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REVIEW

Phenotyping emphysema and airways disease: Clinical value of quantitative radiological techniques

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Author contributions: Turner A designed the review question; Turner A and Subramanian D reviewed the main text; Crossley D wrote the paper.

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Abstract

The pathophysiology of chronic obstructive pulmonary disease (COPD) and Alpha one antitrypsin deficiency is increasingly recognised as complex such that lung function alone is insufficient for early detection, clinical categorisation and dictating management. Quantitative imaging techniques can detect disease earlier and more accurately, and provide an objective tool to help phenotype patients into predominant airways disease or emphysema. Computed tomography provides detailed information relating to structural and anatomical changes seen in COPD, and magnetic resonance imaging/nuclear imaging gives functional and regional information with regards to ventilation and perfusion. It is likely imaging will become part of routine clinical practice, and an understanding of the implications of the data is essential. This review discusses technical and clinical aspects of quantitative imaging in obstructive airways disease.

Key words: Chronic obstructive pulmonary disease; Alpha one antitrypsin deficiency; Computed tomography; Densitometry; Phenotype; Spirometry; Magnetic resonance imaging

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Core tip: Phenotyping emphysematous patients radiologically allow physicians to diagnose and deliver tailored and targeted therapies that are not possible with spirometry. When patients are divided into chronic bronchitis or emphysema on computed tomography (CT), they have significantly different clinical features and spirometry, demonstrating its ability to characterise phenotypic differences. CT offers accurate mapping and measurement of emphysema whereas magnetic resonance imaging (MRI) can provide functional information relating to ventilation and perfusion. This unique feature of MRI can help prognosticate patients in whom surgery is being considered. CT and MRI have both been sufficiently validated clinically and pathologically.

Crossley D, Turner A, Subramanian D. Phenotyping emphysema

and airways disease: Clinical value of quantitative radiological techniques. *World J Respirol* 2017; 7(1): 1-16 Available from: URL: http://www.wjgnet.com/2218-6255/full/v7/i1/1.htm DOI: http://dx.doi.org/10.5320/wjr.v7.i1.1

INTRODUCTION

The pathophysiology of chronic obstructive pulmonary disease (COPD) and Alpha one antitrypsin deficiency (AATD) is increasingly recognised as complex and lung function alone is insufficient for early detection, categorising and dictating management. Up to one third of the lung can be destroyed before respiratory impairment is detected by spirometry[1], meaning those with early disease may remain undiagnosed. Patients with emphysema and airways disease have significant clinical and physiological differences[2,3] and therefore phenotyping radiologically should allow for more individualised treatment with outcomes that are more meaningful to the patient.

The typical clinical phenotype of the patient with emphysema is that of significant breathlessness, hyperinflation and low body mass index. By contrast, the phenotype associated with predominant airways disease, *i.e.*, chronic cough and infective exacerbations, has a different clinical spectrum within the umbrella term of COPD and requires separate recognition. Severity of symptoms and exacerbation rates are factors that directly impact patient's quality of life, and therefore diagnosing and tailoring treatment early on will have the best outcome for symptom resolution and slowing disease progression.

Quantitative imaging techniques can phenotype patients into predominant airways disease or emphysema, providing an objective tool to detect disease earlier and more accurately. This is of increasing significance as targeted treatments beyond inhaled therapy (such as endobronchial valves and alpha one augmentation therapy) become available, which require careful patient selection. Computed tomography (CT) provides detailed information relating to structural and anatomical changes seen in COPD, whereas MRI/nuclear imaging gives functional and regional information with regards to ventilation and perfusion. Optical coherence tomography (OCT) gives microscopic detail of the airway wall where differences in the contribution of active inflammation and airway remodelling could be a useful biomarker and drug target.

This review article discusses these three imaging modalities, how they can be used to phenotype patients radiologically into emphysema and airways disease, and therefore individualise management. The clinical and pathological validation of each is demonstrated as well as the methods of quantification. Their individual merits and how they compare against one another is discussed, and trials that have used imaging as an outcome measure for treatments in COPD already are highlighted. It is the strengths of these techniques make it likely imaging will become part of clinical practice, and an understanding of the implications of the data is therefore essential for healthcare workers.

CT

Phenotyping using CT

Spirometry measures such as the forced expiratory volume in 1 second (FEV1) alone are insensitive to early emphysematous change, and only moderately correlate to quality of life measures[4]. Therefore using symptoms and exacerbations alongside FEV1 to categorise COPD seems logical, which led to adoption of these methods in the most recent GOLD guidelines[5]. However this is not the only conceivable way in which severity could be described; CT scanning has potential to delineate additional phenotypes complementing GOLD severity stage.

Studies have shown measures of airways disease on CT such as increased wall thickening are distinct from those of low density and parenchymal destruction seen in emphysema and therefore can be used to subdivide COPD patients into phenotypes[3,6]. When patients have been classified by CT into emphysema or airways predominant phenotypes, there are significant differences between the groups for lung function, symptoms and exacerbation rates. Table 1 lists relevant trials that have divided patients radiologically and the clinically different variables between the groups. Han *et al*[7] demonstrated differences in the rate of exacerbations between the emphysema and airway predominant phenotypes, and that the risks were independent between the two groups. This adds evidence to the increasing recognition that the two disease states are separate and the driving pathology behind them may be different.

Table 2 summarises the current treatment recommendations from BTS and GOLD once patients have been phenotyped. There is of course overlap between the groups, with those patients with an emphysematous predominant phenotype experiencing more frequent exacerbations, and patients should continue to be evaluated individually. This overlap is highlighted in the table.

Disease distribution

Emphysema as a result of smoking/inhalation of noxious gases most frequently results in the centrilobular distribution of emphysema which begins in the upper zones. However, their relative high V/Q ratio means they contribute significantly less to the overall PFT result and therefore in usual COPD isolated to purely the upper zones, the PFTs may seem relatively normal earlier on. Nakano *et al*[16] showed accordingly that the correlation between FEV1 and %LAA was weakest in the upper zones, but as the emphysema often begins in the upper zones, there is a higher association for DLCO here and centrally rather than peripherally. Similar

Table 1 Summary of studies dividing patients as HRCT defined phenotypes and their significant differences clinical and physiological (P < 0.05)

Ref.	HRCT defined phenotypes	Variables studied	Significant variable difference
Kitaguchi *et al*[8], 2006	A: Little or none of either emphysema or BWT E: Emphysema but no BWT M: Emphysema and BWT	Gas exchange Gas transfer Lung function Response to beta-agonist Response to treatment with ICS Sputum cell differentiation	A: ↑ BMI ↑DLCO ↓ hyperinflation ↑ reversibility ↑response to ICS ↑ % of sputum eosinophils E: No response to ICS M: ↑ response to ICS ↑ % of sputum eosinophils
Fujimoto *et al*[9], 2006	A: Little or none of either emphysema or BWT E: Emphysema but no BWT M: Emphysema and BWT	Exacerbation rates Gas exchange Gas transfer Hospital admissions Lung function Response to beta-agonist Symptoms	M: ↑ volume of sputum, exacerbation rate and admission to hospital
Pistolesi *et al*[10], 2008	From derivation set, created new validation set Group A and B	CT parameters Gas exchange Gas transfer Lung function	A: ↓ FEV1, ↑ TLC ↓ DLCO. ↑ pixel index (threshold -950HU) B: ↑ BMI purulent sputum worse bronchial wall thickening
Han *et al*[7], 2011	Emphysema predominant or Airway predominant	BWT Exacerbation rates lung function % emphysema	Emphysema Predominant (> 35% -950HU): ↓ FEV1 and 6MWD ↑ SGRQ and MRC grade For every 5% ↑ in emphysema, 1.18 fold ↑ exacerbation frequency Airways predominant: For 1 mm ↑ in segmental BWT 1.84 fold ↑ in exacerbation frequency
Subramanian *et al*[3], 2016	Emphysema dominant, airways disease dominant, mixed pathology and mild disease	Blood parameters CT parameters Gas exchange Gas transfer Lung volumes Spirometry	Compared with airway disease dominant group, emphysema dominant group had ↑ lung volumes, ↓ gas transfer ↓ pO_2 + pCO_2 ↓BMI ↑Hb No difference between age, and smoking history between the groups
Da Silva *et al*[2], 2016	Emphysema or airways disease	Clinical + functional evaluation HRCT	Emphysema group: ↑ airflow obstruction ↓ BMI ↓ 6MWD

A: Airways; E: Emphysema; M: Mixed; BWT: Bronchial wall thickening; 6MWD: 6 minute walk distance; CT: Computed tomography; BWT: Bronchial wall thickness; DLCO: Transfer factor for carbon monoxide; ICS: Inhaled corticosteroid; FEV1: Forced Expiratory Volume in 1 second; TLC: Total lung capacity; HU: Hounsfield units; SGRQ: St Georges Respiratory Questionnaire; MRC: Medical reseach council; HRCT: High resolution computed tomography.

findings were demonstrated by Parr *et al*[17] in AATD patients that basal distribution is associated with greater impairment of FEV1 (P = 0.002), but less impairment of gas exchange (P = 0.016), and Aa gradient (P = 0.007). Given the lung function variation between different lung regions the authors warn of using a single physiological parameter as a measure of severity as it may introduce bias.

