03$) with a medium effect size of 0.54 and a mean percentage change of 7.1%. Finally, median fres expiratory was 10.45 (1/s) pre compared to 10.11 (1/s) post exposure ($p=0.01$) with a relatively large effect size of 0.70 and a mean percentage change of -5.8% among participants, showing a decrease after the exposure (Tables 2 and 4).

**Exposure of 20 minutes in 250 $\mu$g/m$^3$**

Statistically significant differences were observed between pre vs post the 20/250 session in $X_5$ inspiratory,

### TABLE 1. Participants' characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total N=20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, n (%)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>9 (45%)</td>
</tr>
<tr>
<td>Female</td>
<td>11 (55%)</td>
</tr>
<tr>
<td>Age (years) (Mean, SD)</td>
<td>30.5 ± 4.4</td>
</tr>
<tr>
<td>Height (cm) (Mean, SD)</td>
<td>176.3 ± 11.6</td>
</tr>
<tr>
<td>Weight (kg) (Median, IQR)</td>
<td>65 (54.5-84)</td>
</tr>
<tr>
<td>Body mass index (BMI) (Mean, SD)</td>
<td>21.9 ± 2.8</td>
</tr>
<tr>
<td>Flow volume</td>
<td></td>
</tr>
<tr>
<td>FEV1% (Mean, SD)</td>
<td>102.1 ± 11.2</td>
</tr>
<tr>
<td>PEF% (Mean, SD)</td>
<td>104.9 ± 14.0</td>
</tr>
</tbody>
</table>

SD: standard deviation, IQR: inter-quartile range

### TABLE 2. IOS parameters pre and post exposure, for the four different exposure sessions

<table>
<thead>
<tr>
<th>IOS parameter</th>
<th>10/250</th>
<th>20/250</th>
<th>10/500</th>
<th>20/500</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±std/ Median (IQR)</td>
<td>Mean±std/ Median (IQR)</td>
<td>Mean±std/ Median (IQR)</td>
<td>Mean±std/ Median (IQR)</td>
</tr>
<tr>
<td>Z5 kPa/(L/s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>0.35±0.08 0.34 (0.30–0.39)</td>
<td>0.34 (0.30–0.43)</td>
<td>0.33 (0.31–0.42)</td>
<td>0.35 (0.31–0.41)</td>
</tr>
<tr>
<td>Post</td>
<td>0.35±0.08 0.32 (0.26–0.38)</td>
<td>0.32 (0.29–0.40)</td>
<td>0.31 (0.28–0.39)</td>
<td>0.33 (0.30–0.39)</td>
</tr>
<tr>
<td>R5 kPa/(L/s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>0.33±0.08 0.32 (0.26–0.38)</td>
<td>0.32 (0.29–0.40)</td>
<td>0.31 (0.28–0.39)</td>
<td>0.33 (0.30–0.39)</td>
</tr>
<tr>
<td>Post</td>
<td>0.34±0.07 0.32 (0.29–0.40)</td>
<td>0.33 (0.30–0.39)</td>
<td>0.31 (0.28–0.39)</td>
<td>0.33 (0.30–0.39)</td>
</tr>
<tr>
<td>R5 inspiratory kPa/(L/s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>0.29 (0.26–0.37) 0.30 (0.27–0.34)</td>
<td>0.30 (0.28–0.37)</td>
<td>0.30 (0.27–0.34)</td>
<td>0.31 (0.28–0.37)</td>
</tr>
<tr>
<td>Post</td>
<td>0.32 (0.27–0.36) 0.30 (0.28–0.37)</td>
<td>0.31 (0.28–0.37)</td>
<td>0.30 (0.27–0.33)</td>
<td>0.30 (0.26–0.38)</td>
</tr>
<tr>
<td>R5 expiratory kPa/(L/s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>0.35±0.09 0.34 (0.30–0.40)</td>
<td>0.34 (0.30–0.41)</td>
<td>0.33 (0.29–0.42)</td>
<td>0.35 (0.30–0.42)</td>
</tr>
<tr>
<td>Post</td>
<td>0.35±0.08 0.34 (0.30–0.41)</td>
<td>0.35 (0.30–0.42)</td>
<td>0.32 (0.29–0.45)</td>
<td>0.31 (0.29–0.45)</td>
</tr>
<tr>
<td>R10 kPa/(L/s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>0.28 (0.26–0.33) 0.29 (0.25–0.34)</td>
<td>0.29 (0.26–0.35)</td>
<td>0.29 (0.26–0.35)</td>
<td>0.3±0.07 0.3</td>
</tr>
<tr>
<td>Post</td>
<td>0.30 (0.26–0.37) 0.30 (0.26–0.37)</td>
<td>0.30 (0.27–0.36)</td>
<td>0.30 (0.27–0.36)</td>
<td>0.31±0.09</td>
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<tr>
<td>R10 inspiratory kPa/(L/s)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>0.26 (0.23–0.30) 0.27 (0.23–0.30)</td>
<td>0.27 (0.25–0.34)</td>
<td>0.27±0.06 0.03</td>
<td>0.28±0.07 0.27</td>
</tr>
<tr>
<td>Post</td>
<td>0.27 (0.24–0.31) 0.27 (0.25–0.34)</td>
<td>0.29±0.07</td>
<td>0.28±0.08</td>
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</tr>
<tr>
<td>R10 expiratory kPa/(L/s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>0.32±0.08 0.31 (0.26–0.38)</td>
<td>0.31 (0.28–0.40)</td>
<td>0.31 (0.28–0.39)</td>
<td>0.31 (0.27–0.44)</td>
</tr>
<tr>
<td>Post</td>
<td>0.32±0.08 0.32 (0.28–0.40)</td>
<td>0.33 (0.29–0.40)</td>
<td>0.31 (0.27–0.44)</td>
<td>0.29 (0.27–0.42)</td>
</tr>
<tr>
<td>R20 kPa/(L/s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>0.29 (0.25–0.33) 0.30 (0.24–0.34)</td>
<td>0.29 (0.27–0.36)</td>
<td>0.29 (0.27–0.36)</td>
<td>0.31±0.07 0.52</td>
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<tr>
<td>Post</td>
<td>0.31 (0.27–0.36) 0.31 (0.25–0.35)</td>
<td>0.32 (0.28–0.36)</td>
<td>0.32 (0.28–0.36)</td>
<td>0.31±0.09</td>
</tr>
<tr>
<td>R20 inspiratory kPa/(L/s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>0.29±0.07 0.28 (0.23–0.31)</td>
<td>0.29±0.06 0.0497</td>
<td>0.29±0.07 0.52</td>
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<tr>
<td>Post</td>
<td>0.29±0.05 0.29 (0.24–0.34)</td>
<td>0.31±0.08</td>
<td>0.30±0.08</td>
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<tr>
<td>R20 expiratory kPa/(L/s)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
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<td>0.33±0.09 0.57</td>
<td>0.32 (0.26–0.38)</td>
<td>0.6</td>
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<td>IOS parameter</td>
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<td>20/250</td>
<td>10/500</td>
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<td>--------</td>
</tr>
<tr>
<td></td>
<td>Mean±std/ Median (IQR)</td>
<td>p-value</td>
<td>Mean±std/ Median (IQR)</td>
<td>p-value</td>
</tr>
<tr>
<td>X5 kPa/(L/s) Pre</td>
<td>-0.10±0.04</td>
<td>0.79</td>
<td>-0.09±0.03</td>
<td>0.13</td>
</tr>
<tr>
<td>Post</td>
<td>-0.10±0.04</td>
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<td>-0.10±0.03</td>
<td></td>
</tr>
<tr>
<td>X5 inspiratory kPa/(L/s) Pre</td>
<td>-0.11±0.04</td>
<td>0.999</td>
<td>-0.09±0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>Post</td>
<td>-0.11±0.04</td>
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<td>-0.10±0.03</td>
<td></td>
</tr>
<tr>
<td>X5 expiratory kPa/(L/s) Pre</td>
<td>-0.10±0.04</td>
<td>0.27</td>
<td>-0.09±0.03</td>
<td>0.35</td>
</tr>
<tr>
<td>Post</td>
<td>-0.09±0.04</td>
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<td>-0.10±0.03</td>
<td></td>
</tr>
<tr>
<td>X10 kPa/(L/s) Pre</td>
<td>-0.004±0.03</td>
<td>0.27</td>
<td>0.01±0.03</td>
<td>0.999</td>
</tr>
<tr>
<td>Post</td>
<td>0.02±0.03</td>
<td></td>
<td>0.01±0.03</td>
<td></td>
</tr>
<tr>
<td>X10 inspiratory kPa/(L/s) Pre</td>
<td>-0.003±0.03</td>
<td>0.36</td>
<td>0.004±0.03</td>
<td>0.999</td>
</tr>
<tr>
<td>Post</td>
<td>0.001±0.03</td>
<td></td>
<td>0.004±0.03</td>
<td></td>
</tr>
<tr>
<td>X10 expiratory kPa/(L/s) Pre</td>
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<td>0.06</td>
<td>0.003±0.03</td>
<td>0.4</td>
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<tr>
<td>Post</td>
<td>-0.01±0.03</td>
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<td>0.001±0.03</td>
<td></td>
</tr>
<tr>
<td>X20 kPa/(L/s) Pre</td>
<td>0.09±0.04</td>
<td>0.03</td>
<td>0.10±0.03</td>
<td>0.38</td>
</tr>
<tr>
<td>Post</td>
<td>0.10±0.04</td>
<td></td>
<td>0.09±0.04</td>
<td></td>
</tr>
<tr>
<td>X20 inspiratory kPa/(L/s) Pre</td>
<td>0.10 (0.07–0.11)</td>
<td>0.16</td>
<td>0.1±0.04</td>
<td>0.75</td>
</tr>
<tr>
<td>Post</td>
<td>0.10 (0.08–0.12)</td>
<td></td>
<td>0.10±0.04</td>
<td></td>
</tr>
<tr>
<td>X20 expiratory kPa/(L/s) Pre</td>
<td>0.085±0.05</td>
<td>0.03</td>
<td>0.10±0.03</td>
<td>0.11</td>
</tr>
<tr>
<td>Post</td>
<td>0.09±0.05</td>
<td></td>
<td>0.09±0.04</td>
<td></td>
</tr>
<tr>
<td>fres [1/s] Pre</td>
<td>9.77 (8.85–12.24)</td>
<td>0.41</td>
<td>9.41 (8.44–11.19)</td>
<td>0.63</td>
</tr>
<tr>
<td>Post</td>
<td>9.89 (8.45–12.70)</td>
<td></td>
<td>9.50 (8.31–11.64)</td>
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</tr>
<tr>
<td>fres inspiratory [1/s] Pre</td>
<td>10.51±2.10</td>
<td>0.65</td>
<td>9.55 (8.67–11.00)</td>
<td>0.54</td>
</tr>
<tr>
<td>Post</td>
<td>10.38±1.89</td>
<td></td>
<td>9.81 (8.66–11.20)</td>
<td></td>
</tr>
<tr>
<td>fres expiratory [1/s] Pre</td>
<td>10.45 (8.94–13.73)</td>
<td>0.01</td>
<td>9.14 (8.17–11.65)</td>
<td>0.48</td>
</tr>
<tr>
<td>Post</td>
<td>10.11 (8.50–12.19)</td>
<td></td>
<td>9.09 (8.40–12.13)</td>
<td></td>
</tr>
<tr>
<td>AX [kpa/L] Pre</td>
<td>0.24 (0.16–0.34)</td>
<td>0.13</td>
<td>0.23 (0.11–0.28)</td>
<td>0.19</td>
</tr>
<tr>
<td>Post</td>
<td>0.25 (0.14–0.31)</td>
<td></td>
<td>0.21 (0.11–0.28)</td>
<td></td>
</tr>
<tr>
<td>AX inspiratory [kpa/L] Pre</td>
<td>0.27±0.15</td>
<td>0.61</td>
<td>0.20 (0.13–0.32)</td>
<td>0.22</td>
</tr>
<tr>
<td>Post</td>
<td>0.26±0.14</td>
<td></td>
<td>0.24 (0.15–0.27)</td>
<td></td>
</tr>
<tr>
<td>AX expiratory [kpa/L] Pre</td>
<td>0.23 (0.16–0.35)</td>
<td>0.12</td>
<td>0.19 (0.10–0.26)</td>
<td>0.11</td>
</tr>
<tr>
<td>Post</td>
<td>0.21 (0.13–0.34)</td>
<td></td>
<td>0.19 (0.11–0.33)</td>
<td></td>
</tr>
<tr>
<td>R5-R20 Pre</td>
<td>0.027±0.04</td>
<td>0.24</td>
<td>0.028±0.04</td>
<td>0.21</td>
</tr>
<tr>
<td>Post</td>
<td>0.023±0.04</td>
<td></td>
<td>0.022±0.04</td>
<td></td>
</tr>
</tbody>
</table>
with a mean value of -0.096 pre compared to -0.10 kPa/(L/s) post exposure (p=0.04) and a medium effect size of 0.50. Mean percentage change of X5 inspiratory among participants was 7.5% (Tables 2 and 4).