Castaldi *et al*[18] found that panlobular rather than centrilobular distribution was associated with stronger associations with lung function and QoL than CT lung density, demonstrating that the distribution of disease has an independent effect on severity. AATD typically occurs in a panlobular distribution with basal predominance, and Dawkins *et al*[19] showed that for these patients, basal distribution carried a higher mortality risk. Finally, in patients randomised to the medical arm of the National Emphysema Treatment Trial, the authors demonstrated that a greater proportion of emphysema in the lower lung zone *vs* upper lung zone was predictive of mortality (P = 0.005)[20].

Lung volume reduction surgery: Using CT measurements both visually and quantitatively allow for more careful selection of COPD patients when considering lung volume reduction surgery (LVRS). Selecting patients appropriately to either medical or surgical treatments can reduce the associated mortality. The National Emphysema Treatment Trial randomised 1218 severe emphysema patients to either LVRS or medical management[21]. They visually scored the CT scans of patients as being either predominantly upper lobe or lower lobe, and assessed exercise capacity. They found that in a carefully selected population of those with upper lobe emphysema and a low exercise capacity, those in the surgical treatment arm had a significantly lower mortality (RR for death 0.47, P = 0.005). However, in those with predominantly lower lobe emphysema but a

Table 2 Treatment of chronic obstructive pulmonary disease as defined by computed tomography phenotypes

CT phenotype	CT defining features	Clinical features	Findings	Treatments	Ref.
Emphysema	↓ Perc15 Emphysema Centrilobular Panlobular Paraseptal Bullous	Health status	↓ BMI[2] ↑ SGRQ + MRC[7]	Rehabilitation Nutritional support Palliative care	GOLD 2016[5]
		Exercise tolerance	↓ 6MWD[2] ↓ pO_2 ↓ pCO_2[3]	Rehabilitation Maintenance of physical activity Oxygen	GOLD 2016[5]
		Lung function	↑ TLC ↓ KCO ↓ FEV1/FVC	LAMA/LABA LVRS/BVLS Transplant Bullectomy[11] LVRS[11]	GOLD 2016[5] NICE 2010[11]
		Symptoms	↑ Hb[3] No significant response to ICS[8]	Theophylline Rehabilitation typically MRC > 3	GOLD 2016[5] NICE 2010[11]
Airways disease		Exacerbation frequency/ severity	↑ Exacerbations hospital admissions[7]	LABA/phosphodiesterase-4 inhibitor LAMA/phosphodiesterase-4 inhibitor Mucolytics Add in ICS Prophylactic antibiotics	GOLD 2016[5] NICE 2010[11] Brown *et al*[12], 2007 Fabbri *et al*[13], 2009 Calverley *et al*[14], 2009 Herath *et al*[15], 2013
	Lower wall area/body Surface area ratio (WA/BSA) Lower luminal area/BSA Higher %WA	Symptoms	Significant response to ICS+ Significantly higher % of sputum eosinophils[8] Peribronchial thickening[10] Air trapping	Physiotherapy and active breathing techniques Mucolytics Roflumilast Bronchodilators	NICE 2010[11]

6MWD: 6 minute walk distance; CT: Computed tomography; ICS: Inhaled corticosteroid; FEV1/FVC: Forced expiratory volume in 1 second/forced vital capacity; TLC: Total lung capacity; HU: Hounsfield units; SGRQ: St georges respiratory questionnaire; MRC: Medical reseach council; HRCT: High resolution computed tomography; Perc15: 15th percentile point; KCO: Gas transfer co-efficient; LVRS: Lung volume reduction surgery; LAMA: Long acting muscarinic antagonist.

high exercise capacity, those randomised to the surgical arm did worse (RR for death 2.06, P = 0.02). Therefore, LVRS confers a survival advantage in carefully selected patients, but there is associated higher mortality with no significant increase in functional status in those with non-upper zone predominant disease. Gierada *et al*[22] have also demonstrated that those upper lobe predominant emphysema, in a heterogeneous have a two-fold or more average increase in FEV1 following LVRS.

Predicting post-operative FEV1: CT density masking to quantify the severity of emphysema is linked to favourable post-operative outcomes. Sverzellati *et al*[23] applied a density mask to 9 COPD patients awaiting lobectomy for lung cancer, along with spirometry. With specific equations, they predicted the post-operative FEV1 using both values and found quantitative CT was superior to lung function (r = 0.9). Gierada *et al*[24] used various LAA measurements and determined that a 75% LAA or greater for -900HU threshold, or 25% at -950HU were associated with improved outcomes post-operatively including a > 50% improvement in FEV1 and 2 fold increased six minute walk distance.

Finally the ratio of upper to lower lobe emphysema is of particular importance in assessing predicted post-operative FEV1 following bilateral LVRS. Consistent with the fact that upper lobe predominance is associated with better outcomes, Flaherty *et al*[25] found that the CT emphysema ratio (CTR) was the best single predictor of a successful 12% increase in FEV1 (absolute value 200 mL). Importantly, the highest CTR scores (> 2.5) were associated with a greater than 90% specificity at each time point up to 36 mo, although the sensitivity was low. The positive predictive value of this threshold was at least 75% up to 36 mo after surgery. The negative predictive value remained moderate at all thresholds throughout 36 mo of follow-up.

Quantification of emphysema

CT densitometry is the method of quantifying the severity of emphysema using dedicated software. Figure 1 demonstrates how the CT images are digitally produced. X-rays are emitted and passed through the subject and received by detectors that calculate how much the intensity has been reduced by the tissue. These attenuation co-efficients are then converted into a digital image in the form of a matrix consisting of many small data sets. Each small square in the matrix is a pixel, and in 3D with volume adjustment is a voxel. Each pixel is assigned a value in hounsfield units (HU) from -1000 representing the least possible density/ attenuation, *i.e.*, air and 1000 representing the highest,

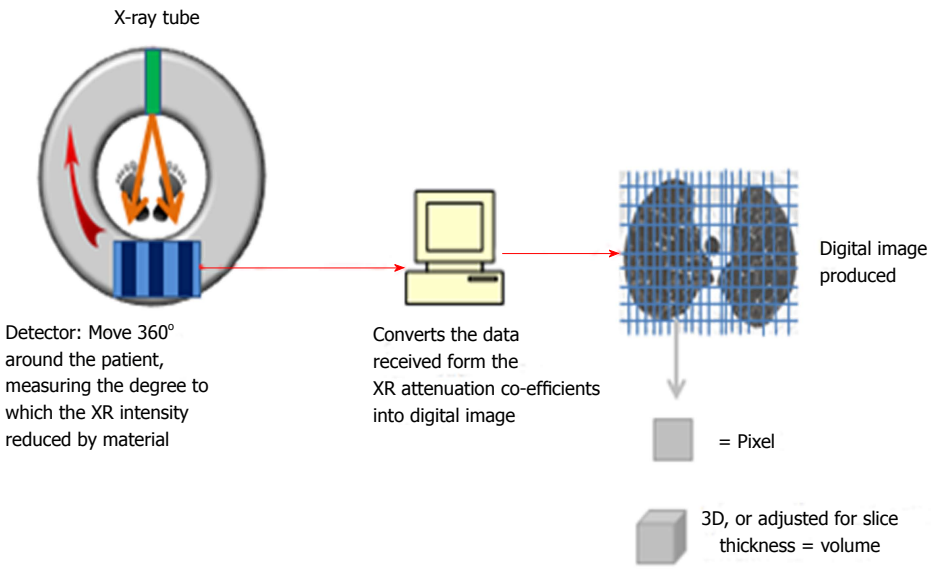

Figure 1 The process of computed tomography scanning. X-rays are passed from the source through the subject laid on the table, and received by the detectors that rotate 360° around the patient. The reduction of the intensity of the XR beam passed through the subject is calculated as an attenuation co-efficient, which from all the slices is reformatted into a digital image.

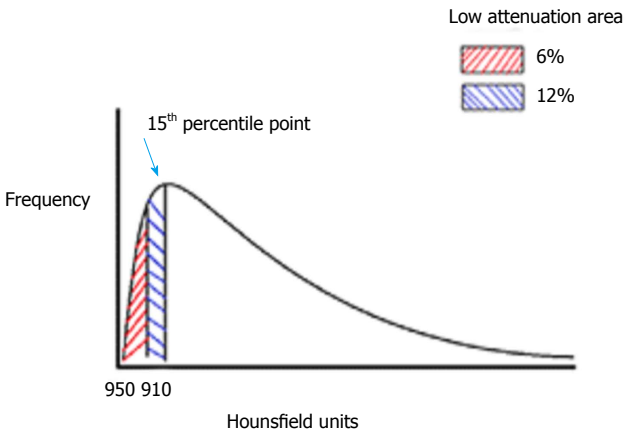

Figure 2 Calculation of densitometric indices. Example of a density histogram, and how the area under the curve at a given threshold is calculated. In this figure, with a threshold of -910HU, 12% of the pixels are between -910 and -1000HU.

Table 3 Table to summarise studies performed in alpha one antitrypsin deficiency and chronic obstructive pulmonary disease directly comparing the most accurate measure of computed tomography density

Condition	Type of study	910	950	Perc15	Conclusion of superior measure	Ref.
Alpha one	RCT	x	x		950	Parr *et al*[29]
	RCT		x	x	950 and Perc15	Parr *et al*[30]
	RCT	x	x	x	Perc15	Parr *et al*[26]
	Review	x	x	x	Perc15	Hogg *et al*[28]
Chronic obstructive pulmonary disease	RCT	x	x	x	Perc15	Shaker *et al*[31]
	Review	x		x	Perc15	Dirksen *et al*[27]
	RCT		x	x	950	Chong *et al*[32]

Variables tested, type of study and conclusion of the most superior measure shown. RCT: Randomised controlled trial.

i.e., solids. These pixels or voxels can be plotted on a histogram as shown in Figure 2. There are two ways of reading the severity from this histogram. The first is the value of where the 15th percentile point lies on the curve (Perc15) and is the most preferred value in trials quoting density, as it is most accurate and sensitive to change[17,26-28]. The second method is to calculate the percentage under the curve that represents the low attenuation area for a selected threshold, *e.g.*, -910HU or -950HU. These and other values are used in studies quoting density, and Table 3 demonstrates trials that have sought to ascertain the most valid method in both AATD and COPD.

Validation

Pathological correlations: The ability of density analysis to accurately assess the degree of emphysema has been validated on pathological studies. Müller *et al*[33] in 1988 showed a strong correlation between density mask results and an assigned emphysema pathology score (1 to 100) in 28 patients who had undergone lobar resection for a lung tumour (r = 0.83, P < 0.001). In a larger group of patients who had undergone resection for similar reasons, Gould *et al*[34] also demonstrated a strong correlation between emphysema measures quantitatively on imaging and that on resected specimens (r = 0.77)[35,36].

Clinical correlations: Numerous studies have shown significant correlations between CT measures of emphysema (Perc15 and %LAA 950) and FEV1 and DLCO[37-40], as well as measures of exercise tolerance, *e.g.*, MRC grade and 6 min walk distance (6MWD)[41-45]. There are also significant correlations with frequency

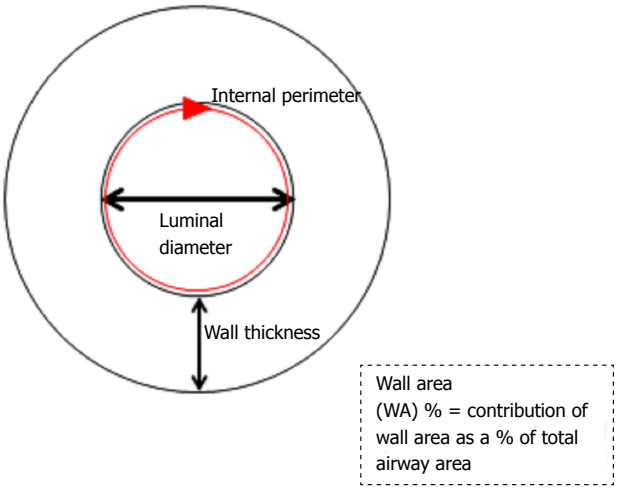

Figure 3 Airways disease measurements. Diagram to demonstrate various values calculated in assessing either the luminal or wall contribution to airway thickening.

of exacerbations and ultimately mortality[19,41,46-48]. In the NELSON trial (Dutch and Belgium Lung Cancer Screening Trial), Mohamed Hoesein *et al*[35,36] have shown smokers who normal lung function demonstrated evidence of emphysema on CT concluding that CT is a more sensitive in detecting emphysema than PFTs. However, the R^2 value between CT density and FEV1 even when adjusted for other variables remains 0.3-0.68 indicating that the parenchymal disease detected by CT density only contributes for 30% to 68% of the total variation[18,49-51]. Therefore other factors including small airways disease must additionally contribute to the altered lung function seen.

Airways disease

Quantification: Luminal area (LA) and the wall area (WA) (expressed as a percentage (%WA = WA/LA + WA*100)[52] can be derived from CT measurements, as well as bronchial wall thickness (BWT) as the square root of WA adjusted for the internal perimeter[53,54] (Figure 3). Airway measurements are often based on the full width at half maximum principle[55,56]. However, this method is known to overestimate the value of wall thickness and various algorithms for quantification are modifications are of this[57,58].

Validation: Nakano *et al*[52,55] demonstrated on histology slices that those airways with an internal diameter of greater than 0.75 cm could accurately predict the dimensions of small airways with an internal diameter of 1.27 mm (r = 0.57, P < 0.01) and in particular measurements from the right S1 segmental bronchus. Airway wall thickening as measured by CT is related to obstructive spirometry[59-62], and chronic sputum production is associated with increased likelihood of an exacerbation leading to a hospital admission[63], and death from a pulmonary infection[64]. Chronic bronchitis (cough and sputum production for at least > 3 mo in 2 consecutive years)[5] has a greater mean %WA and internal perimeter, and is associated with higher exacerbation and mortality rates[53,65,66].

CT quantification variability: The potential pitfall of CT analysis is that the various components must all be equal in order to compare like for like. These factors include using the same software programme[67], the same reconstruction algorithm[68-70], appropriately calibrating the scanner[26,29] and adjusting for volume[27,32,71]. If CT density logistics are standardised, then scans may be compared longitudinally to measure treatment effect, and combined from different centres. A detailed review of CT noise reduction by Dirksen *et al*[27] 2008 recommended using a soft reconstruction algorithm, with a slice thickness of 3-5 mm, at a low radiation dose using a phantom. As for volume adjustments, there is no general consensus as to which method is preferable, though physiologically adjustment using the patient's own volume measurements seems more intuitive.

Trials: CT has been used as an alternative outcome measure in therapeutic trials for patients with emphysema. When performing power calculations in the EXACTLE study using CT density as a measure of response to alpha one augmentation therapy, the author's calculated 494 patients would need to be recruited in each treatment arm for 3 years using FEV1 as the primary outcome measure[72]. In the RAPID trial however, they calculated 180 patients distributed over the two treatment arms would provide a power of at least 80% using two sided P value of 0.05[73].

CT has been used to measure response in both usual COPD and in Alpha 1 anti-trypsin deficiency and the summary detailing CT measure used, outcomes and the strengths and weaknesses of each study are presented in Table 4. Notably, in AATD the recent RAPID trial was the first RCT to demonstrate a significant improvement in lung density with alpha one augmentation therapy. Stockley *et al*[74] pooled the data from the two RCTs by Dirksen et al in 1999 and 2009 (EXACTLE), and with the increase in statistical power , augmentation therapy increased the lung density as measured by 2.997 g/L in comparison to the placebo arm (95%CI: 0.669 to 3.926, P = 0.006).

MAGNETIC RESONANCE IMAGING

Magnetic resonance imaging (MRI) measures the behaviour of protons once a strong magnetic force is applied. The lungs have therefore been notoriously difficult to image due to the abundance of air and low proton density. However, technology has advanced so that MRI may capture changes in a much shorter time window and use inhaled gases (oxygen and hyperpolarised helium/xenon) that alter the proton behaviour in different ways, so that disease and heterogeneity in the lung may be detected. The benefits of MRI over CT and PFTs are the ability to acquire functional information with regards to ventilation, perfusion and alveolar diffusion,

Table 4 Summary of interventional drug trials using computed tomography measures as an outcome measure

Ref.	Study design	Pt N°	Duration	CT measure	Drug	Result
Usual COPD						
Shaker *et al*[75]	RCT	254	2-4 yr	Perc15 and -910HU	Budesonide or placebo	Annual fall in Perc15 ↑in the placebo arm *vs* budesonide (P = 0.09) Annual increase in -910HU↓in the budesonide arm (P = 0.02)
Hoshino *et al*[76]	RCT	54	16 wk	%WA, LA, BWT	Tiotropium, Indacaterol or both	Combination therapy resulted in a ↓in %WA and wall thickness (P < 0.01)
Nordenmark *et al*[77]	RCT	36	12 wk	BWT, air trapping index and %WA	Reversible neutrophil elastase inhibitor 60 mg BD	No difference
Shimizu *et al*[78]	Inter-ventional trial	23	1 wk	Airway inner luminal area	SFC	Ct detected the significant change in airway inner luminal area r = 0.65, P < 0.001
Alpha 1 Antitrypsin deficiency						
Stolk *et al*[79]	RCT	262	1 yr	Perc15	Parlovarotene	No benefit on lung density
Mao *et al*[80]	RCT-pilot study	20	9 mo	-910HU	ATRA	No benefit
Roth *et al*[81]	RCT feasibility study	148	9 mo	-910HU	Patients received ATRA either LD, HD, 13-cRA or placebo	No definitive clinical benefits
Dirksen *et al*[82]	RCT	32	3 yr	Perc15	Alpha1-antitrysin	CT analysis showed a non-significant trend towards a favourable effect. CT lung density twice as sensitive as PFTs
Dirksen *et al*[72] (EXACTLE)	RCT	77	2-2.5 yr	Perc15	Prolastin	CT densitometry more sensitive measure for the detection of emphysema progression than PFTs or health status indices
Chapman *et al*[73]	RCT	180	2 yr	Perc15	Alpha 1 proteinase inhibitor	Annual rate of density decline at TLC ↓in treatment group (P = 0.03)

CT: Computed tomography; WA: Wall area; LA: Luminal area; BWT: Bronchial wall thickening; SFC: Salmeterol/fluticasone; LD: Low dose; HD: High dose; 13-cRA: 13-cis retinoic acid; ATRA: All trans retinoic acid; RCT: Randomised controlled trial; TLC: Total lung capacity.

and any regional differences. MRI therefore could offer an attractive solution to evaluating underlying pathology and targeting treatment.