**Exposure of 10 minutes in 500 μg/m³**

Statistically significant differences were observed between pre vs post the 10/500 session in R10 inspiratory, R10 expiratory, R20 and R20 inspiratory. Mean R10 inspiratory pre was 0.27 kPa/(L/s) compared to 0.29 kPa/(L/s) post exposure (p=0.03), with a medium effect size of 0.51 and a mean percentage change of 7.7%. Median R10 expiratory pre was 0.31 kPa/(L/s) compared to 0.33 kPa/(L/s) post exposure (p=0.03), with a medium effect size of 0.52 and a mean percentage change of 6.5%. Median R20 pre was 0.29 kPa/(L/s) compared to 0.32 kPa/(L/s) post exposure (p=0.03), with a medium effect size of 0.45 and a mean percentage change of 6.8% among participants. Finally, mean R20 inspiratory was 0.29 kPa/(L/s) pre compared to 0.31 kPa/(L/s) post exposure (p=0.0497), with a medium effect size of 0.47 and a mean percentage change of 6.9% among participants (Tables 2 and 4).

**Exposure of 20 minutes in 500 μg/m³**

There were no statistically significant differences observed between measurements pre and post this exposure session among participants (Table 2).

Thirteen additional individuals participated in this exposure session to assess if differences were to be observed with more participants. The 13 additional participants had similar characteristics with the rest of our sample; 8 (62%) were males and 5 (38%) females, with a mean age of 31 years old and a mean BMI of 24 (data not shown). After the addition of the 13 participants, statistically significant differences were observed between pre vs post the 20/500 session in X5 inspiratory, with a median of -0.1 kPa/(L/s) pre compared to -0.11 kPa/(L/s) post exposure (p=0.03) and a small effect size of 0.27. The mean percentage change of X5 inspiratory among participants was -15.9%, showing a decrease in X5 inspiratory post exposure (Table 3).

Statistically significant differences were observed between the exposure sessions of 20/250 and 10/250 and the IOS parameters R10 inspiratory, X20, X20 expiratory, fres expiratory, AX and AX expiratory. In particular R10

<table>
<thead>
<tr>
<th>IOS parameter</th>
<th>Mean ± std / Median (IQR)</th>
<th>p-value</th>
<th>Mean change (mean % change)</th>
<th>Effect size (absolute)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z5 kPa/(L/s)</td>
<td>Pre: 0.33 (0.29 - 0.40)</td>
<td>0.28</td>
<td>0.01 (3.1%)</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>Post: 0.35 (0.30 - 0.42)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R5 kPa/(L/s)</td>
<td>Pre: 0.32 (0.28 - 0.38)</td>
<td>0.19</td>
<td>0.01 (3.8%)</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>Post: 0.33 (0.28 - 0.39)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R5 inspiratory kPa/(L/s)</td>
<td>Pre: 0.30 (0.26 - 0.33)</td>
<td>0.34</td>
<td>0.01 (3.2%)</td>
<td>0.22</td>
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<tr>
<td></td>
<td>Post: 0.30 (0.27 - 0.35)</td>
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</tr>
<tr>
<td>R5 expiratory kPa/(L/s)</td>
<td>Pre: 0.35 (0.29 - 0.42)</td>
<td>0.51</td>
<td>0.01 (2.7%)</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>Post: 0.34 (0.29 - 0.43)</td>
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</tr>
<tr>
<td>R10 kPa/(L/s)</td>
<td>Pre: 0.29 (0.25 - 0.34)</td>
<td>0.13</td>
<td>0.01 (3.6%)</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>Post: 0.29 (0.26 - 0.36)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>R10 inspiratory kPa/(L/s)</td>
<td>Pre: 0.26 (0.22 - 0.30)</td>
<td>0.16</td>
<td>0.01 (4.3%)</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>Post: 0.27 (0.24 - 0.32)</td>
<td></td>
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<tr>
<td>R10 expiratory kPa/(L/s)</td>
<td>Pre: 0.31 (0.27 - 0.39)</td>
<td>0.49</td>
<td>0.01 (2.4%)</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>Post: 0.31 (0.27 - 0.41)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>R20 kPa/(L/s)</td>
<td>Pre: 0.30 (0.25 - 0.34)</td>
<td>0.46</td>
<td>0.01 (2.5%)</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>Post: 0.31 (0.25 - 0.35)</td>
<td></td>
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<tr>
<td>IOS parameter</td>
<td>Mean ± std / Median (IQR)</td>
<td>p-value</td>
<td>Mean change (mean % change)</td>
<td>Effect size (absolute)</td>
</tr>
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<td>-------------------------------</td>
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</tr>
<tr>
<td>R20 inspiratory kPa/(L/s) Pre</td>
<td>0.28 (0.24 - 0.32)</td>
<td>0.44</td>
<td>0.01 (3.5%)</td>
<td>0.18</td>
</tr>
<tr>
<td>R20 inspiratory kPa/(L/s) Post</td>
<td>0.28 (0.25 - 0.33)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R20 expiratory kPa/(L/s) Pre</td>
<td>0.32 (0.26 - 0.36)</td>
<td>0.79</td>
<td>-0.001 (0.3%)</td>
<td>0.03</td>
</tr>
<tr>
<td>R20 expiratory kPa/(L/s) Post</td>
<td>0.32 (0.27 - 0.35)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X5 kPa/(L/s) Pre</td>
<td>-0.11 ± 0.05</td>
<td>0.45</td>
<td>0.002 (-0.8%)</td>
<td>0.13</td>
</tr>
<tr>
<td>X5 kPa/(L/s) Post</td>
<td>-0.11 ± 0.04</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X5 inspiratory kPa/(L/s) Pre</td>
<td>-0.10 (-0.12 - -0.09)</td>
<td>0.03</td>
<td>0.01 (-15.9%)</td>
<td>0.27</td>
</tr>
<tr>
<td>X5 inspiratory kPa/(L/s) Post</td>
<td>-0.11 (-0.12 - -0.07)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>X5 expiratory kPa/(L/s) Pre</td>
<td>-0.11 ± 0.05</td>
<td>0.35</td>
<td>0.004 (-2%)</td>
<td>0.16</td>
</tr>
<tr>
<td>X5 expiratory kPa/(L/s) Post</td>
<td>-0.10 ± 0.05</td>
<td></td>
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</tr>
<tr>
<td>X10 kPa/(L/s) Pre</td>
<td>-0.01 (-0.02 - 0.01)</td>
<td>0.89</td>
<td>0 (-10.9%)</td>
<td>0</td>
</tr>
<tr>
<td>X10 kPa/(L/s) Post</td>
<td>0 (-0.02 - 0.02)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X10 inspiratory kPa/(L/s) Pre</td>
<td>3.68E-19 ± 0.03</td>
<td>0.17</td>
<td>-0.004 (18.5%)</td>
<td>0.24</td>
</tr>
<tr>
<td>X10 inspiratory kPa/(L/s) Post</td>
<td>-0.004 ± 0.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X10 expiratory kPa/(L/s) Pre</td>
<td>0 (-0.02 - 0.02)</td>
<td>0.88</td>
<td>-0.0003 (10.6%)</td>
<td>0.02</td>
</tr>
<tr>
<td>X10 expiratory kPa/(L/s) Post</td>
<td>0 (-0.02 - 0.02)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X20 kPa/(L/s) Pre</td>
<td>0.09 ± 0.04</td>
<td>0.79</td>
<td>-0.001 (0.19%)</td>
<td>0.05</td>
</tr>
<tr>
<td>X20 kPa/(L/s) Post</td>
<td>0.09 ± 0.04</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X20 inspiratory kPa/(L/s) Pre</td>
<td>0.10 ± 0.03</td>
<td>0.93</td>
<td>0.0003 (0.6%)</td>
<td>0.02</td>
</tr>
<tr>
<td>X20 inspiratory kPa/(L/s) Post</td>
<td>0.10 ± 0.04</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X20 expiratory kPa/(L/s) Pre</td>
<td>0.09 ± 0.04</td>
<td>0.84</td>
<td>-0.001 (-1.1%)</td>
<td>0.04</td>
</tr>
<tr>
<td>X20 expiratory kPa/(L/s) Post</td>
<td>0.09 ± 0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fres [1/s] Pre</td>
<td>10.54 (8.88 - 12.61)</td>
<td>0.94</td>
<td>0.27 (3%)</td>
<td>0.17</td>
</tr>
<tr>
<td>fres [1/s] Post</td>
<td>10.63 (8.87 - 12.71)</td>
<td></td>
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</tr>
<tr>
<td>fres inspiratory [1/s] Pre</td>
<td>10.24 (9.27 - 11.83)</td>
<td>0.79</td>
<td>0.14 (1.2%)</td>
<td>0.12</td>
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<tr>
<td>fres expiratory [1/s] Pre</td>
<td>9.48 (8.76 - 13.58)</td>
<td>0.82</td>
<td>0.4 (4.6%)</td>
<td>0.20</td>
</tr>
<tr>
<td>fres expiratory [1/s] Post</td>
<td>11.37 (8.72 - 13.21)</td>
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<tr>
<td>AX [kpa/L] Pre</td>
<td>0.22 (0.15 - 0.37)</td>
<td>0.82</td>
<td>0.01 (2.4%)</td>
<td>0.07</td>
</tr>
<tr>
<td>AX [kpa/L] Post</td>
<td>0.19 (0.15 - 0.38)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AX inspiratory [kpa/L] Pre</td>
<td>0.24 (0.17 - 0.30)</td>
<td>0.79</td>
<td>0.003 (0.3%)</td>
<td>0.04</td>
</tr>
<tr>
<td>AX inspiratory [kpa/L] Post</td>
<td>0.23 (0.19 - 0.37)</td>
<td></td>
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<tr>
<td>AX expiratory [kpa/L] Pre</td>
<td>0.18 (0.14 - 0.39)</td>
<td>0.97</td>
<td>0.01 (6.4%)</td>
<td>0.04</td>
</tr>
<tr>
<td>AX expiratory [kpa/L] Post</td>
<td>0.21 (0.15 - 0.39)</td>
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<tr>
<td>R5-R20 Pre</td>
<td>0.03 ±0.04</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R5-R20 Post</td>
<td>0.03 ±0.04</td>
<td></td>
<td></td>
<td></td>
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<td>IOS parameter</td>
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<td>20/250</td>
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<td>20/500</td>
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<tr>
<td></td>
<td>Mean change (mean % change)</td>
<td>Effect size (absolute)</td>
<td>Mean change (mean % change)</td>
<td>Effect size (absolute)</td>
</tr>
<tr>
<td>Z5 [kPa/(L/s)]</td>
<td>0.002 (1.5%)</td>
<td>0.05</td>
<td>0.02 (5.2%)</td>
<td>0.35</td>
</tr>
<tr>
<td>R5 [kPa/(L/s)]</td>
<td>0.004 (2.1%)</td>
<td>0.08</td>
<td>0.01 (4.9%)</td>
<td>0.31</td>
</tr>
<tr>
<td>R5 inspiratory [kPa/(L/s)]</td>
<td>-0.004 (0.5%)</td>
<td>0.10</td>
<td>0.01 (5.5%)</td>
<td>0.31</td>
</tr>
<tr>
<td>R5 expiratory [kPa/(L/s)]</td>
<td>-0.002 (0.5%)</td>
<td>0.04</td>
<td>0.02 (5.1%)</td>
<td>0.28</td>
</tr>
<tr>
<td>R10 [kPa/(L/s)]</td>
<td>0.003 (2.2%)</td>
<td>0.06</td>
<td>0.02 (6.6%)</td>
<td>0.41</td>
</tr>
<tr>
<td>R10 inspiratory [kPa/(L/s)]</td>
<td>-0.01 (-0.2%)</td>
<td>0.14</td>
<td>0.02 (6.8%)</td>
<td>0.41</td>
</tr>
<tr>
<td>R10 expiratory [kPa/(L/s)]</td>
<td>-0.004 (0.3%)</td>
<td>0.07</td>
<td>0.02 (7.1%)</td>
<td>0.37</td>
</tr>
<tr>
<td>R20 [kPa/(L/s)]</td>
<td>0.01 (3.9%)</td>
<td>0.17</td>
<td>0.02 (7.3%)</td>
<td>0.43</td>
</tr>
<tr>
<td>R20 inspiratory [kPa/(L/s)]</td>
<td>0.001 (1.8%)</td>
<td>0.01</td>
<td>0.02 (7.3%)</td>
<td>0.49</td>
</tr>
<tr>
<td>R20 expiratory [kPa/(L/s)]</td>
<td>0.004 (2.9%)</td>
<td>0.08</td>
<td>0.01 (5.9%)</td>
<td>0.30</td>
</tr>
<tr>
<td>X5 [kPa/(L/s)]</td>
<td>0.001 (0.3%)</td>
<td>0.06</td>
<td>-0.005 (5%)</td>
<td>0.35</td>
</tr>
<tr>
<td>X5 inspiratory [kPa/(L/s)]</td>
<td>0 (2.1%)</td>
<td>0</td>
<td>-0.01 (7.5%)</td>
<td>0.50</td>
</tr>
<tr>
<td>X5 expiratory [kPa/(L/s)]</td>
<td>0.01 (-5.1%)</td>
<td>0.25</td>
<td>-0.005 (5.6%)</td>
<td>0.21</td>
</tr>
<tr>
<td>X10 [kPa/(L/s)]</td>
<td>0.004 (9.8%)</td>
<td>0.25</td>
<td>0 (-22.1%)</td>
<td>0</td>
</tr>
<tr>
<td>X10 inspiratory [kPa/(L/s)]</td>
<td>0.004 (-13.3%)</td>
<td>0.21</td>
<td>0 (-11.7%)</td>
<td>0</td>
</tr>
<tr>
<td>X10 expiratory [kPa/(L/s)]</td>
<td>0.01 (2.5%)</td>
<td>0.46</td>
<td>-0.003 (-19.7%)</td>
<td>0.19</td>
</tr>
<tr>
<td>X20 [kPa/(L/s)]</td>
<td>0.01 (18.5%)</td>
<td>0.53</td>
<td>-0.004 (-4.7%)</td>
<td>0.20</td>
</tr>
<tr>
<td>X20 inspiratory [kPa/(L/s)]</td>
<td>0.01 (11.1%)</td>
<td>0.40</td>
<td>-0.002 (-1.3%)</td>
<td>0.07</td>
</tr>
<tr>
<td>X20 expiratory [kPa/(L/s)]</td>
<td>0.01 (7.1%)</td>
<td>0.54</td>
<td>-0.01 (-9.6%)</td>
<td>0.37</td>
</tr>
<tr>
<td>fres [1/s]</td>
<td>-0.19 (-0.7%)</td>
<td>0.15</td>
<td>0.35 (3.3%)</td>
<td>0.26</td>
</tr>
<tr>
<td>fres inspiratory [1/s]</td>
<td>-0.13 (-0.3%)</td>
<td>0.10</td>
<td>0.24 (2.2%)</td>
<td>0.24</td>
</tr>
<tr>
<td>fres expiratory [1/s]</td>
<td>-0.79 (-5.8%)</td>
<td>0.70</td>
<td>0.5 (4.9%)</td>
<td>0.27</td>
</tr>
<tr>
<td>AX [kPa/L]</td>
<td>-0.03 (-4.1%)</td>
<td>0.33</td>
<td>0.02 (11.9%)</td>
<td>0.33</td>
</tr>
<tr>
<td>AX inspiratory [kPa/L]</td>
<td>-0.01 (7.4%)</td>
<td>0.12</td>
<td>0.02 (11.4%)</td>
<td>0.24</td>
</tr>
<tr>
<td>AX expiratory [kPa/L]</td>
<td>-0.05 (-10.4%)</td>
<td>0.38</td>
<td>0.03 (11.7%)</td>
<td>0.39</td>
</tr>
</tbody>
</table>
inspiratory had a higher increase in the exposure session of 20/250 (6.8% increase post vs pre exposure) compared to the exposure session of 10/250 (-0.2% decrease post vs pre exposure), a difference marginally statistically significant (p=0.05). A decrease in X20 was observed in the exposure session of 20/250 (-4.7% decrease post vs pre exposure) compared to an increase of 18.5% (post vs pre exposure) in 10/250, (p=0.04). Similarly, X20 expiratory decreased in 20/250 exposure session (-9.6% decrease post vs pre exposure) while it increased in 10/250 (7.1% increase post vs pre exposure), (p=0.02). On the contrary, fres expiratory increased by 4.9% (post vs pre exposure) in 20/250 while it decreased by -5.8% in 10/250 (post vs pre exposure) (p=0.02). Also, AX increased by 11.9% (post vs pre exposure) in 20/250 exposure session while it decreased by -4.1% (post vs pre exposure) in 10/250 (p=0.04). Similarly, AX expiratory increased by 11.7% in 20/250 (post vs pre exposure) while it decreased by -10.4% in 10/250 (p=0.03) (Tables 4 and 5).