Phenotyping with MRI

Airways disease: MRI is already used to visualise airway changes in more detail in cystic fibrosis, *e.g.*, inflammation, mucus plugging and bronchiectasis[83]. In this capacity, MRI is superior over CT with its ability to more accurately differentiate soft tissue, *e.g.*, remodelling/inflammation[84,85]. The increased airway resistance seen in small airways disease in asthma has also been evaluated by MRI. Where bronchoconstriction has resolved clinically MRI assessment of ventilation demonstrated focal, fixed obstructive defects that may be reversible with targeted therapies, *e.g.*, broncho-thermoplasty[86]. The ability of MRI to accurately measure the resultant degree of hyperinflation and air trapping has obvious potential clinical applications in COPD, *e.g.*, endobronchial coils/LVRS.

Emphysema: The apparent diffusion co-efficient (ADC) measured in MRI is a reflection of the amount of measured molecular movement, with more movement in emphysema where there are larger air sacs and destroyed alveolar walls[87]. Therefore a high ADC indicates more severe emphysema and could be used either diagnostically or for assessment longitudinally. As there is increased interest in using CT density as a direct measure of parenchymal response to augmentation therapy in AATD, ADC would be another potential option of measuring alveolar changes.

Vascular remodelling secondary to hypoxic vasoconstriction is likely part of a more systemic process associated with COPD. Perfusion studies, *i.e.*, dynamic contrast enhanced MRI may therefore act as another useful imaging biomarker to detect and prevent further disease[88]. For example, where there is a perfusion defect with preserved ventilation, then this maybe a target for bronchial dilators. Similarly where there is preserved perfusion, up to 20% have emphysematous regions which therefore may act as a map for targeted interventional therapies, *e.g.*, Bronchoscopic Lung Volume Reduction Surgery (BVRS)[89]. Jobst *et al*[90] showed the association between oxygen enhanced MRI and contrast enhanced MRI r value is 0.52 therefore there is a link but there are other factors in play such that one is not a surrogate for the other. A summary of how MRI can help phenotype COPD is given in Table 5.

Clinical validation: MRI findings from the various modalities have been correlated with lung function and CT density in numerous studies (Table 6), R values for FEV1 ranging from 0.61-0.72 and 0.45-0.9 for DLCO.

Pathological validation: One of the pathological hallmarks of emphysema is the destruction of alveolar walls and dilatation of respiratory bronchioles[103,104].

Table 5 Magnetic resonance imaging modalities to phenotype and treat chronic obstructive pulmonary disease

Phenotype	MRI modality	Findings	Suggested treatments
Airways disease	Hyperpolarised MRI	Detailed anatomical information of airway inflammation, oedema and mucus plugging[84,85] Regional information re. lung volumes, *e.g.*, focal bronchoconstriction	Nebulised antibiotics Chest clearance techniques[83] Broncho-thermoplasty[91] BVRS
Emphysema	Hyperpolarised MRI	Global high ADC[87] Low PaO_2[92]	Early disease detection Future alpha one augmentation therapy[1]
	Oxygen enhanced MRI	↑↓Relative enhancement signal[93,94]	Targets for resection Early emphysema detection
	Dynamic contrast MRI	Global microvascular reduction blood flow[95] Focal defects, small pulmonary emboli Increased pulmonary pressure	Lifestyle moderation Anticoagulation Treat as pulmonary hypertension

Potential treatments based on the phenotypes identified by the technique, but that have not yet been tested are noted by [1]in the table. MRI: Magnetic resonance imaging; BVRS: Bronchoscopic volume reduction surgery; ADC: Apparent diffusion co-efficient; KCO: Transfer co-efficient.

Table 6 Studies correlating magnetic resonance imaging with other clinical variables

MRI modality	FEV1	Gas transfer	CT density (LAA% 950HU)
Hyperpolarised gas	-0.632-0.76[38,86,92,96,97]	-0.45-0.82[38,92,98,99]	0.8-0.9[96,100]
O_2 enhanced	-0.74[93]	DLCO: 0.911[94] KCO: 0.66[93]	
DCE-MRI	[1]0.677[101]		
UTE-MRI[2]		0.6[102]	0.72[102]

[1]Dynamic contrast measured by the signal intensity perfusion defect (SIpd); [2]Ultra-short echo time-MRI. CT: Computed tomography; DLCO: Transfer factor for carbon monoxide; FEV1: Forced expiratory volume in 1 second; UTE-MRI: Ultra-short echo time-MRI; DCE-MRI: Dynamic contrast enhanced magnetic resonance imaging measured by the signal intensity perfusion defect (SIpd).

Histologically this may be measured by the surface area to volume ratio (SA/V) and this was compared with MRI findings in five patients who had undergone bilateral lung transplant for end-stage COPD. Using He-MRI and measuring the ADC, the correlation between histology and MRI findings was very strong (r = 0.96)[105]. Morino *et al*[106] in an animal model measured the correlation between dynamic contrast MRI and alveolar enlargement as defined by the mean linear intercept (Lm) and this demonstrated a slightly weaker correlation though still significant (r = -0.77, P < 0.001).

Quantification of emphysema using MRI

Oxygen enhanced MRI: Proton MRI measures the longitudinal and transverse relaxation times (T1 and T2 respectively) after the strong magnetic force has been applied[85]. Oxygen molecules shorten the T1 relaxation time, and mapping the degree of change can depict the heterogeneity of ventilation within the lungs[107]. The mean wash in time maps of oxygen created significantly correlates to FEV1 and FEV/FVC ratio (-0.74 for both) demonstrating its strong relationship to current measures of ventilation[93]. The degree of altered signal change as depicted by the mean relative enhancement signal has a stronger correlation with gas transfer (r = 0.83)[94] and therefore as well as acting as a map of ventilation, oxygen enhanced MRI may also reflect alveolar-capillary gas transfer 4214[93]. O_2 MRI has also been demonstrated to be able to separate emphysematous patients from asymptomatic smokers[92].

Benefits of offering oxygen enhanced MRI particularly over other inhaled gases acting as a contrast is that it may technically be implemented at most centres without the need for specialist equipment but would require specialist software[85]. There is no breath holding manoeuvres required which is preferable in COPD patients, the signal artefacts are relatively low as is the overall cost. However, the scanning time is considerably longer (30 min *vs* 5 min) and the repeatability has not yet been confirmed[108].

Hyperpolarised MRI

ADC: Using spin technology to hyperpolarise inhaled gases through polarised laser light, the signal enhancement is amplified and then measured[107]. The larger the range of movement of the gas particles, the higher the ADC. Therefore in emphysematous alveoli where there is destruction of attachments, there will be more movement, and a higher ADC[87]. For this reason ADC can give information about alveolar anatomy unlike HRCT. ADC correlates with lung function, and is sensitive at detecting differences between emphysematous and non-emphysematous patients[109].

Helium MRI of alveolar partial pressure

PaO_2: Based on the rate of polarised helium decay in relation to regional oxygen concentration, and the diffusion across alveolar membranes, the alveolar partial pressure of PaO_2 can be calculated[87,110]. This can detect changes in asymptomatic current smokers, as well as correlating with lung function, SGRQ and 6MWD[92].

Helium ventilation MRI: Following a breath hold, the thoracic volume can be calculated together with He ventilated images in order to calculate the percentage ventilated volume and ventilation defect volume%

Table 7 Summary of studies comparing magnetic resonance imaging and computed tomography in chronic obstructive pulmonary disease

Ref.	Year	Pt No.	Variables	Results
Ley *et al*[96]	2004	13	ADC and EI *vs* FEV1	ADC *vs* FEV1, $R = 0.7$ EI *vs* FEV1, $R = 0.5$ MLD *vs* FEV1, $R = 0.4$
Ohno *et al*[93]	2008	71	O_2 enhanced MRI (mean wash in time and relative enhancement ratio), CT defined lung volumes *vs* lung function	Mean wash in time *vs* FEV1, $r = -0.74$ Relative Enhancement Ratio *vs* KCO, $r = 0.66$ CT lung volume *vs* FEV1, $r = 0.61$ CT lung volume *vs* KCO, $r = 0.56$
Van Beek *et al*[98]	2009	94	ADC and MLD *vs* FEV1/FVC and DLCO	ADC *vs* Fev1/fvc, $r = 0.5$ MLD *vs* Fev1/fvc, $r = 0.52$ ADC *vs* DLCO, $r = 0.59$ MLD *vs* DLCO, $r = 0.29$
Diaz *et al*[38]	2009	27	ADC and EI *vs* FEV1 and DLCO	ADC *vs* FEV1, $r = 0.67$ EI *vs* FEV1, $r = 0.55$ ADC *vs* DLCO, $r = -0.82$ Perc15 *vs* DLCO, $r = 0.6$
Quirk *et al*[114]	2011	30	Hyperpolarised He *vs* CT density in at risk smokers	Lung morphometry *vs* %LAA 950: Significant difference seen in those still smoke, not on CT
Xia *et al*[101]	2014	55	+ve rate of Perfusion defects *vs* CT changes	Early COPD: MRI detected 8/8, *vs* CT 3/8 $P = 0.003$ Mod. COPD: MRI detected 9/9, *vs* CT 7/9 $P = 0.47$
Hueper *et al*[95]	2015	144	DCE-MRI *vs* CT density	PMBF *vs* %LAA 950: Evidence of non-linearity, $P = 0.015$

ADC: Apparent diffusion co-efficient; EI: Emphysema index; FEV1: Forced expiratory volume in 1 second; MLD: Mean lung density; MRI: Magnetic resonance imaging; DLCO: Transfer factor for carbon monoxide; KCO: Transfer co-efficient; COPD: Chronic obstructive pulmonary disease; DCE-MRI: Dynamic contrast enhanced-magnetic resonance imaging.