A statistically significant difference was observed in

<table>
<thead>
<tr>
<th>IOS parameter</th>
<th>20/250 vs 10/250</th>
<th>10/500 vs 10/250</th>
<th>20/500 vs 10/250</th>
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<tbody>
<tr>
<td></td>
<td>β (se)</td>
<td>p-value</td>
<td>β (se)</td>
</tr>
<tr>
<td>Z5 kPa/(L/s)</td>
<td>0.014 (0.01)</td>
<td>0.25</td>
<td>0.011 (0.01)</td>
</tr>
<tr>
<td>R5 kPa/(L/s)</td>
<td>0.011 (0.01)</td>
<td>0.38</td>
<td>0.012 (0.01)</td>
</tr>
<tr>
<td>R5 inspiratory kPa/(L/s)</td>
<td>0.019 (0.01)</td>
<td>0.15</td>
<td>0.02 (0.01)</td>
</tr>
<tr>
<td>R5 expiratory kPa/(L/s)</td>
<td>0.017 (0.01)</td>
<td>0.24</td>
<td>0.018 (0.01)</td>
</tr>
<tr>
<td>R10 kPa/(L/s)</td>
<td>0.015 (0.01)</td>
<td>0.20</td>
<td>0.014 (0.01)</td>
</tr>
<tr>
<td>R10 inspiratory kPa/(L/s)</td>
<td>0.022 (0.01)</td>
<td>0.05</td>
<td>0.025 (0.01)</td>
</tr>
<tr>
<td>R10 expiratory kPa/(L/s)</td>
<td>0.023 (0.01)</td>
<td>0.11</td>
<td>0.023 (0.01)</td>
</tr>
<tr>
<td>R20 kPa/(L/s)</td>
<td>0.012 (0.01)</td>
<td>0.36</td>
<td>0.012 (0.01)</td>
</tr>
<tr>
<td>R20 inspiratory kPa/(L/s)</td>
<td>0.020 (0.01)</td>
<td>0.13</td>
<td>0.019 (0.01)</td>
</tr>
<tr>
<td>R20 expiratory kPa/(L/s)</td>
<td>0.011 (0.02)</td>
<td>0.50</td>
<td>0.004 (0.02)</td>
</tr>
<tr>
<td>X5 kPa/(L/s)</td>
<td>-0.006 (0.00)</td>
<td>0.25</td>
<td>0.001 (0.00)</td>
</tr>
<tr>
<td>X5 inspiratory kPa/(L/s)</td>
<td>-0.006 (0.00)</td>
<td>0.27</td>
<td>-0.002 (0.00)</td>
</tr>
<tr>
<td>X5 expiratory kPa/(L/s)</td>
<td>-0.011 (0.01)</td>
<td>0.14</td>
<td>0.001 (0.01)</td>
</tr>
<tr>
<td>X10 kPa/(L/s)</td>
<td>-0.004 (0.00)</td>
<td>0.42</td>
<td>-8.67e-19 (0.00)</td>
</tr>
<tr>
<td>X10 inspiratory kPa/(L/s)</td>
<td>-0.004 (0.00)</td>
<td>0.40</td>
<td>-0.003 (0.00)</td>
</tr>
<tr>
<td>X10 expiratory kPa/(L/s)</td>
<td>-0.009 (0.01)</td>
<td>0.09</td>
<td>-0.004 (0.01)</td>
</tr>
<tr>
<td>X20 kPa/(L/s)</td>
<td>-0.012 (0.01)</td>
<td>0.04</td>
<td>-0.009 (0.01)</td>
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<tr>
<td>X20 inspiratory kPa/(L/s)</td>
<td>-0.009 (0.01)</td>
<td>0.12</td>
<td>-0.004 (0.01)</td>
</tr>
<tr>
<td>X20 expiratory kPa/(L/s)</td>
<td>-0.017 (0.01)</td>
<td>0.02</td>
<td>-0.01 (0.01)</td>
</tr>
<tr>
<td>fres [1/s]</td>
<td>0.533 (0.4)</td>
<td>0.21</td>
<td>0.189 (0.4)</td>
</tr>
<tr>
<td>fres inspiratory [1/s]</td>
<td>0.372 (0.32)</td>
<td>0.24</td>
<td>0.148 (0.32)</td>
</tr>
<tr>
<td>fres expiratory [1/s]</td>
<td>1.288 (0.54)</td>
<td>0.02</td>
<td>0.843 (0.54)</td>
</tr>
<tr>
<td>AX [kPa/L]</td>
<td>0.05 (0.02)</td>
<td>0.04</td>
<td>0.015 (0.02)</td>
</tr>
<tr>
<td>AX inspiratory [kPa/L]</td>
<td>0.026 (0.02)</td>
<td>0.21</td>
<td>0.002 (0.02)</td>
</tr>
<tr>
<td>AX expiratory [kPa/L]</td>
<td>0.082 (0.04)</td>
<td>0.03</td>
<td>0.043 (0.04)</td>
</tr>
</tbody>
</table>
R10 inspiratory between the exposure sessions of 10/500 compared to 10/250. In particular, R10 inspiratory had a higher increase in the exposure session 10/500 (7.7% increase post vs pre exposure) compared to the exposure session 10/250 (-0.2% reduction post vs pre exposure) (p=0.03) (Tables 4 and 5).

**DISCUSSION**

The current study showed for the first time that a brief 10–20 minutes exposure of healthy non-smokers to SHS, resulted in measurable changes of respiratory mechanics during tidal breathing. Parameters of Impedance, Resistance and Reactance, showed changes post exposure in all sessions and for all individuals. Central airways alterations were observed mainly in inspiration, whereas peripheral airways alterations prevailed in expiration.

From the comparison between sessions, the 20/250 exposure was the session depicting significant alterations in several parameters; specifically, Resonant Frequency and AX area increased, while high frequency Reactance X20 decreased. Following the intra-breath analysis, alterations were also observed in the expiratory components of Resonant Frequency, AX and X20 as well as in the inspiratory component of Resistance R10. Keeping PM$_{2.5}$ concentration constant at 250 µg/m$^3$ and examining 10 and 20 minutes of exposure respectively, revealed significant changes in the 20/250 session in comparison to the 10/250, an indication it was likely the effect of prolonged duration that was associated with findings. Resonant Frequency represents the frequency where the sum of the two components of Reactance, Elastance and Inertance, equals 0, since their measures are equal and opposite in sign. In high frequencies (above fres) it is the inertive pressure of the large airways that predominate, while in the lower frequencies the elastic properties of the lung periphery prevail. The triangular area below the Resonant Frequency, AX area, expresses the respiratory Elastance, the reciprocal of Compliance and is a marker of airway closure; increased AX, as was found in the 20/250 exposure session, expresses the increased respiratory Elastance and consequently reduced Compliance. Increased fres and AX indicate alteration of the elastic properties in the lung periphery and in association with a more negative, decreased X5 express the expiratory flow limitation of the small airways. While R10 is not usually included in the IOS interpretation in the adult clinical settings, it is worth noting that Komarow et al found that R10 showed a better ability to differentiate between children with and without asthma; thus it is suggested that the increased R10 taken together with the also increased fres, AX and AX expiratory post exposure in the present study, could reflect the likely broncho constrictive response to the irritative SHS compounds.

The 10/500 session showed increased central and medium airway Resistance (R20, R20 inspiratory, R10, R10 inspiratory, R10 expiratory) in addition to a higher increase in R10 inspiratory than the 10/250 session. The acute exposure to an irritative inhalant including SHS, induces chemesthesis, expressed by the sensory irritation of eyes, nose, and the large upper airways; sensory irritation is mediated by the trigeminal, glossopharyngeal and vagus nerves respectively; this response may explain the increased central airways Resistance found in the 10/500 session. However, while in the 10/500 session increased central airway Resistance (R20) was observed, no changes were depicted in the 20/500 to verify the effect of duration. The addition of another 13 individuals in this session, led to a significantly more negative inspiratory component of Distal Capacitive Reactance (X5) post exposure; these findings indicate that the same PM$_{2.5}$ concentration, in the brief 10 minute exposure led to increased central airway Resistance, while the prolonged, double the duration, 20 minutes exposure, led to decreased Distal Capacitive Reactance, a marker of lung periphery. Distal Capacitive Reactance, X5, reflects the elastic properties of the lung periphery, and indirectly the dimension of peripheral airways. X5 in reflecting the elastic recoil of the small airways, takes more negative values in disorders that lead to both reduced lung elasticity and hyperinflation; the more negative X5 observed post exposure in the present study, points out to the small airways being the site of the immediate alterations induced by SHS.

It is worth noting that while not significant, the 20/500 session in addition to the significant X5 inspiratory reduction, also showed a trend for Z5, R5, fres and AX to increase, a combination that describes the peripheral airway obstruction pattern, characteristic of the Chronic Obstructive Pulmonary Disease (COPD), known to be causally related to active and passive smoking. Furthermore, this trend, captured following a brief 20 min exposure to SHS, could be interpreted as the likely initial footprint of the long, insidious process that precedes the spirometric detection of the FEV$_1$ decline associated with smoking and chronic exposure to SHS.

In line with a previous study by Mangnussen, cur-
rent study did not show a consistent response to the SHS exposure, neither did document a clear association with PM$_{2.5}$ concentration; we did however observe that increased exposure duration led to changes of respiratory mechanics post exposure at 20 minutes, those changes being stronger in the 20/250 compared to the 20/500 session.

While these findings may appear conflicting and not consistent, it is worth noting that Shusterman et al in their review suggest that there are three types of concentration/time relationships in regards to sensory irritation, i) the (c×t=k) relationship described under Haber’s law, that has only been experimentally documented in humans for certain compounds such as war gases, ii) a (c$^a$×t=k) relationship valid for certain time intervals within the exposure duration and iii) a plateau reaching relationship, followed by waning or reversing of the time effects; the authors concluded that further studies using physiologically based pharmacokinetic models (PBPK) are needed. In the case of the present study it is likely that a plateauning (concentration/duration) relationship leading to waning of effects could explain why it was the 10/500 session that revealed significant changes in contrast to the longer 20/500 exposure. Furthermore, it is suggested that SHS aging and temporal effect (hygroscopic growth, particle coagulation, deposition on surfaces), could partly explain why contrary to what was anticipated, weaker changes were observed in the higher exposure (20/500)$^2$.

Our study has some limitations

As participants were able to detect the presence and smell of smoke, the possible psychological effect of this knowledge was not accounted for. To overcome the within individual confounding factors, we examined the same participants in all four sessions. We did not however measure the respiratory and heart rate of participants which could vary between and within individuals across sessions and could therefore lead to the inhalation of different SHS quantities. We did use the same exposure room with constant volume and ventilation conditions across all sessions although the actual air flow and ventilation rate were not directly measured. Finally, the fact that we did not perform a control session meant that we could only test for differences between the exposure sessions having one of the sessions as our reference category.

CONCLUSION

Present study is the first to show that acute exposure of healthy non-smokers to SHS, equivalent to that produced by one and two cigarettes respectively, leads to alterations of resting breathing mechanics, successfully captured by IOS. Alterations were expressed by increased Resistance of peripheral and central airways; specifically, mainly the expiratory components of the peripheral airways and the inspiratory component of central airways Resistance increased, findings suggestive of a likely broncho-constrictive response to the irritative inhalant, bearing the potential for airflow limitation. A mild, linear, effect of exposure duration was found, while no clear effect was observed for the level of exposure. Further research is needed to establish the exact impact of exposure determinants in the pathophysiology of the SHS induced disease.

CONFLICTS OF INTEREST
None.

FUNDING OR GRANT SUPPORT
All authors declare that no funding was received for the work described in this manuscript.
Εισαγωγή: Περιορισμένες έρευνες έχουν εξετάσει τις συνέπειες της έκθεσης σε παιδικό κάπνισμα (SHS) στην αναπνευστική λειτουργία· η πλειοψηφία έχει χρησιμοποιήσει τις κλασσικές τεχνικές λειτουργικού ελέγχου που απαιτούν βίαιες αναπνευστικές δοκιμασίες. Σκοπός της παρούσας μελέτης ήταν να εκτιμήσουμε τις άμεσες επιπτώσεις της έκθεσης σε SHS στη μηχανική της ήρεμης αναπνοής. 

Μεθοδολογία: 20 υγιείς, μη καπνιστές, 18-45 ετών συμμετείχαν σε 4 συνεδρίες έκθεσης σε SHS· 10 λεπτά σε 250 μg/m 3 PM2.5 (10/250), 20 λεπτά σε 250 μg/m 3 PM2.5 (20/250), 10 λεπτά σε 500 μg/m 3 PM2.5 (10/500) και 20 λεπτά σε 500 μg/m 3 PM2.5 (20/500). Παράμετροι Παλμικής Ταλαντωσιμετρίας (IOS) μετρήθηκαν πριν και αμέσως μετά από κάθε συνεδρία. Οι διαφορές των παραμέτρων IOS για κάθε συνεδρία (προ/μετά) εκτιμήθηκαν με paired t-tests ή Wilcoxon tests. Οι διαφορές μεταξύ των συνεδριών εκτιμήθηκαν με mixed linear μοντέλα. 

Αποτελέσματα: Στατιστικά σημαντικές διαφορές παρατηρήθηκαν στις παραμέτρους IOS στις τέσσερις συνεδρίες, με τις περισσότερες διαφορές στην 10/500 και τις λιγότερες στην 20/500. Η ανάλυση μεταξύ των συνεδριών έδειξε σημαντικές διαφορές σε πολλές παραμέτρους IOS μεταξύ 20/250 και 10/250, ενώ η 10/500 διέφερε σημαντικά από την 10/250 ως προς την έκθεση ησυχίας R10. 

Συμπεράσματα: Προκείται για την πρώτη μελέτη που διαπιστώνει ότι η οξεία έκθεση υγιών μη καπνιστών σε SHS, οδηγεί σε μεταβολές της μηχανικής της ήρεμης αναπνοής. Η μέθοδος IOS αποτυπώνει αυξημένη αντίσταση περιφερειακών και κεντρικών αεραγωγών, ευρήματα ενδεικτικά βρογχοσύσπασης, πιθανώς ως απόκριση στον εισπνεόμενο ερεθιστικό παράγοντα. Το αποτέλεσμα της έκθεσης φαίνεται να συσχετίζεται κυρίως με τη διάρκεια της, ενώ δεν παρατηρήθηκε σαφής επίδραση του επιπέδου ρύπανσης.

Λέξεις - Κλειδιά: Αντίσταση αναπνευστικού συστήματος, Μηχανική της αναπνοής, Παλμική Ταλαντωσιμετρία, Παθητικό κάπνισμα, Δευτερογενές παθητικό κάπνισμα

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A comparative study of Gene Xpert MTB/RIF to AFB smear microscopy in the diagnosis of pulmonary tuberculosis in India

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ABSTRACT

BACKGROUND: To determine the specificity and sensitivity of Gene Xpert MTB/RIF assay and compare it against acid-fast bacillus (AFB) smear microscopy in the diagnosis of pulmonary tuberculosis.

METHODS: The study retrospectively compared the results of Gene Xpert MTB/RIF, AFB smear microscopy and AFB culture on respiratory specimens collected from ‘presumptive’ pulmonary tuberculosis patients from June 2014 to September 2015. Only patients for whom all the three test results were available were included in the study. The result of AFB smear and Gene Xpert MTB/RIF were compared to the results of AFB culture which was taken as the reference standard.

RESULTS: The sensitivity & specificity of Gene Xpert MTB/RIF was found to be superior to smear microscopy (90.5% & 99.7% against 62.8% & 97.8% respectively). Additionally, 27 patients were detected as having rifampicin resistance using the Gene Xpert assay. Diabetes mellitus, HIV, smoking and presence of cavity(ies) on chest x-ray were identified as important risk factors for multi-drug resistant TB (MDR-TB). CONCLUSION: Gene Xpert outperformed the more commonly used AFB smear microscopy in terms of specificity, sensitivity, positive predictive values (PPV) and negative predictive values (NPV). It was also able to identify drug resistant TB at an early stage. Hence, it has the potential to replace AFB smear microscopy as an initial diagnostic test in “presumptive” pulmonary TB patients. Pneumon 2020, 33(3):131-138.

INTRODUCTION

According to the WHO, it is estimated that globally there were around 10 million new tuberculosis (TB) cases in 2018. India, China, Indonesia, Philippines, Pakistan, Nigeria, Bangladesh, and South Africa together accounted for two-thirds of the new cases. The WHO statistics for India in 2018 gave

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an estimated incidence of 2.69 million cases of TB with 4,40,000 deaths (excluding HIV+TB deaths)\(^1\). In 2014, TB surpassed HIV to become the leading cause of death due to a single infectious agent\(^2\).

Traditionally, the diagnosis of pulmonary tuberculosis relies on the detection of acid fast bacillus by smear microscopy or culture with each method having its own advantages and limitations. AFB smear microscopy has been the most commonly used method for the microbiological diagnosis of TB but its utility is hindered by a poor sensitivity (22% - 80%) and the fact that the acid fast stain cannot differentiate between mycobacteria species necessitating concomitant culturing for species identification and drug susceptibility testing\(^3\). As per the Revised National Tuberculosis Control Programme (RNTCP) guidelines of India, sputum smear examination using Auramine O stains and LED-based fluorescent microscopy forms the mainstay tool in the diagnosis of TB owing to less cost, rapidity and its simplicity. AFB culture due to its high specificity and sensitivity is considered as the "gold standard" for TB diagnosis and is used to follow up patients on treatment for drug-resistant TB. AFB culture, however, takes 2 to 8 weeks' time to yield a final result and is not helpful for making an early diagnosis.

A major breakthrough in TB diagnostics was the introduction of Xpert MTB/RIF assay which is a cartridge based nucleic acid amplification test (CB-NAAT) that was first approved by the WHO in 2010\(^4\). Potential advantages of the test include a rapid turn-around time of 2 hours, good sensitivity and specificity for pulmonary samples, ability to detect \textit{M. Tuberculosis} and rifampicin resistance directly from the collected specimen and use of a closed PCR system with a low risk of cross-contamination\(^3\). Currently, in India, RNTCP advocates the up front use of Gene Xpert in the diagnosis of TB in \textit{key populations} like presumptive HIV associated TB, presumptive pediatric TB and extra-pulmonary cases where conventional AFB smear microscopy is likely to be negative\(^5\). The programme is now extending the use of rapid molecular tests i.e. Cartridge Based Nucleic Acid Amplification Test (CBNAAT) and Line Probe Assay (LPA) to \textit{all the diagnosed and notified} TB cases as a part of its Universal Drug Susceptibility Testing (UDST) strategy\(^6\).

This study aims to determine the sensitivity and specificity of Gene Xpert MTB/RIF assay and compare its performance with AFB smear examination in the diagnosis of pulmonary tuberculosis in the Indian population. It also aims to find the association of MDR-TB occurrence with clinico-demographic variables like age, sex, smoking status, past history of treated TB, HIV, neoplasia, chronic renal failure, diabetes mellitus and cavitary lung lesion on chest radiograph.

**MATERIALS AND METHODS**

1. **Patients and study design**

This record based retrospective study was conducted using data from teaching hospitals affiliated to Kasturba Medical College, Mangalore. This study was approved by the Institutional Ethics Committee of Manipal University with a waiver of informed consent (IEC KMC MLR 02-15/26) and performed in accordance with the principles of the Declaration of Helsinki.

Case records of patients who either attended the outpatient clinic or were inpatients, during the period from June 2014 to September 2015 were included on satisfying the following inclusion and exclusion criteria.

**Inclusion criteria:**

1. Patients aged ≥12 years suspected to be suffering from pulmonary TB whose sputum or bronchial aspirates were subjected to testing by acid fast bacillus (AFB) smear microscopy, AFB culture and Gene Xpert.

**Exclusion criteria:**

1. Pulmonary TB suspects whose sputum or bronchial aspirates were not tested using \textit{all} the three methods i.e. AFB smear microscopy, AFB culture and Gene Xpert.

2. Patients whose case sheets or reports provided incomplete information leading to absence of crucial data.

3. Patients initiated on anti-tuberculosis treatment prior to evaluation of their respiratory samples.

Archived case records of patients satisfying all the inclusion and exclusion criteria, as described above, were chosen using a non-random convenience sampling technique. Their number came up to 936 in the study (n=936).

2. **Data Collection**

Requisite permissions were obtained from the District Medical Officer (DMO) of Government Wenlock Hospital, Mangalore and the Medical Superintendent (MS) of Kasturba Medical College (KMC) Hospitals, Mangalore in order to access the hospital records. Smear microscopy for AFB was done using the LED fluorescence technique in all patients. Gene Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) was done as per the manufacturer’s instruction
for all the samples. Liquid-media mycobacterial culture of all samples was done using the Mycobacterial Growth Indicator Tube (MGIT) method as a reference standard.

All the laboratory reports pertaining to TB diagnosis i.e. Gene Xpert MTB/RIF assay, AFB smear microscopy and AFB culture and the clinico-demographic data as recorded in case sheets with chest radiographs of the study patients were retrieved and information recorded therein was used to fill a ‘Study Performa’ from which the results were subsequently analyzed. Confidentiality of the patients was protected throughout the study.