(VDV%)[85,111]. This was able to discriminate between healthy smokers and those with COPD in a 2015 trial, but there was no significant correlation with spirometry[111].

The main drawbacks of hyperpolarised helium MRI are that hyperpolarised helium is in limited supply and expensive. The technique requires specialist centres with appropriately trained radiologists[85], and patients are required to breath hold for around 20 s, which is very challenging for patients with COPD. However, hyperpolarised MRI has no radiation dose and gives high spatial resolution. It provides detailed regional information about gas exchange and ventilation, and its repeatability has been established[108].

Perfusion: Detecting early changes in the vascularity of patients at risk of developing emphysema could potentially act as another early biomarker of disease. Dynamic Contrast Enhanced MRI involves injecting contrast and measuring the amount of time taken for the contrast to pass through the pulmonary circulation, *i.e.*, the longer the time taken, the more flow restriction there must be. Transit time of blood through the pulmonary circulation is notoriously rapid, though MRI with ultra-fast capabilities is able to capture this[112,113]. Not only is this technique feasible it also correlates to clinical parameters. Hueper *et al*[95] demonstrated this is possible on a microvascular scale, and demonstrated evidence of disease in patients with COPD in areas of lung not emphysematous on CT.

Trials: Multiple studies have demonstrated that MRI correlates more strongly with PFTs than CT does (Table 7). However at this early stage it still remains unclear if MRI is more sensitive, as the literature is not as advanced.

Nuclear imaging

Nuclear imaging techniques provide useful information regarding ventilation and perfusion which can be used for assessing emphysematous lungs and regional contributions. There is no significant scope for information regarding soft tissue and fine anatomical measurements, and therefore whilst can measure the severity of emphysema to a certain degree, it is not able to phenotype in the same way as CT/MRI.

Positron emission tomography

Positron emission tomography (PET) measures gamma rays emitted from molecules labelled with radioisotopes, and an image of where the molecules concentrated is created. Most commonly PET is used in oncology to look for the extent and spread of malignant disease by using labelled glucose, and determining metabolically active sites. There has been increased recognition of the role of increased neutrophil activity in COPD. 18-FDG has been used as a surrogate marker of neutrophilic inflammation in order to ascertain if it could be a useful biomarker[115]. The authors found uptake was significantly higher in the upper zones in those with COPD compared with healthy controls ($P = 0.009$) and correlated with lung function. They additionally tried to

Table 8 Practical considerations for positron emission tomography *vs* single photon emission computed tomography

Modality	Advantages	Disadvantages
PET	Increased resolution	Cyclotron and radiopharmaceutical preparation Rapid repeat testing not possible[87]
SPECT	Lower cost More widely available. Dynamic SPECT give time course of ventilation	Lower spatial and contrast resolution

PET: Positron emission tomography; SPECT: Single photon emission computed tomography.

Table 9 Studies correlating single photon emission computed tomography with other clinical variables

Modality	*R* value	Ref.
DCE-MRI	0.50-0.67	Molinari *et al*[127]
FEV1	-0.64	Bajc *et al*[121] Jögi *et al*[122]
FEV/FVC	-0.63, 0.67	Bajc *et al*[121] Jögi *et al*[122]
He-MRI	0.45	Stavngaard *et al*[128]
DLCO	0.57	Sandek *et al*[123]

DCE-MRI: Dynamic contrast enhanced magnetic resonance imaging; FEV1: Forced expiratory volume in 1 second; FEV1/FVC: Forced expiratory volume in 1 second/forced expiratory volume; He-MRI: Helium-magnetic resonance imaging; DLCO: Transfer co-efficient of carbon monoxide.

use PET-CT as an outcome measure for augmentation therapy in patients with AATD but found no significant difference in readings before and after treatment.

Vidal Melo *et al*[116] labelled and injected nitrogen (13-NN-labelled saline) in 15 patients with COPD. Nitrogen has very low solubility in blood and therefore in the lungs diffuses rapidly in to alveolar space[117]. PET scanning with this method exploits these features of nitrogen so that areas where there is high concentration of nitrogen in the lung initially must be well perfused. Furthermore, once the patient breathes, nitrogen is washed out and therefore areas with retained nitrogen are less well ventilated.

Single photon emission CT

Using this method, the labelled radioisotope emits one rather than two gamma rays during the decay process, and for this reason has less radiation but subsequently less resolution. Labelled agents are inhaled (*e.g.*, xenon) and injected (*e.g.*, technetium DTPA) and the contributions of ventilation/perfusion ascertained. The merits of both tests are summarised in Table 8. The clinical application of single photon emission CT in COPD are largely sub-divided into pre-operative assessment for those considered for lung volume reduction surgery (including bullectomy), and for the early detection of emphysema.

SURGICAL ASSESSMENT

Assessing V/Q mismatch can give functional information about regions of inadequate ventilation not visible on CT, and is cheaper and more convenient than MRI. Suga *et al*[118] demonstrated its usefulness particularly in the pre-operative assessment for bullectomy, and the valuable information gained regarding function of lung tissue within and surrounding the bullous before it is resected. A retrospective analysis was performed on patients who had undergone endobronchial valve placement (EBVs) and perfusion as measured by perfusion scintigraphy. They found that those with lower baseline local perfusion benefitted from EBV placement independent of the lobe, summarising that assessing a patients perfusion pre-operatively may be a method of calculating predicted benefit[119]. Finally, Sudoh *et al*[120] compared PET/CT to PPO segment counting in predicting post-operative outcomes but found no superiority.

EARLY DISEASE

The pathobiological theory that COPD is a systemic disorder with ongoing inflammation and microvascular changes is exploited in assessment of V/Q mismatch. Changes in perfusion may well precede visible changes on CT and certainly lung function, and has therefore potential to diagnose and initiate treatment earlier if required[121,122].

Validation

A summary of correlations between SPECT and various other clinical measures is shown in Table 9. There is moderate-strong correlation with FEV1 but less so with gas transfer and MRI (0.45-0.67)[123]. With regards to sensitivity and specificity for emphysema diagnosis, MRI would seem superior to perfusion scintigraphy[124]. There is a very small amount of work regarding pathological validation and nuclear imaging, but so far these are animal models only[125,126].

OCT

OCT works through a bronchoscope and using near infra-red rays instead of soundwaves (used in ultra-sound), can give extremely precise image of the airway. Using two light beams with one shone onto a mirror to act as a standard measure, the other beam is directed into the tissue and the pattern and the amount that is reflected back is interpreted as an image[129]. It can visualise around 2-3 mm and gives almost a histological

Table 10 Demonstration of how optical coherence tomography could phenotype in chronic obstructive pulmonary disease

Condition	OCT method	Findings	Suggested treatments
Chronic bronchitis	Endoscopic	Increased volume of submucosal glands; central airway inflammation[133-135]	Investigations directed towards asthma overlap syndrome; targeted inhaled steroids
Emphysema	Anatomical OCT	Can visualise collapsibility dynamically[136]	Bronchodilators; smoking cessation

OCT: Optical coherence tomography.

view of the airway wall[130]. Unlike ultrasound which requires a water medium and direct contact to operate, the OCT probe doesn't need to be pressed against the airway wall. Better than CT or MRI, OCT can give a clear view of the airway wall components, *i.e.*, the submucosa, the smooth muscle, and cartilage[131]. In asthma and COPD where there is ongoing inflammation and subsequent airway remodelling, OCT would serve a purpose to view the causes of airway wall thickening and intra-luminal narrowing. The technology is already used in ophthalmology and cardiology, but in respiratory despite having promising capacity, it is still in its research phase.

PHENOTYPING

OCT can only image as far as the device carrying it (usually a bronchoscope) can go. Therefore this technology is limited to the airways and not the parenchyma. However, through creating a pleural window, and miniaturised devices within a 30 gauge needle, the probe can be inserted through the chest wall[132]. The potential for phenotyping patients in COPD could be assessing the amount of active inflammation, airway remodelling/fibrosis to assess why there are regional problems with sputum production or bronchiectasis. Those in favour of OCT have optimistic views that assessing airway pathology would make way for targeted therapeutic interventions (Table 10). OCT is in its infancy however, and more trials are needed.

CLINICAL VALIDATION

There have been two studies that have compared OCT to FEV1, both from the same group in 2008 and then 2014[137]. They find the correlation in these two studies between FEV1 and OCT to be strong (-0.75 and -0.78 respectively) though the 2014 study only found a significant correlation in the male subjects. The slope of the line plotted between OCT and FEV *vs* CT and FEV1 was steeper, and therefore the authors concluded OCT's potential superiority over CT for assessing small airways disease.