3. Statistical Analysis

Data obtained was analyzed using Statistical Package for the Social Sciences (SPSS) version 17.0 and then represented as frequencies and percentages. Proportion of tuberculosis cases diagnosed was calculated with respect to all the three tests employed for TB diagnosis i.e. Gene Xpert MTB/RIF, AFB culture and AFB smear microscopy. The sensitivity, specificity, Negative Predictive Value (NPV) and Positive Predictive Value (PPV) of Gene Xpert and AFB smear microscopy for the diagnosis of pulmonary tuberculosis was compared using AFB culture as the reference standard, i.e. samples that were positive on culture were considered as ‘true positive’ while those that were negative on culture were considered as ‘true negative’.

The number of MDR-TB patients diagnosed using the Gene Xpert assay was analyzed and a possible association of MDR-TB occurrence with parameters like age, sex, tobacco smoking, past history of TB, HIV, neoplasia, chronic renal failure, diabetes mellitus, and presence of cavitary lesion on chest radiograph was derived using the Chi square test. Only those associations which had a "p" value of less than 0.05 were considered to be statistically significant.

RESULTS

The sample population in this study consisted of 936 pulmonary tuberculosis suspects who were evaluated by AFB smear microscopy, AFB culture and Gene Xpert of their sputum or bronchial aspirate.

We analyzed the data calculating the proportion of TB cases diagnosed using the three tests (viz. smear, culture and Gene Xpert). It was found that of the 936 cases, 234 cases were diagnosed as pulmonary tuberculosis on the basis of a positive AFB culture (25%), 162 cases were positive on AFB smear microscopy (17.30%) whereas 214 cases could be diagnosed on a positive Gene Xpert result (22.86%) (Table 1).

In order to determine the sensitivity, specificity, PPV and NPV of Gene Xpert as a diagnostic tool for pulmonary TB, the results of Gene Xpert on respiratory samples were compared to the results obtained from culture reports (the ‘reference’ standard). Among the 936 participants who were tested for pulmonary TB, 214 patients tested positive for TB by Gene Xpert (722 subjects tested negative for TB on Gene Xpert). In comparison, the AFB culture was positive in 234 patients. When a two by two contingency table was constructed with the above data we found that of the 214 TB positives given by Gene Xpert only 212 patients truly had the disease (‘true positive’) while the remaining 2 subjects were falsely labelled as positive for TB (‘false positive’). Similarly, out of the 722 patients who tested negative for TB using Gene Xpert only 700 patients were truly free from the disease (‘true negative’) while 22 patients were falsely labelled as negative for TB while they actually had the disease (‘false negative’). Hence, the present study shows that in our sample population the sensitivity of Gene Xpert was 90.5%, specificity was 99.7%, PPV was 99% and the NPV was 97% (Table 2).

The results of AFB smear microscopy of the 936 pulmonary TB suspects were compared against their corresponding culture results (the ‘reference’ standard) to determine the efficiency of smear examination as a diagnostic tool. 162 patients tested positive for TB on smear microscopy (774 subjects tested negative for TB). However, as per the results of AFB culture, 234 patients actually suffered from the disease. When a two by two contingency table was constructed with the above data we found that of

| TABLE 1. Proportion of tuberculosis (TB) cases diagnosed using AFB smear microscopy, culture and Gene Xpert MTB/RIF (n=936) |
|---------------------------------|-----------------|-----------------|-----------------|
| **Sample**                     | **Test**        | **Positive for TB** | **Negative for TB** | **Total** |
| Pulmonary                      | Culture         | 234 (25%)         | 702 (75%)         | 936      |
| (Sputum/Bronchial aspirate)    | Gene Xpert      | 214 (22.86%)      | 722 (77.14%)      | 936      |
|                                | Smear microscopy| 162 (17.31%)      | 774 (82.69%)      | 936      |
the 162 TB positives given by smear microscopy only 147 patients truly had the disease ('true positives') while the remaining 15 subjects were falsely labeled as positive for TB ('false positive'). Similarly, out of the 774 patients who tested negative for TB using smear microscopy only 687 patients were truly free from the disease ('true negative') while 87 patients were falsely labeled as negative for TB though they actually had the disease ('false negative'). Thus, when used as a diagnostic tool in pulmonary TB, the sensitivity, specificity, PPV and NPV of AFB smear microscopy was seen to be 62.8%, 97.8%, 90.7% and 88.75% respectively (Table 3).

A comparison of the sensitivity, specificity, PPV and NPV of Gene Xpert and AFB smear shows that Xpert MTB/RIF outperformed smear microscopy in all the performance parameters (Table 4).

Of the 936 respiratory samples tested in the study, 27 samples showed rifampicin resistance on the Xpert MTB/RIF assay implying a prevalence of MDR/RR-TB of 2.88% in the sample population. Table 5 represents the clinical and demographic profile of these MDR/RR-TB patients.

The study revealed statistically significant associations between the occurrence of MDR-TB and diabetes mellitus (p= 0.039), HIV (p= 0.019), cavitary lung

TABLE 2. Performance of Gene Xpert in the detection of pulmonary tuberculosis (n=936).

<table>
<thead>
<tr>
<th>Culture positive for TB</th>
<th>Culture negative for TB</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene Xpert positive for TB</td>
<td>212 (99.06%)</td>
<td>2 (0.93%)</td>
</tr>
<tr>
<td>Gene Xpert negative for TB</td>
<td>22 (3.04%)</td>
<td>700 (96.95%)</td>
</tr>
<tr>
<td>Total</td>
<td>234</td>
<td>702</td>
</tr>
</tbody>
</table>

TABLE 3. Performance of AFB smear microscopy in the detection of pulmonary tuberculosis (n=936).

<table>
<thead>
<tr>
<th>Culture positive for TB</th>
<th>Culture negative for TB</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smear positive for TB</td>
<td>147 (90.74%)</td>
<td>15 (9.26%)</td>
</tr>
<tr>
<td>Smear negative for TB</td>
<td>87 (11.24%)</td>
<td>687 (88.76%)</td>
</tr>
<tr>
<td>Total</td>
<td>234</td>
<td>702</td>
</tr>
</tbody>
</table>

TABLE 4. Comparison of acid fast smear and Gene/Xpert MTB/RIF tests.

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid fast smear</td>
<td>62.8%</td>
<td>97.8%</td>
<td>90.7%</td>
<td>88.75%</td>
</tr>
<tr>
<td>Gene Xpert MTB/RIF</td>
<td>90.5%</td>
<td>99.7%</td>
<td>99%</td>
<td>97%</td>
</tr>
</tbody>
</table>

TABLE 5. Demographic and clinical profile of MDR-TB patients in the study (n= 27).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number (n)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>22</td>
<td>81.48</td>
</tr>
<tr>
<td>Female</td>
<td>05</td>
<td>18.52</td>
</tr>
<tr>
<td>Age (in years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;30 years</td>
<td>4</td>
<td>14.81</td>
</tr>
<tr>
<td>30-60 years</td>
<td>16</td>
<td>59.26</td>
</tr>
<tr>
<td>&gt;60 years</td>
<td>7</td>
<td>25.93</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>18</td>
<td>66.67</td>
</tr>
<tr>
<td>No</td>
<td>09</td>
<td>33.33</td>
</tr>
<tr>
<td>Past history of TB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>23</td>
<td>85.18</td>
</tr>
<tr>
<td>No</td>
<td>04</td>
<td>14.82</td>
</tr>
<tr>
<td>Years since TB treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5 years</td>
<td>14</td>
<td>51.85</td>
</tr>
<tr>
<td>5-10 years</td>
<td>05</td>
<td>18.51</td>
</tr>
<tr>
<td>&gt;10 years</td>
<td>04</td>
<td>14.81</td>
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<tr>
<td>Diabetes Mellitus</td>
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<td></td>
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<tr>
<td>Yes</td>
<td>08</td>
<td>29.63</td>
</tr>
<tr>
<td>No</td>
<td>19</td>
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</tr>
<tr>
<td>HIV</td>
<td></td>
<td></td>
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<tr>
<td>Yes</td>
<td>01</td>
<td>3.70</td>
</tr>
<tr>
<td>No</td>
<td>26</td>
<td>96.30</td>
</tr>
<tr>
<td>Cancer</td>
<td></td>
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</tr>
<tr>
<td>Yes</td>
<td>00</td>
<td>0.0</td>
</tr>
<tr>
<td>No</td>
<td>27</td>
<td>100.0</td>
</tr>
<tr>
<td>Renal Failure</td>
<td></td>
<td></td>
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<tr>
<td>Yes</td>
<td>02</td>
<td>7.40</td>
</tr>
<tr>
<td>No</td>
<td>25</td>
<td>92.60</td>
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<tr>
<td>Lung cavity on chest X-ray</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>20</td>
<td>74.07</td>
</tr>
<tr>
<td>Absent</td>
<td>07</td>
<td>25.93</td>
</tr>
</tbody>
</table>
lesion on chest radiograph \((p = 0.022)\) and smoking \((p = 0.001)\) as compared to patients without these conditions. Our study also showed that the occurrence of MDR-TB was higher among males \((81.48\%)\) & those patients who had been previously treated for TB \((85.18\%)\) - especially within the past 5 years \((51.8\%)\). However, these associations were not found to reach statistically significant proportions (Table 6).

**DISCUSSION**

In this study, among the patients who were screened for presumptive pulmonary TB symptoms, nearly one-fourth were detected to have TB using culture \((25\%)\) and nearly an equal number were diagnosed using Gene Xpert \((22.86\%)\). Both the tests outperformed the traditional AFB smear microscopy which could only detect 17.3% of the TB cases. An evaluation of the performance of Gene Xpert MTB/RIF in diagnosing pulmonary TB in China showed a detection rate of 36.6% for the Gene Xpert assay and 34% by BACTEC MGIT 960 culture with no significant difference in between the two methods which is similar to the observation in our study\(^7\). Studies from the Indian subcontinent have reported that Xpert MTB/RIF was superior to both AFB smear microscopy and AFB culture in the detection of mycobacterium in respiratory samples\(^8,9\). Hence, the present study corroborates the observations made in the earlier studies that Xpert MTB/RIF is at least as good as, if not better than AFB culture in the diagnosis of pulmonary tuberculosis.

A Cochrane review assessing the diagnostic accuracy of Xpert MTB/RIF as an initial diagnostic test replacing smear microscopy in pulmonary TB concluded that, in adults, irrespective of their HIV status, Xpert MTB/RIF is both highly sensitive \((89\%)\) and specific \((99\%)\). Further, it had higher sensitivity for TB detection in smear-positive than in smear-negative patients and could increase TB detection in culture confirmed cases by 23%\(^10\). Similar observations were also made in Ethiopia a country with a high prevalence of TB like ours where the use of Gene Xpert MTB/RIF increased the case detection rate by 31%\(^11\). A large multicenter implementation study involving 6648 participants concluded that a one-off MTB/RIF test has a sensitivity of 90.3%, specificity of 99%, PPV of 96.8% and a NPV of 96.8%\(^11\). The present study showed that Gene Xpert MTB/RIF had a sensitivity of 90.5%, specificity of 99.7%, a PPV of 99% and a NPV of 97% in pulmonary specimens which is in broad agreement with the above studies.

A comparison of the smear microscopy and Gene Xpert results in our patients showed that smear microscopy had a lower sensitivity \((62.8\% \text{ v/s } 90.5\%)\), PPV \((90.7\% \text{ v/s } 99\%)\) and NPV \((88.75\% \text{ v/s } 97\%)\) as compared to Gene Xpert, whereas the specificity of the two tests were comparable \((97.8\% \text{ v/s } 99.7\%)\). The high specificity

<table>
<thead>
<tr>
<th>No.</th>
<th>Characteristic</th>
<th>Prevalence of MDR-TB n (%)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><strong>Smoking status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Smokers</td>
<td>18 (12.67)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Non-smokers</td>
<td>09 (12.50)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td><strong>Diabetes Mellitus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Patients with DM</td>
<td>08 (13.33)</td>
<td>0.039</td>
</tr>
<tr>
<td></td>
<td>Patients without DM</td>
<td>19 (12.33)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td><strong>HIV</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Patients with HIV</td>
<td>01 (11.10)</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td>Patients without HIV</td>
<td>26 (12.68)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td><strong>History of past TB</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Patients who had TB in the past</td>
<td>23 (13.45)</td>
<td>0.536</td>
</tr>
<tr>
<td></td>
<td>Patients who did not have TB in the past</td>
<td>04 (9.30)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td><strong>Lung cavity on chest x-ray</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lung cavity present</td>
<td>20 (12.42)</td>
<td>0.022</td>
</tr>
<tr>
<td></td>
<td>Lung cavity absent</td>
<td>07 (13.20)</td>
<td></td>
</tr>
</tbody>
</table>

*where p value <0.05 considered as a statistically significant association.
of smear microscopy could be attributed to the use of LED fluorescence microscopy for smear examination and regular laboratory quality supervision at our DOTS microscopy center. A systematic review on the different screening tools for TB concluded that smear microscopy had a lower sensitivity and PPV in comparison to Xpert, whereas the specificity and NPV were at par\textsuperscript{13}. Authors from other TB endemic countries have also reported a higher sensitivity of Xpert than smear microscopy in the detection of TB\textsuperscript{7,8,11,14,15}. The observation that the sensitivity and specificity of Gene Xpert is much greater than that of smear microscopy seems to be logical since the Gene Xpert assay requires the presence of only 131 CFU/ml whereas smear microscopy requires 5×10\textsuperscript{7} to 10×10\textsuperscript{4} bacilli/ml in the collected specimen to be reported as a positive smear\textsuperscript{16,17}. The study results reveal that 22 culture confirmed cases were reported as negative by the Gene Xpert assay ('false negative'). This number is much less than AFB smear microscopy where 87 'false negative' cases were reported. Hence, the use of Xpert MTB/RIF reduces the scope of human and laboratory errors and the number of cases incorrectly diagnosed as 'non-TB' by relying on smear reports alone. The observed false negatives in Gene Xpert assay could be due to various factors like poor specimen quality or low bacillary load i.e. below lower limit of detection by Gene Xpert (131 CFU/ml) but above limits of detection by culture methods (10-100 bacilli/ml). Studies have shown that the quality of sputum sample (i.e. salivary, mucoid, purulent or blood stained) is an important determinant which influences the diagnostic performance of the Gene Xpert MTB/RIF assay. Also, the presence of heme compounds and other endogenous PCR inhibitors could inhibit DNA amplification resulting in false negatives results which is reported to be in range of 3\% - 15\%\textsuperscript{18,19}. The present study reported 2 cases which were false positive on the Gene Xpert assay. A possible explanation is that Xpert does not discriminate live, viable AFB from DNA of dead AFB persisting in lung tissue and hence may be positive in patients with previously treated TB; sputum culture, however, will be negative in such cases. Friedrich SO et al observed that during the course of anti-TB treatment the positivity rate of Xpert MTB/RIF assay declines more slowly than that of sputum smear microscopy and culture methods. In fact, even at treatment completion, 27\% of their patients remained Xpert positive whereas 4\% were positive on liquid culture\textsuperscript{20}. Using Gene Xpert, the proportion of patients found to have MDR-TB in our study population was 2.88 \% which is much less than the predicted estimates for India. Globally, the prevalence of MDR-TB is estimated to be 4.1\% among the new TB cases and 19\% among the previously treated cases\textsuperscript{1}. India’s First National Anti-TB Drug Resistance Survey (NDRS) reports the prevalence of MDR-TB as 6.19\% among all patients, with 2.84\% among new and 11.60\% among previously treated TB patients\textsuperscript{21}. The present study showed that among the 27 MDR-TB cases, majority of the patients were males and were between the ages of 30-60 years. A study undertaken in Belarus showed history of previous treatment of TB, age <35 years, HIV, smoking, alcohol abuse, imprisonment and disability to be independent risk factors associated with MDR-TB\textsuperscript{22}. Smokers in our study were more likely to develop MDR-TB than their non-smoking counterparts and this association was statistically significant. A systematic review and meta-analyses to assess the association between drug-resistant TB & tobacco smoking found substantial evidence that tobacco smoking (both past and current) is strongly associated with increased risk of DR-TB\textsuperscript{23}. Diabetes mellitus was the most common co-existing medical condition identified in patients with MDR-TB. Of special concern is the fact that both diabetes mellitus and tuberculosis individually have a high prevalence in the Indian population and a convergence of the two diseases in the future could be catastrophic. A possible explanation for this association may involve ‘immune impairment’ linked to poor glucose control making diabetic patients prone to infection by MDR-TB strains which are otherwise relatively less virulent and less likely to cause active disease in non-diabetic patients\textsuperscript{24}. The data associating HIV infection with MDR-TB is conflicting. A systematic review assessing HIV infection as a risk factor for drug resistant TB failed to demonstrate an overall association between MDR-TB and HIV\textsuperscript{25}. On the contrary, a more recent meta-analysis indicated that HIV is a risk factor for MDR-TB. This was attributed to immunosuppression due to the disease itself as well as drug-related factors like malabsorption leading to development of drug resistance and treatment failure in co-infected patients\textsuperscript{26}. The present study also showed HIV infection to be a statistically significant risk factor for MDR-TB in Indian patients. Earlier studies have shown history of previous TB treatment to be an important risk factor for MDR-TB\textsuperscript{22,26-28}. A similar observation was made in the present study although this association was not strong which could be attributed to an effective implementation of DOTS strategy across India which has brought uniformity in the regimens, dosages and patient adherence by using the
directly observed treatment strategy. It can be assumed that a well implemented DOTs strategy reduces the risk of acquired drug resistance thus weakening the association between past treatment history of TB and DR-TB.

A large number of MDR-TB patients in the study had cavities on their chest X-ray. Previous studies have shown that the radiological finding of cavitary lesion(s) - especially thick walled, multiple and size ≥30 mm could predict multi-drug resistance TB. The possible explanations for this association could be the increased spontaneous mutations due to the high mycobacterial load within the cavities, the reduced exposure of AFB to host defenses and the poor penetration of drug across thick cavity walls leading to sub-inhibitory drug concentrations within the cavities.

A limitation of the present study was that the observations were drawn from a sample population limited to a single city and hence cannot be generalized to the large country like India. Furthermore, the findings in the present study are not novel in itself, but these could help validate existing evidence regarding the role of Gene Xpert in the diagnosis of pulmonary TB in a high burden setting.

CONCLUSION

The results of the present study suggest that Gene Xpert MTB/RIF with its higher sensitivity (90.5%) when compared to AFB smear microscopy (62.8%) could increase the overall number of TB cases detected. Hence, the inclusion of Gene Xpert as an initial test in the RNTCP diagnostic algorithm will ensure that TB cases, and MDR-TB in particular are diagnosed early so that treatment could be initiated during the early stages of the disease itself.

A practical application of the knowledge of risk factors associated with drug resistant TB identified in this study is that it could help medical practitioners to identify these during initial patient assessment and take steps either to minimize their effects (eg. regular monitoring & controlling blood sugars in diabetic patients, stringent monitoring of smear conversion in cavitary TB etc.) or avoid the risk factor altogether (eg. enforcing smoking cessation strategies in active smokers).

ACKNOWLEDGEMENTS

The study was supported by the Indian Council of Medical Research short term studentship grant (ICMR-STS), 2015 (Reference I.D.: 2015-01746).

CONFLICTS OF INTEREST

The authors of the study do not have any potential conflict of interest relevant to this article.

FUNDING SOURCE

The study was supported by the Indian Council of Medical Research short term studentship grant (ICMR-STS), 2015 (Reference I.D.: 2015-01746).

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Case Report

Metastatic lung cancer in oral cavity
A case report and literature review

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Key words:
- Metastatic tumours
- Gingival mucosa
- Lung adenocarcinoma

INTRODUCTION
Tumors are complex heterogeneous and self-organized biological systems where homeostatic mechanisms are dysregulated leading in rapid proliferation of cells and invasion of surrounding tissues⁴. The uncontrollable proliferation leads in cells to detach from the primary tumor, travel through the blood or lymph system, and form new tumor sites in other organs or tissues of the body⁴. Metastasis is also a complex process regulated by several signaling pathways and requires cell motility, invasion, survival, proliferation, and evasion of the immune system. Metastatic tumors to the oral region may manifest in the soft tissues or in the jawbones however are extremely rare, accounting for approximately 1-2% of malignant oral tumors¹⁰. Lung cancer represents approximately the 12% of the total cancer incidence burden and the 18% of cancer-related death worldwide placing it at the first place in terms of mortality ranking¹. Although metastatic tumors in the oral cavity are rare, literature describes that lung cancer as the most common malignancy metastasizing to the oral cavity, followed by breast, kidney, and liver tumors¹⁰. Metastasis to the oral cavity, although can occur at any age, is most common in elderly individuals aged 60 years and older. Only in a few cases the oral/jaw bones lesions are the first clinical manifestation of primary tumor spread. Here we present a very unusual incidence of a metastatic adenocarcinoma of lung in the oral cavity as first manifestation. Dentist must conscious and suspicious for such a diagnosis as it mimics odontogenic infections or other benign conditions especially in elderly with history of heavy smoking.

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ABSTRACT
Metastatic tumors to the oral cavity are rare and they may be localized in the oral soft tissues or jaw bones. Here we present a very unusual incidence of a metastatic adenocarcinoma in the oral cavity originated from lung as first manifestation. Dentists must be aware and suspect such a diagnosis as it mimics odontogenic infections or other benign disorders especially in high-risk patients such as elderly with history of heavy smoking.
CASE REPORT

An 86-year-old Caucasian male with heavy smoking history record presented in Dental clinic of General Hospital of Agios Nikolaos in Crete, Greece with a painful swelling in the left upper back teeth region of the jaw in the past 2 months. The patient was referred in the clinic by a private dentist with an initial diagnosis of a periapical abscess caused by residual teeth roots of #24#25 (Left Premolars).

Physical examination revealed a marked swelling on the left posterior maxillary gingival mucosa (left premolar region) (Figure 1, A). Dental periapical x-ray revealed no residual roots but findings of radio lucent area within the lesion (Figure 1, B). A biopsy obtained under local anesthesia without complications and was sent for histology examination. The histological and immunohistochemistry analysis of the specimen revealed a positivity of TTF1 and a metastatic invasion of poorly differentiated lung adenocarcinoma confirming the chest x-ray image of possible non-small cell lung cancer (Figure 1, C). Treatment for the patient was only supportive as his general condition was burdened and his physical performance was extremely poor.

DISCUSSION

Metastasis is a complex process, often accompanied with dormancy periods prior to any initiation of tumor growth in metastatic organ. Briefly, the metastatic cascade of events involves invasion of the primary tumor lesion to the surrounding extracellular matrix (ECM) from malignant cells that acquire a mesenchymal phenotype with lose cell-cell adhesion, induction of angiogenesis due to hypoxia mechanisms that usually forms immature vessels, inflammation and invasion of ECM with mediation of matrix metalloproteinases (MMPs). This allows malignant metastatic tumor cells to intravasate into circulation stream, travel, and anchor to endothelial cells of another tissue, extravasate and adapt to the new microenvironment and establish a metastatic colony. The infiltrated cells proceed to overt metastasis reinitiating their proliferative cell cycles at the new tissue, and finally form metastatic colonies that are clinically diagnosed.

Metastatic tumors to the oral cavity are uncommon and available literature is mostly based on sporadic case reports or case series. Possible routes of metastasis to the oral cavity are assumed to be through arterial, venous, and lymphatic circulation where Circulating Tumor Cells (CTCs) circumvent the filtration of the lungs through the valveless vertebral venous plexus or through the arteriovenous shunts in the lungs to move to other organs. Oral lesions can be distinguished in two types, mucosal and jawbone primary tumors or metastatic sites as in this case report. Metastasis in jawbone has been related with remnants of hematopoietic marrow that still exists in some elder in the posterior parts of the mandible and can serve as a favorable niche for metastatic CTCs.

Interestingly, mandible is more often involved than maxilla with molar area to be the most frequent site over premolar and angle-ramus. Regarding mucosal metastasis, gingiva is the most common site in oral soft tissues and chronic inflammation has been associated as a co-factor. A chronically inflamed microenvironment in gingiva with expression of cytokines such as interleukin IL-1 and tumor necrosis factor TNF-a, can facilitate metastasis by stimulate processes that attract CTCs as a promising pre-metastatic niche to engraft and proliferate.

Due to its rarity and lack of specific characteristics, oral metastasis may be mistakenly diagnosed as a periapical abscess or a benign primary oral disease. Generally,
several studies try to assess dentists' awareness, knowledge, attitudes, and practices to recognize or diagnose potential tumor lesions in oral cavity (primary or metastatic)\textsuperscript{2,6,11,19}. Most of these studies report a reduced awareness in the diagnosis of possible tumor lesions (metastatic or primary) attributed to several factors mainly the rarity of incidence of oral metastasis or primary tumors and lack of experience as a routine diagnostic procedure for dentists. Visual screening though could potentially reduce mortality in high-risk individuals, hence dentists, as front-line healthcare professionals for oral health, could advance their competencies regarding knowledge and attitudes to screen high-risk patients for oral tumor lesions\textsuperscript{16}. Although the presence of metastatic lesions in the oral cavity usually is sign of extensive metastasis and of very poor prognosis, as was also in this case, palliative care to improve the quality of life and prolong as much as possible is the main goal.

CONFLICT OF INTEREST
None.

FUNDING OR GRANT SUPPORT
This work did not receive any funding or grant support.