PATHOLOGICAL VALIDATION

Tsuboi took 7 human lungs immediately resected for lung cancer, and placed the OCT camera down. They showed that the images of the airway and of the alveolus taken from OCT matched though seen on histology, *i.e.*, definition between submucosa, smooth muscle and cartilage, and then the structure of the alveoli and its adjacent bronchial wall. In a small number of subjects, no statistical analyses were performed but the results are visually convincing[131,138].

CONCLUSION

Quantitative imaging techniques provide sensitive, repeatable and accurate information in COPD patients, and are likely to be used increasingly for both diagnosis and measuring the response to treatment. There are differences in the application of each modality and common pitfalls to be recognised, and standardising each of them is necessary before they can become a bigger player in clinical practice.

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REVIEW

Advance of antioxidants in asthma treatment

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Abstract

Asthma is an allergic disease, characterized as a recurrent airflow limitation, airway hyperreactivity, and chronic inflammation, involving a variety of cells and cytokines. Reactive oxygen species have been proven to play an important role in asthma. The pathogenesis of oxidative stress in asthma involves an imbalance between oxidant and antioxidant systems that is caused by environment pollutants or endogenous reactive oxygen species from inflammation cells. There is growing evidence that antioxidant treatments that include vitamins and food supplements have been shown to ameliorate this oxidative stress while improving the symptoms and decreasing the severity of asthma. In this review, we summarize recent studies that are related to the mechanisms and biomarkers of oxidative stress, antioxidant treatments in asthma.

Key words: Asthma; Oxidative stress; Reactive oxygen species; Antioxidants

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Core tip: Oxidative stress plays an important role in the pathogenesis of asthma. The imbalance of oxidative and anti-oxidative system is caused by exogenous and endogenous reactive oxygen species. Some elevated substances could be served as oxidative or antioxidative biomarkers. Different kinds of treatments showed antioxidative role, including diet, vitamins and food supplements; natural extracts; magnetic field and laser, *etc*. However, no antioxidants were applied in first-line therapy of asthma now. More works are needed, especially clinical trial, to clarify the clinical value of antioxidant therapy.

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Asthma is a chronic inflammatory lung disease that is induced by cellular mechanisms that result in airway

hyper-reactivity and airflow limitation[1]. Patients with asthma suffer from a variety of symptoms, including dyspnea, recurrent coughing, chest tightness, shortness of breath and sporadic, frequent wheezing[2]. Previous studies have indicated that oxidative stress plays an important role in the development of asthma[3]. Reactive oxygen species (ROS) present in asthmatic airways are derived from many sources, including exposure to environmental peroxidants, infiltration of inflammatory cells in the airway, metabolic disorders, and decreased levels of cellular antioxidants. Airway oxidative stress also has been associated with declining disease status, poor lung function, and epigenetic changes[4].

Antioxidative treatment, such as food supplements[5,6] and vitamins[7], is a potential therapy for asthma, but has not been denoted as a first-line method because current results are not consistent with clinical data. In this review, we have summarized the recent literature related to oxidative stress characteristics and antioxidants treatments in asthma.

OXIDATIVE STRESS RESPONSE IN ASTHMA

Sources of ROS and asthma

Exposure to exogenous ROS and asthma: Airway epithelial cells are in close contact with the external environment in humans. When asthmatic patients are exposed to exogenous ROS-such as environmental tobacco smoke[8], airborne pollution[9], home dust mites[10] or sulfar mustard[11]-in the air, which may trigger symptoms of asthma.

Outdoor air pollution is definitely associated with the incidence of asthma[12]. As primary air pollutants, exposure to O_3 or NO_2 can cause inflammation and repair, as indicated by secretion of chemokines and cytokines[13,14]. O_3 exposure may increase the *NK-1R* gene expression and then induce subsequent acute oxidant stress[15]. Inhalation of Cl_2 results in oxidative lung injury by ROS and low-molecular-weight hyaluronan, which then activates the RhoA and Ca^{2+} channels of airway smooth muscle cells, resulting in airway hyper-responsiveness (AHR)[16]. Particulate matter (PM), a major component of air pollution, includes diesel soot, welding fumes, carbon black, coal or oil fly ash. Diesel exhaust inhalation may increase airway responsiveness[17], decrease total cysteine levels, increase cystine and s-glutathionylated cysteine in bronchoalveolar lavage fluid (BALF)[18], increase nitrite and decrease pH in exhaled breath condensate (EBC)[19,20]. Further studies have demonstrated that an assay of oxidative potential was more closely associated with lung function than PM2.5 mass by measuring dithiothreitol levels[21]. Cigarette smoking is also a high risk factor for asthma. Passive smoking could impair histone deacytylase-2 function *via* PI3K signaling activation, which reduces histone deacetylase-2 protein expression[8]. Cigarette exposure can induce the expression of glutathione peroxidase-1-protein tyrosine phosphatase-1B-protein phosphatase-2A, which may induce the destruction of lung tissue[22].

Indoor pollution cannot be ignored. Dermatophagoides species produce O_2^- by converting the dehydrogenase form of XOR to the oxidase form[10], which may be associated with increased levels of DNA repair proteins and apoptosis[23]. Hexabromocyclododecane and phthalates are indoor pollutants that may enhance inflammatory cytokines expression[24,25]. Urinary 2-phenanthrene, 1-pyrene and Di- (2-ethylhexyl) phthalate have been found to be associated with asthma diagnoses[26,27]. Observations in cleaning workers have revealed decreases in non-reversible lung function and a total increase in IgE levels[28]. These chemicals and acrolein induce the production of ROS and malodialdehyde while decreasing glutathione (GSH) levels[24,25,27,29].

Endogenous ROS and asthma: Endogenous ROS is associated with enzymes produced by inflammatory or epithelial cells, which is induced by inflammation during the immune response to pathogens or allergenic substances. The main source of intracellular ROS is from mitochondrial respiration, produced primarily by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, as well as the xanthine/xanthine oxidase system. O_2^- is produced by these enzymes in activated cells, such as eosinophils or macrophages, and O_2^- also can generate $ONOO^-$ from either NO or by transfer onto H_2O_2 *via* superoxide dismutase (SOD). H_2O_2 may cause the generation of the more oxidative OH^- by the Fenton reaction when Fe^{2+} is present[30]. Macrophages produce ROS *via* other enzymes, such as heme peroxidase, myeloperoxidase (MPO) or eosinophilic peroxidase (EPO). Hypochlorous acid and hypobromous acid can be generated by these enzyme-mediated chain reaction in the presence of Cl^- or Br^-, which are more oxidative and toxic[31].

The formation of hypohalite and hypobromite results in increasing NO levels, which is produced by epithelial inducible nitric oxide synthase (iNOS). Reactive nitrogen species (RNS) then may quickly be formed in the presence of ROS[32]. High levels of protein nitration, such as bromotyrosine adducts, have been observed in inflammatory airways and are associated with low control of asthma[33].

The role of oxidative stress and defense mechanisms in asthma

Increases in ROS levels are strongly related to the severity of asthma in patients[34]. There are higher amounts of ROS and RNS in asthmatic patients, which leads to airway inflammation[35]. ROS/RNS activate nuclear factor-κB (NF-κB), mitogen-activated protein kinase (MAPK), activator protein-1, and other transcription factors, which result in lung inflammation[35-39]. These redox-sensitive transcription factors promote the expression of many proinflammatory cytokines-

such as interleukin (IL)-6, IL-8, and tumor necrosis factor alpha (TNF-α) - which then induce activation of inflammatory cells in the airways[40,41], further leading to lung tissue damage and destruction[22]. CysLTrl(-/-) mice had abnormal antioxidant response and increased susceptibility to oxidative damage[42]. Deficiency in mannose-binding lectin could notably diminish pulmonary inflammation after exposure to O_3[43].

Nuclear factor erythroid 2-related factor 2 (Nrf2) is an NF-E2-related factor of exogenous toxins and oxidative stresses that plays an important role in oxidative stress defense mechanisms[44]. As a vital factor in the antioxidant pathway, Nrf2 activates antioxidant enzymes that catalyze ROS into non-toxic substances and that are water soluble, which is conducive to balancing the oxidative and antioxidative system in the body[45]. Nrf2-deficient mice had elevated levels of oxidative stress, inflammation, mucus, and AHR, which resulted in a higher incidence of asthma[40].

Biomarkers of oxidative stress in asthma: As mentioned previously, O_2^- and NO are mainly produced by NADPH oxidase and iNOS, respectively, while O_2^- is dismutated to form H_2O_2. These oxidative species are all found at high levels in the EBC of asthmatic patients[46]. Some parameters that are indirect measurements of ROS-including carbonys, nitrotyrosine, isoprostanes, and 8-hydroxydeoxyguanosine-can provide important information regarding the overall oxidant load. Oxidative stress lipid peroxides are end products formed during ROS-mediated attacks on cell membranes, and these include pentane, ethane, isoprostanes, MDA, and thiobarbituric acid reactive substances (TBARS). 8-isoprostanes are novel biomarkers that can be used to evaluate oxidative stress, and are significantly elevated in the sputum, EBC, plasma, and lung tissues of asthma objects[47-50]. However, concentration of 8-isoprostanes in EBC did not increase after bronchial provocation or were not altered between asthmatic and healthy patients[51,52]. Asthmatic patients had increased levels of MDA in their plasma, EBC, and sputum[53-58]. Higher blood levels of TBARS were found in asthmatic children[59]. DNA can be attacked by ROS, and 8-oxo-deoxyguanosine is a biomarker for DNA damage. NADPH oxidase-4 is overexpressed in asthmatic patients and the level of 8-oxo-deoxyguanosine was more highly elevated in patients with neutrophilic asthma than those with non-neutrophilic asthma[60]. Amino acid oxidation of the protein backbone occurred by ROS *via* a MPO/EPO catalyzed reaction with halide ions or nitrite on tyrosine, causing nitrotyrosine, bromotyroxine, chlorotyrosine, and carbonyl modifications. For example, 3-nitrotyrosine was found to have a negative correlation with percentage predicted forced expiratory volume in one second (FEV1 % pred)[54], and levels of 3-nitrotyrosine in maternal blood and cord blood were notably elevated in allergic asthma[61]. Total oxidant status (TOS) was used as a direct parameters to evaluate whole body oxidative status, and this was significantly higher in asthma patients[62].

Oxidative stress and airway inflammation in asthma: Oxidative stress has been implicated in the pathogenesis of asthma. Endogenous H_2O_2 may increase the activity of matrix metalloproteinase-9, an important inflammation biomarker. In asthmatic patients, matrix metalloproteinase-9 activity and 8-isoprostane levels were significantly increased under acute exacerbation and were decreased in remission, but were still higher than in healthy controls relative to the plasma levels of total matrix metalloproteinase-9[48]. TNF-α levels were increased in the plasma and lung tissues of both ovalbumin (OVA)-sensitized guinea pigs and obese mice[63,64]. TNF-α may induce mitochondria to generate endogenous ROS[65]. Th2-cytokine response (like IL-4 or IL-5) was observed to be higher in asthma exacerbations, whereas O_3 could notably induce IL-6, IL-8 expression[13,66]. After H_2O_2 inhalation, Th17-related pro-inflammatory markers were upregulated in both liver and vasculature, and this result suggested that ROS inhalation may cause systemic inflammation[67].

ROS are related to airway inflammation in asthma. Cell signals are activated by DNA repair-mediated oxidation, which results in gene expression from epithelial and submucosal tissues, leading to smooth muscle contractions of the airway[68]. Lim *et al*[69] discovered that pulmonary eosinophilia, AHR, mucus hypersecretion and iNOS were significantly elevated in OVA-induced asthma mice. This phenomenon could be suppressed using SRS27, an NF-κB inhibitor. H_2O_2 may reduce epithelial resistance, induce epithelial damage and decrease epithelial responsiveness and suppress the anti-inflammation role of corticosteroids[70]. Changes in the ultramicrostructure and reduction of mitochondrial respiratory membrane protein complex protein in airway epithelial cells are associated with the recruitment of inflammatory cells caused by an oxidizing environment. Allergens may exacerbate eosinophil infiltration in airway epithelial cells, cause mitochondrial dysfunction and affect the balance between Th1 and Th2 cell immune response[71]. Aquarporin-3(-/-) mice were reduced in airway inflammation after decreasing chemokine (C-C motif) ligand (CCL)24 and CCL22 levels *via* reduced levels of cellular H_2O_2[72].

Biomarkers of antioxidation in asthma: With respect to TOS, total antioxidant status (TAS) or total antioxidant capacity (TAC) are used to assess overall non-enzymatic antioxidant potential. Some studies have demonstrated that TAS or TAC was notably higher in asthma than in healthy controls[55,62], although several studies have reported conflicting results. Fatani *et al*[53] observed that TAC levels were significantly decreased in emergency asthmatic patients in contrast to outpatient. In asthmatic children, TAC was found to be lower in recurrent wheezing children than healthy children, and the numbers of wheezing episodes in the last 6 mo were negatively correlated with serum TAC,

hair Zn, and Se levels[73]. Yoon *et al*[74] observed that serum TAC levels were positively correlated to forced expiratory volume in 1 second (FEV1) at baseline. After adjusting for related factors, the results were not significantly different after a sufficient observation duration.

Enzymatic antioxidants-such as SODs, catalase, and GPxs-may reduce ROS and hydroperoxides to less harmful and water-soluble products. SOD activities were decreased in asthmatic patients, while CuZnSOD activity was also found to be significantly lower in asthma patients when compared to healthy controls[55,75,76]. Serum level and activity of GPx was remarkably lower in asthmatic individuals[77,78]. Paraoxonase 1 (PON1) is an esterase enzyme that displays antioxidant characteristics. The PON1 activity in the asthmatic patients was significantly lower compared to healthy controls. Interestingly, PON1 presented an area under roc curve of 0.679 for the identification of uncontrolled asthma[62,79].

Non-enzymatic antioxidants include glutathione proteins, sulfhydryls, and vitamin C. There were remarkably lower levels of total thiols, protein sulfhydryls, ascorbic acid and NO in asthma patients[53,55,75,77]. As a novel inflammation-associated biomarker, clusterin is a sensitive cellular biosensor of oxidative stress. Hong *et al*[80] discovered that CCL20 secretion was negatively associated with clusterin expression in EBC, while clusterin also reduced intracellular ROS levels. Expression of clusterin in the sputum of asthmatic children was higher than in healthy children, and clusterin was more elevated in eosinophil-dominant sputum than in non-eosinophilic sputum. Furthermore, clusterin levels were associated with asthma severity, but these levels were lower when asthma was exacerbated[81,82].

Genetic association and oxidative stress in asthma

Gene polymorphisms can be involved in the oxidative stress response. Glutathione S-transferase (GST) is a key enzyme that acts in the initial step of binding in glutathione-catalyzed reactions, which occurs primarily in the cytosol. *GST* genes control π-class GST activity. Further work has revealed that the genotype of Ile105Val and the allele frequency of Val105 in GSTP1 were higher than in healthy controls, and these features are linked to the severity of airway dysfunction and airway hyperreactivity[83,84]. The risk of asthma diagnosis is increased when GSTP1 with an AA genotype is accompanied by supplementation with low intake of vitamin A[85]. Mice that were null for GSTT1 had an associated increased amount of recurrent wheezing and risk of asthma[86,87]. Lower threshold concentrations of allergen could produce bronchoconstriction in GSTM1 wild-type asthma but GSTM1 wild-type asthma was not associated with risk of asthma[87,88]. *GSTA1* (C/T) and *GSTO2* genes were found to be related to allergies and risk factors for asthma[89]. The RR genotype of *PON1* gene gave a higher risk of asthma, whereas the TT genotype of the catalase gene and T allele of resistance-1 gene more frequently appeared in asthma patients[79,90,91].

ANTIOXIDATIVE TREATMENTS IN ASTHMA

Based on the oxidative stress reaction and defense mechanism, the following antioxidant therapies may be effective in asthma.

Diet, vitamins and food supplements

Foods and nutrients could be utilized to protect airways and lung tissue from oxidative damage through a variety of mechanisms. Vitamin E, a fat-soluble vitamin, is a major defense against ROS, which is the primary source of oxidant-induced membrane damage in the human body. Vitamin C, a water soluble vitamin, is responsible for maintaining the antioxidant capacity in the aqueous phase, while also contributing to the membrane-bound oxidative regeneration of vitamin E. Similarly, vitamin A and vitamin A carotenoids-such as α-carotene, β-carotene, β-cryptoxanthin, lutein/zeaxanthin, and lycopene-have antioxidant properties. Selenium is incorporated into the antioxidant enzyme GPx, which reduces the organic peroxide H_2O_2, thereby preventing peroxidation of cell membrane lipids and subsequent instability. Zinc is present in all cells and is an essential trace element in thousands of catalytic proteins and transcription factors, and this metal can be used as an antioxidant. All of these vitamins and nutrients can be found in fruits, vegetables, seeds, seafood, seed oils, nuts and beef[92,93]. Asthmatic adults with low-antioxidant diets have lower FEV1 scores, lower percentage predicted forced vital capacity, higher plasma C-reactive protein and more frequent exacerbation than those on a high-antioxidant diet[94], whereas supplementation notably improved both symptoms and lung function in exercise-induced asthma[95]. Vitamin A, vitamin E, and Se were found significantly lower in asthma than in controls, and vitamin E was negatively impacted by FeNO and MDA. FeNO level was significantly decreased during a study involving nutraceutical supplements[5,54]. The vitamin E isoform γ-Tocotrienol increased the level of Nrf2 by blocking NF-κB, and inhibited oxidative damage by promoting endogenous antioxidant production in the lung[96]. More important, γ-Tocotrienol improved acetylcholine- or methacholine-induced AHR, while also reducing lipopolysaccharides (LPS)-induced neutrophil infiltration[96-98]. In contrast, food supplements did not upregulate glutathione or oxidized glutathione and were irrelevant to the incidence of asthma[99,100].

Vitamin D is from dietary intake or synthesized in the skin during exposure to UVB. Severe asthma patients that were deficient in vitamin D had lower FEV1 values compared to patients with sufficient vitamin D during exacerbation. The absence of vitamin D3 could enhance ROS and DNA damage *via* TNF-α release and NF-κB expression. Vitamin D3 supplementation was able to reverse this phenomenon[7]. Treatment with

vitamin D3 reduced OVA-induced airway inflammation, immunoglobulin E overexpression, expression of α-smooth muscle actin, collagen deposition, and goblet cell hyperplasia, but does enhance activation of the Nrf2/HO-1 pathway[101].