REFERENCES
Case Report

Tuberculous cervical lymphadenitis after BCG immunotherapy for bladder cancer
A case report and review of the literature

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Key words:
- Bacille Calmette-Guerin
- Bladder cancer immunotherapy
- Cervical lymphadenitis
- Abscess
- Lemierre’s syndrome

ABSTRACT
Since its introduction in 1976, Bacille Calmette-Guerin (BCG) has become the standard of care in high risk non-muscle-invasive bladder cancer. Apart from its efficacy, BCG immunotherapy is also considered to be safe. However, in addition to its common adverse effects, which are mild, it can rarely cause serious adverse effects, which can be life threatening. In this report, the case of an 85-year-old male, who developed tuberculous cervical lymphadenitis and abscess after BCG immunotherapy is described. The patient presented with acute renal failure. During his hospitalization he also developed hepatitis because of the anti-tuberculous therapy and eventually passed away, probably by septic emboli originated from his right jugular vein which was infiltrated by his abscess. Tuberculous cervical lymphadenitis accompanied with abscess is a quite rare complication of BCG immunotherapy, but clinicians should be aware of it, as it can be proved fatal.

INTRODUCTION
Bacillus Calmette-Guérin (BCG), apart from vaccine for Tuberculosis, can also serve as a bladder cancer immunotherapeutic vaccine.1 When BCG is infused into the urinary bladder, it causes an inflammatory reaction with monocyte infiltration and class-II major histocompatibility complex expression in malignant cells. Subsequently, the malignant cells are targeted by lymphokine-activated killer cells and BCG antigen-presenting cells.2 BCG immunotherapy for bladder cancer is not only effective but also safe, since its most common adverse effects are easily manageable. However, some more serious, but uncommon, adverse reactions have also been reported. To date, limited cases of tuberculous lymphadenitis following BCG immunotherapy for bladder cancer have been reported3 and to the best of our

Abbreviations
BCG: Bacillus Calmette-Guérin
CT: computed tomography

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knowledge cervical lymph nodes were involved in only one case that was treated successfully. We report a case of an 85-year old male, who developed tuberculous cervical lymphadenitis and abscess after BCG immunotherapy for bladder cancer and eventually died.

CASE REPORT

The patient presented with acute renal failure [Urea:224mg/dl, Creatinine: 4.39mg/dl], while his baseline creatinine level was about 1.50mg/dl. From his recent history, he presented an abscess in his right cervix, which had appeared ten days earlier, accompanied with low fever. Three days later, he had been subjected to surgical drainage of the abscess and had received antibiotic therapy with Amoxicillin/Clavulanic. From his past history, he presented type II diabetes mellitus and bladder papillomas, while twelve months before, he had developed bladder cancer for which he had been treated with BCG immunotherapy.

On admission, his white blood cells were normal [8,420/μL (62.0% neutrophils, 24.6% lymphocytes)] and his arterial blood gases were pH=7.42, PaCO₂=31.6mmHg, PaO₂=67.2mmHg and HCO₃⁻=19.8mEq/L on room air, while his c-reactive protein was elevated (9.14mg/dl). Treatment with fluids was initiated for his renal failure and low blood pressure (85/55mmHg). Intravenous antibiotic therapy for his abscess with Piperacillin/Tazobactam was also initiated while pus from the abscess was sent for culture. A neck ultrasound revealed multiple swollen lymph nodes which maintained their shape and vascularization, in a phase of acute inflammation, accompanying a spindle-like, sub-sounded inflammatory region, with increased anarchic internal vascularization, ending in a tube-like outgrowth beneath the skin like fistula (Figures 1a–b).

Four days later, the patient’s blood pressure was stabilized, his renal function improved, maintaining a continuous diuresis, but his low fever persisted, while his pus culture was negative for common bacteria. Thus, a neck computed tomography (CT) was performed, which showed swollen lymph nodes with central melting and the abscess, surrounding the patient’s right jugular vein, which appeared fine-spun (thin with decreased inner diameter) with a lack of repletion after contrast agent infusion, an image that set the suspicion of tuberculous lymphadenitis and of right jugular vein thrombosis (Figure 2). Therefore, a new pus sample was collected from his abscess and sent for Xpert MTB/RIF assay (Cepheid, Sunnyvale, California, USA), acid fast staining and culture for mycobacteria, something that was not done with the first sample. The new sample was positive for Mycobacterium Tuberculosis complex nucleid acid and resistance to Rifampicin was not detected. Acid fast staining was negative. Having optimized the patient’s renal function, his antibiotic therapy with Piperacillin/Tazobactam was
stopped and standard anti-tuberculosis treatment (Isoniazid, Rifampicin, Pyrazinamide and Ethambutol) was initiated. A chest CT was also performed, which did not reveal any signs of active or past pulmonary tuberculosis. Furthermore, the patient was not aware of coming in close contact with any person having pulmonary tuberculosis in the past, while before his BCG immunotherapy, he was subjected to Mantoux test, which was negative. His close relatives were also checked with Mantoux test and were all negative. Because of his recent history, BCG immunotherapy was considered to be responsible for the patient’s tuberculous lymphadenitis. Unfortunately molecular confirmation was not possible. The culture of the last pus sample also came back positive for the Mycobacterium Tuberculosis complex six weeks later. Although the subtype of the Mycobacterium could not be identified, the strain was resistant to Pyrazinamide.

Nine days after anti-tuberculous treatment initiation, the patient developed transaminasaemia and hyperbilirubinemia, which were attributed to the anti-tuberculous regimen. Treatment was discontinued and the liver biochemistry profile started to improve. However six days later and before reinitiation of treatment, the patient presented acute chest pain with dyspnea and developed a rapidly evolving type I respiratory failure with respiratory alkalosism and a respiratory rate of 34 breaths/min. He also showed hemodynamic instability unresponsive to fluid and vasopressor therapy. Having not presented elevated troponin levels, changes in his electrocardiogram compared to that of his admission, apart from tachycardia (132 beats/minute), or any other pathological findings, it was suspected that the patient suffered from septic emboli originating from his right jugular vein, which was infiltrated by his abscess. However, CT Pulmonary Angiogram was not performed due to the patient’s rapid deterioration and eventual death within one hour.

DISCUSSION

Since its introduction in the treatment of high risk non-muscle-invasive bladder cancer, BCG has become the standard of care. BCG immunotherapy has been shown to be an effective treatment for the superficial bladder carcinoma, since its efficacy in the prevention of recurrence is estimated at 70-99%, which is higher than for local chemotherapy. Despite the adverse effects that have been reported, it is generally considered to be safe, since its most common adverse effects, such as cystitis (>90% of adverse effects), fever (2.9%), hematuria (1%), prostatitis (0.9%), and arthralgia or arthritis (0.5%), are mild and non-life-threatening. However, serious adverse events such as spondylitis with adjacent mycotic aortic aneurysm, spondilodysitis with medullary and spinal abscess, granulomatous pneumonia, tuberculous epididymitis, parotid tuberculosis and miliary tuberculosis have also been reported. Immunocompromisation and organ inflammation after BCG immunotherapy is considered to be caused through hematogenous spread. In the only case of cervical lymphadenitis after BCG treatment for bladder cancer that has been reported in the literature, the age of the patient was 68 years and the infection occurred two years after BCG treatment, whereas in the case presented here, the patient was 85 years old and the infection developed one year after his BCG treatment. The main limitation of this case report is the inability to identify BCG from culture. Nevertheless, there were many factors that indicated a BCG infection.
recent BCG immunotherapy that the patient received for his bladder cancer. Secondly, the lack of history of exposure based on his negative mantoux test and thirdly the absence of signs of active or past pulmonary tuberculosis in the chest CT scan. Most importantly the isolated strain was resistant to Pyrazinamide and BCG is typically resistant to Pyrazinamide. Since monoresistance to pyrazinamide is not common for “typical” tuberculosis this finding along with the patient’s history may be considered as an indirect confirmation of BCG. It is also worth mentioning, that there are several cases of suspected BCG infection after treatment for bladder cancer in the literature, in which identification of BCG was not possible.3,12-16 According to a 2014 review, in BCG suspected infections after BCG bladder treatment, acid-fast staining, mycobacterial culture and PCR-based assays were only positive in 25.3%, 40.9%, and 41.8% of cases, respectively.17 The higher sensitivity of PCR and culture can explain the negative acid fast staining observed in the present case.

Another limitation of this case is the patient’s unidenti- fied cause of deterioration and death. Transaminasaemia and hyperbilirubinemia, which forced the discon- tinuation of the anti-tuberculous therapy, were probably caused by the combination of three hepatotoxic drugs. His acute chest pain, accompanied by dyspnea, tachypnea and rapidly evolving type I respiratory failure with respiratory alkalosis and his severe hemodynamic instability, are common in pulmonary embolism. The findings of his neck CT, showing a fine-spun right jugular vein, with lack of repletion after contrast agent infusion, surrounded by the abscess, set the suspicion of Lemierre's syndrome, since there were no other pathological findings indicating an alternative diagnosis. Septic emboli due to tuberculous pathogens are rare,18 and so is the Lemierre's syndrome. Lemierre's syndrome is the infectious thrombophlebitis of the internal jugular vein, which may lead to further systemic complications such as septic emboli. In a 2020 systematic review of Lemierre’s syndrome, there were no cases of the syndrome that were caused by tuberculous pathogens,19 something that also reflects the findings of earlier systematic reviews. To our knowledge there is only one published case, in which the syndrome was caused by a coinfection by Levinea sp and Mycobacterium tuberculosis.20

This is the first case of tuberculous cervical lymphadenitis after BCG immunotherapy for bladder cancer that proved lethal. Clinicians should always suspect Tuberculosis in a patient with infection after BCG immunotherapy for bladder cancer, regardless the localization of the infection, and act timely, in order to diagnose and treat it, taking into account the various complications that could emerge in the process.

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CONFLICT OF INTEREST
None.

FUNDING
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Tracheal diverticulum
Case report of an asymptomatic male patient

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Key words:
- Diverticulum
- Tracheal
- Paratracheal cyst
- Congenital
- Acquired

INTRODUCTION

The paratracheal air cysts include various entities. The physician’s suspicion is orientated in most cases to the digestive system, although the cause could be some rare anomalies arising from the respiratory system. One of these is tracheal diverticulosis. Others include tracheocele, lymphoepithelial cysts, and bronchogenic cysts. Tracheal diverticulum is a type of paratracheal air cyst with an incidence of 2.4%⁵. It is asymptomatic in most of the cases and is usually detected incidentally by imaging methods or post-mortem. It consists of small collections of air in the paratracheal region lined by ciliated columnar epithelium. Tracheal diverticulum is most commonly located at the right posterolateral region of the trachea in about 97% of cases⁵. The main reason is, that on the left of the trachea is the esophagus, which provides support, thus making the wall of the right side more buoyant.

CASE PRESENTATION

A 75-year old male patient was hospitalized after presenting possible respiratory infection with productive cough and without fever. He presented additional breath sounds on physical examination of the lungs. The patient was a smoker and has a history of chronic obstructive pulmonary disease. He was subjected to chest X-ray and a CT scan of the chest. Chest CT scan
was performed with 3 mm sections without intravenous contrast agent administration. Axial images of the chest showed an air-filled formation located at the upper mediastinum, with a maximum diameter of 3.1 cm, which seems to communicate with the trachea by means of a peduncle (Figure 1).

**EPIDEMIOLOGY**

Tracheal diverticulum is a rare and benign entity defined by one or more sachet cavities outpouching from the wall of the trachea. It was first described by Rokitansky in 1838. A report of the largest series of 64 cases, carried out by Goo et al, appears in up to 1% of autopsies. Buterbaugh and Erly estimated that they can occur in approximately 3.7% of the population. It is usually located on the right aspect of the trachea at the level between the vertebrae T1 and T3. The average size of the diverticulum is 4 mm. Its wall may be either thin or thick. The mean age of patients with tracheal diverticulum is 58 years, while the average age of patients with paratracheal air cysts is 55 ± 16.6. It is more common in males (64%) than in females (36%). However, paratracheal cysts are more common in women than in men. There are many different studies in the literature that evaluate the relationship between tracheal diverticulum and bronchial or lung diseases, such as COPD (chronic obstructive pulmonary disease).

**CLASSIFICATION**

The tracheal diverticulum is divided into two types, congenital and acquired.

Congenital is more common in men than women, it is small in diameter and it communicates through a small tract with the tracheal lumen. It is histologically similar to the trachea and represents a supernumerary tracheal branch 4-5 cm below the vocal cords or just above the carina. The congenital tracheal diverticulum arise either from a defect in the differentiation of the endoderm during the development of the posterior tracheal wall membrane or from a defect in the development of tracheal cartilage during the sixth week of fetal life. It affects the entire anatomy of the trachea (respiratory epithelium, smooth muscle and cartilage) and often fills with mucus. It is rarely associated with other congenital malformations, such as tracheoesophageal fistula.

Acquired can occur at any level of the trachea. Moreover, it can be multiple, broader and larger in size than congenital. Histologically, it includes only respiratory epithelium and not smooth muscle or cartilage. Usually there are outpouchings on the tracheal wall located between the extra- and intrathoracic trachea. Long-term increased intratracheal pressure due to chronic cough or chronic obstructive pulmonary disease (COPD) in association with a weakened wall can cause acquired diverticulosis. Acquired may also occur in consequence of complications from surgical procedures or tracheomalacia.

Mounier-Kuhn’s syndrome is a rare disease and is characterized by multiple tracheal diverticula, major bronchial and tracheal stretching, bronchiectasis and recurrent episodes of lower respiratory tract infection.

**CLINICAL PRESENTATION**

Although it is mostly asymptomatic, its presence in symptomatic patients has been associated with chronic cough, recurrent episodes of hiccups, voice hoarseness, wheezing, cough, dyspnoea, odynophagia and dysphagia, respiratory infections and difficulty in placement of tracheal tube (three cases have been described worldwide). Tracheal diverticulum can also cause dysphonia due to recurrent paralysis caused by direct compression of it. If it is infected it can also lead to paratracheal abscess.

There is a case report of pneumomediastinum as a result of perforation of a tracheal diverticulum caused by tracheal intubation.
DIAGNOSIS

Computed tomography examination, including thin sections or reconstructed images, is the proper imaging method for the study of tracheal diverticulum. It is useful for locating, calculating the size, contour and thickness of the tracheal tract wall. It can also demonstrate the connection between the diverticulum and the tracheal lumen. Especially sagittal images are particularly useful. Furthermore, it can be used to distinguish congenital and acquired ones, depending on the presence or absence of cartilage and the size of the neck of the diverticulum. For detailed imaging of tracheal diverticulum the thickness of slices of chest CT should be less than 1mm\(^4\).

Bronchoscopy can also establish diagnosis, but it is an invasive procedure. In addition, diverticula with a narrow opening or just a fibrous connection with the trachea can be bronchoscopically missed\(^10\).

The diverticulum can be infected due to recurrent upper respiratory tract infections. This infected diverticulum can lead to paratracheal abscess. If it is infected, it can be enhanced after intravenous contrast agent administration. Infection may also progress into empyema or subphrenic abscess. Differential diagnosis of Zenker’s infected diverticulum is difficult, and endoscopic upper gastrointestinal system may be required if communication with the trachea in chest CT is not apparent.

TREATMENT

Treatment is not indicated in asymptomatic patients. In symptomatic patients therapeutic approach should be based on age, co-morbidity, and clinical presentation. The treatment of choice in symptomatic patients is usually surgical resection. This can be done without thoracotomy but with lateral cervical access. In addition, endoscopic cauterizations with laser or electrocoagulation are used.

For elderly patients conservative treatment is preferred with use of antibiotics, mucolytic agents and respiratory physiotherapy\(^1\).

Particularly for the acquired diverticula, surgical treatment is not always beneficial. The prevention of infections is usually preferred in patients with multiple tracheal and wide-spread diverticula. For congenital, the surgical resection is preferred due to the long-term accumulation of mucus inside them and potential risk of infection. However, for patients with congenital tracheal diverticulum who undergo surgery there is a risk of injury of the esophagus or the laryngeal nerve\(^3\).

Patients with paratracheal abscess and respiratory distress should have an emergency intubation and surgical drainage.

CONCLUSIONS

The tracheal diverticulum should be included in the differential diagnosis of paratracheal air cysts. CT chest scan is the imaging method of choice for its diagnosis. However, predisposing factors (more common in men, COPD or chronic cough) should also be considered. The therapeutic approach depends on the existence of symptoms, age and co-morbidity. In this patient, a possible association of tracheal diverticulum with respiratory infection or COPD should be considered. Lastly, it should also be taken into account whether the type is congenital or acquired, as the surgery is not in all cases beneficial.

CONFLICT OF INTEREST

None.

FUNDING

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ΠΕΡΙΛΗΨΗ

Εκκόλπωμα τραχείας. Περίπτωση ασυμπτωματικού άρρενου γηριατρικού ασθενούς

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Το εκκόλπωμα τραχείας είναι μια σπάνια δυσπλασία. Ανιχνεύεται συνήθως με τις απεικονιστικές μεθόδους και πρέπει να συμπεριληφθεί στην διαφορική διάγνωση οποιασδήποτε παρατραχειακής κύστης
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Massive tension Pyopneumothorax and pneumoperitoneum in a Covid-19 patient

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A formerly healthy 55-year-old woman presented in the emergency room with fever, weakness, and dizziness. The PCR test for SARS-CoV-2 turned positive and she was at first admitted to the internal medicine department and subsequently to the Intensive Care Unit because of respiratory failure. She was intubated on day 7 and was extubated on day 15. Nevertheless, she was reintubated on day 17. Aspergillus fumigatus was isolated in bronchial secretions and treated with isavuconazole, while she was submitted to a tracheotomy on day 30. Her course was complicated by several episodes of ventilator-associated pneumonia caused by multi-resistant gram-negative bacteria treated accordingly. On day 40 after a hemodynamic deterioration she was submitted to a change of central venous catheter and an empirical change of antimicrobial treatment. On day 45, after further deterioration and the presence of subcutaneous emphysema, a tension pneumothorax on the right side was revealed. The insertion of the chest tube drainage showed the presence of a purulent pleural fluid along with the presence of air. The chest X-ray showed free air in the peritoneum, so she was submitted to an emergency C/T scan of the chest and abdomen, confirming the presence of a large quantity of free air in the peritoneum (Figures 1,2) that was attributed to the spreading of the air from the lung. Pseudomonas aeruginosa was cultured in the pleural fluid but despite the proper treatment, the patient expired on day 49.

FIGURE 1. C/T scan of the chest. Presence of residual free air in right pleural space. The thoracostomy tube is in place.

FIGURE 2. C/T scan of the abdomen. Presence of large quantity of free air in peritoneum.
Drug-induced hepatotoxicity of antituberculosis drugs

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Key words:
- Drugs
- Tuberculosis
- Hepatotoxicity

A 35 years-old male smoker was admitted for anorexia, nausea, vomiting, sweating and fatigue for over three days. The patient was on antituberculosis treatment, since 2 months, with isoniazid, rifampicin, pyrazinamide and ethambutol for cavitary pulmonary tuberculosis (right upper lobe cavitary lesion) (Figure 1). The blood test showed serum alanine aminotransferase and aspartate aminotransferase levels three times the upper limit of normal and also a lower hemoglobin level of 10.9 grams/dL. Computed tomography with contrast showed liver and spleen enlargement (Figure 2). The patient undergone liver biopsy that revealed large lipid vacuoles within hepatocytes (steatosis) (Figure 3). After ten days of treatment withdrawal, isoniazid was reintroduced, increasing sequentially, adding further rifampicin, pyrazinamide, and ethambutol without liver toxicity. The patient is under regular follow up.

FIGURE 1. Chest radiography.

FIGURE 2. Computed tomography of the abdomen.

FIGURE 3. Histological sample.
A 60 years-old male smoker presented with dyspnoea, dry cough, fatigue, and night sweats lasting over 6 months, without any improvement despite various treatments. Sputum culture revealed acinetobacter baumannii. Chest radiography (Figure 1) was highly suggestive for a pulmonary tumor with. A chest computed tomography (Figure 2) showed a pulmonary mass at the posterior segment of the right upper lobe (50/40/60 millimeters) with linear extensions into the adjacent parenchyma, multiple nodular calcifications and bronchiectasis. After exploratory thoracotomy, a biopsy showed thickened pleura, with granulation tissue and areas of chronic perivascular inflammatory infiltrate. In some sections, marginally, blood exudate along with polymorphonuclear inflammatory cells was observed, consistent with chronic infectious fibrinous pleuritis (Figure 3). Administration of intravenous antibiotics, according to the antibiogram, had a favorable outcome (Figure 4).

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COVID-19 and congenital long QT syndrome

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Key words:
- SARS-CoV-2
- COVID-19
- Coronavirus

A 40 years-old male, presented with four days nausea, loose stools, vomiting, without respiratory symptoms. He has a history of congenital long QT syndrome. On physical examination hippocratic fingers and normal vital signs. Lab results: total cholesterol serum 221 mg/dl, triglycerides 226 mg/dl, glucose 118 mg/dl, a white blood cell count of 5.81 10⁹/Liter, neutrophils 4.22 10⁹/Liter, lymphocytes 39.93%, D-dimers 612 ng/mL, creatine kinase-MB 22 U/L, lactic acid dehydrogenase 816 U/L, fibrinogen 497 mg/dL, mildly elevated transaminase levels due to alcohol abuse, modified thrombin clotting time, normal renal function, troponin T 7.2 ng/L, ferritin 392 ng/mL, a C reactive protein level of 19 ug/L and erythrocyte sedimentation rate of 45 mm/hr. Electrocardiogram revealed prolongation of the QT interval in excess of 0.49 sec. Normal spirometry values. Negative nasal swab for flu. Nasopharyngeal swab tested positive by SARS-CoV-2 RT-PCR. Chest computed tomography revealed multiple and bilateral patchy ground glass opacities (Figure 1). He received Tocilizumab 8 mg/kg administered as a single 60 minute intravenous infusion, and heparin. After two negative RT-PCR tests at 24 hours interval and thirteen days after onset of symptoms, he was considered clinically recovered and discharged without any complications (Figure 2).

![FIGURE 1. Initial Chest CT.](image1)

![FIGURE 2. F/U Chest CT.](image2)
A practical lesson in clinical communication

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Key words:
- Bronchiectasis
- Bronchography
- Clinical communication
- Medical history

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My new patient is in his early eighties, in reasonably good health. He has a long-standing pulmonary problem which has been fairly stable; as this was recently noticed in a chest film he had for some other reason, he decided to have it checked. He tells me this was first picked up when he was doing his mandatory army service, and has a document to prove it. Out of the plastic cover of his insurance booklet he draws out a folded hand-written note. There is no date on the piece of paper, a page torn from a military hospital notepad, which has a few lines of text on both sides and a crude, yet pretty accurate drawing of the patient’s problem [see Figure below]. A branched tree-like structure fills a hemithorax, bearing ‘fruit’ that looks like black olives. An icon of bronchiectasis, as it would appear on bronchography, the state-of-the-art imaging modality of the 1960’s.

The patient provides me with the necessary background history. As a soldier he had been admitted with pleurisy, underwent bronchography (a not particularly pleasant procedure, which has virtually vanished from everyday practice with the advent of computed tomography), and this brought to light the underlying pathology. The treating physician gave him the appropriate instructions for regular postural drainage and antibiotics in case of exacerbation, but also took the additional step of providing a written record for posterity. As compact disks or even typewritten discharge summaries were unimaginable in those days, he used what he had ready to hand: pen and paper. His brief note is as informative today as it was six decades ago. I am sure he never imagined that it would last that long.

The signature of the doctor on the note is indecipherable. However I can make out the text, and here is its translation:

Bronchography was performed on the right side, and this showed saccular-cystic bronchiectasis in the posterior segment of the upper lobe and the apical of the lower, and cylindrical (bronchiectasis) in the basal and anterior (?) segments. The middle lobe bronchus is probably occluded (middle lobe syndrome?).

Therapeutic instructions: In case of exacerbation postural drainage is recommended, as well as use of broad spectrum antibiotics following sensitivity testing, or, failing that, chloramphenicol 1.5g daily for 10 days along with vitamin B complex supplements.

At the mention of chloramphenicol my mind takes another flight back in time. I am not sure how many of the readers have ever used it, but I recall that as late as 1989 we were advised by our consultants to prescribe it in difficult cases of chest infection (bronchiectasis or exacerbations of COPD) in the late Monsall Hospital for Infectious Diseases in Manchester. There was
a fear of side effects (especially bone marrow suppression and aplastic anemia), which we had been taught in third-year pharmacology, but thankfully we had never seen it, and our patients usually did well. I have not met or prescribed it again, and modern reference books only mention it in the formulation of eye ointment, and as a rarely used treatment for exotic infections such as plague and tularemia. I suspect it was pushed out of fashion by newer, fancier and more expensive antibiotics with a better safety profile. I ask my patient whether he had ever needed to take the drug: the answer is negative.

With these thoughts I scan and save the note for my patient record, and also for its historical value, and bless the memory of the unknown army physician for his very practical communication skills. And congratulate my patient for meticulously preserving this ‘holy relic’ of medical history.

**Postscript:** A few days later, modern imaging confirms the patient’s history and adds fine detail to the hand-drawn bronchographic sketch. The CT scan report states that there are confluent cystic bronchiectases in the right upper and lower lobe as well as local pleural thickening on the right side. Sixty years later, the initial diagnosis is still valid, and the patient remains well.
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Acknowledge the persons who provided a true contribution and who endorse the data and conclusions. Acknowledge any funding sources.

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