Cockroach extract-immunized mice significantly increased AHR. Combined supplementation with choline chloride, vitamin C, and selenium could potentially reduce AHR, inflammation and oxidative stress by inducing IL-10 expression *via* FOXP3(+) signaling[102]. A comparison between organic selenium (Pro-Se) and inorganic selenium has implicated that Pro-Se might significantly elevate serum SE levels and restore endogenous antioxidant enzyme levels. Furthermore, Pro-Se does not accumulate in liver and kidney and, thus, has lower toxicity[103]. Resolvin-D1 inhibited H_2O_2 and IL-8 both in mRNA and protein level in 16 human bronchial epithelial cells stimulated by cigarette smoke extract through degradation and NF-κB activation[104].

Thiol antioxidants

In antioxidant therapy, thiol antioxidants are popular supplements to cause glutathione conversion. N-acetyl cysteine (NAC) is the most commonly used thiol precursor. Challenge with OVA increases airway inflammation and vascular inflammation, while treatment with NAC significantly inhibits ROS and lipid peroxides[105]. NAC supplementation reduces baseline airway responsiveness when irritated by diesel exhaust inhalation and also reduces use of bronchodilators[17]. In mice BALF, NAC application remarkably decreases inflammatory cytokines (IL-13, IL-5), neutrophil and eosinophil numbers[106].

Natural extracts

Natural extracts contain antioxidant compounds derived from plants, such as benzoic acid, cinnamic acid, coumarin, gallnut tannic acid, and flavonoids. Sakuranetin is a flavonoid and treatment with sakuranetin can attenuate AHR, while decreasing 8-isoprostane, Th2 pro-inflammatory cytokines, IgE, and vascular endothelial growth factor levels, as well as remodeling airways by inhibiting NF-κB activation[107,108]. Astragalin, another flavonoid, suppresses eosinaphilia infiltration stimulated by LPS and H_2O_2 *via* the Toll-like receptor 4-PKCβ-NADPH signaling pathway[109].

Resveratrol is a polyphenolic compound that is mainly found in peanuts, grapes (red wine), *Polygonum cuspidatum*, mulberry, and other plants and is a strong natural biological polyphenol. OVA-challenged obese mice had more eosinophil infiltration in lung tissue than in lean mice, and resveratrol decreases p47phox expression and ROS production, increases SOD levels and reverses elevated TNF-α and iNOS in the lung tissues[63]. Resveratrol treatment in allergic mice decreases oxidative stress and significantly restores mitochondrial function. In asthma, resveratrol probably downregulates the phosphoinositide 3-kinase-protein kinase B pathway by upregulating inositol polyphosphate 4 phosphatase[110].

Morin, an active ingredient obtained from Moracease plants, which attenuates the extensive trafficking of inflammatory cells into BALF in OVA-challenged mice, inhibiting the inflammation infiltration into lung tissue. Morin abolished intracellular ROS and MAPK[111]. Ethyl acetate fraction from Sonchus asper extract, Boerhavia procumbens in toluene diisocyanate and Esculentoside A inhibited oxidative stress pathways, reducing anti-inflammatory response and improving lung injury[112]. Ethyl acetate fraction and Esculentoside A treatment significantly upregulated Nrf-2 expression, increased SOD activity and intracellular glutathione levels[113,114]. Oral treatment with Capsicum annuum L. methanolic extract remarkably decreased the pathophysiological signs of allergic airway disease, reducing ROS levels of BALF in mice and inhibiting Th-2 cytokines *via* attenuated NF-κB activation[115].

In addition to maintaining the balance between oxidative and antioxidative systems in lung tissues and airways, these substances also suppress mucous gland hypertrophy, goblet cell hyperplasia, collagen deposition and airway remodeling, including the extracts of Sinomenine, Morin, Tinospora cordifolia and Gleditsia sinesis[111,116-118].

LPS is commonly found in the environment, causing and potentially exacerbating airway inflammation, which leads to an increase in IgE levels, Th2-cytokines response, histamine release, and EPO and MPO activation. Intranasal curcumin could significantly improve asthma exacerbation induced by LPS[66]. Carissa opaca fruit extracts can restore the activities of antioxidant enzymes and GSH, while the amount of TBARS and DNA fragmentation also decreased[119]. Such phenomenon was partly observed when using tomato juice treatment[120].

Antioxidant synthetics

Y-27632, a Rho-kinase inhibitor, is able to control airway inflammation, airway responsiveness, remodeling and oxidative stress. Y-27632 treatment in guinea pigs induced by allergens provoked decreased FeNO levels, while inflammation, extracellular matrix remodeling, and oxidative stress in the lung were also attenuated[121,122].

Nitric oxide synthases (NOS), H_2S and arginases are thought to be involved in lung allergy disease. Treatment with 1400W (an iNOS-specific inhibitor), nor-HOHA (an arginase inhibitor) or NaHS (a H_2S donor sodium hydrosulfide) reduces the expression of arginase 2, 8-isoprostane and NF-κB in distal lung tissue. These inhibitors also decreased eosinophil infiltration in lung tissues, subsequently improving tissue resistance and elastance[49,123].

Some compounds could act on ROS signaling pathways directly and indirectly to cause antioxidant effects. HYDAMTIQ is a new poly (ADP-ribose) polymerase inhibitor that prevents airway damage in asthma. Treat-

ment with HYDAMTIQ reduces MDA, 8-hydroxy-2′-deoxyguanosine, the amount of eosinophils and other leucocytes in lung tissue, while also reducing smooth muscle and globlet cell hyperplasia, whereas the mast cells of HYDAMTIQ-treated animals have reduced histamine release *in vitro* when exposed to OVA[124]. Phosphorylation of histone 3 at serine 10 is related to oxidant-associated inflammation. P38α MAPK and IκB kinase 2 signaling pathway may be affected by ROS, as the combined usage of p38α MAPK and IκB kinase 2 inhibitors, reduced histone 3 at serine 10, inflammatory gene expression in monocytes and lung macrophages from asthmatic patients[36]. Angiotensin-I converting enzyme 2 (ACE2) is an enzyme that protects against asthma. An ACE2 activator, diminazene aceturate, prevents asthmatic lung that is induced by cytokine expression and elevated levels of ACE2 and IκB. Diminazene aceturate could also decrease carbachol (as an oxidative parameter), attenuate oxidative stress, reverse airway remodeling and right ventricular hypertrophy[125]. Diallyl sulfide decreases infiltrated inflammatory cell counts and Th2 proinflammatory cytokines in BALF in OVA-induced mice *via* Nrf2 activation by regulating microRNA-144, -34a, and -34b/c[126].

Mice treated with S-adenosylmethionine, a potent methyl donor, had decreased amounts of Th-2 pro-inflammatory cytokines and 4-hydroxy-2-nonenal in lung tissues, while airway inflammation and fibrosis is suppressed by in mice[127]. Pituitary adenylate cyclase-activating polypeptide reverses vanadate-induced AHR, principally through bronchodilator activity and counteraction of proinflammatory and prooxidative effects[128].

Metals and new materials

Tiron treated mice could significantly attenuate OVA-induced oxidative stress, by reducing pulmonary MDA and increasing GSH and SOD levels. Tiron could also minimize immunoreactivity of NF-κB in these mice, and down regulate levels of NOx, IL-13 and TGF-β1[129].

Nanoparticles are proved to be antioxidative objects. Gold nanoparticles treated mice, the levels of pro-inflammatory cytokines and ROS were inhibited, mucus production, peribronchiolar fibrosis and AHR induced by allergens were also attenuated[130]. Vitamin D(VD)-loaded nanoemulsions treatment could effectively decrease MPO activity, oxidative stress, C3 protein level and other proinflammatory cytokines than common forms of VD[131]. A new series of fully biodegradable Hydroxybenzyl alcohol-incorporated polyoxalate (HPOX) was noticed to its inhibition role to airway inflammation. HPOX nanoparticles reduced intracellular oxidative stress generation and suppression proinflammatory mediators by clearing hydrogen peroxide[132]. The microparticles of vanillyl alcohol-containing copolyoxalate could reduce oxidative stress, suppress the levels of pro-inflammatory cytokines (like TNF-α) and iNOS in the lung tissue of OVA challenged asthmatic mice[133].

Non-drug treatment

Living organisms exposed to a static magnetic field (SMF) may have affects on ROS levels. The ragweed pollen extract may induce allergic inflammation in mice after SMF-exposure; the TAC in mouse airways increased and allergic inflammation decreased; this reaction was time-dependent. Furthermore, SMF could stimulate cellular ROS-eliminating mechanisms[134]. Low-level laser therapy (LLLT) has been proven to be an anti-inflammatory therapy and after treatment with LLLT exposure the activity of histone deacetylase of U937 cells could be depressed by activating protein kinase A *via* inhibition of PI3K, which is not reversed by H_2O_2[135]. This result suggests that LLLT could be a potential antioxidative therapy.

Antioxidant effects of current drugs in asthma

Corticosteroids are widely used to treat asthma *via* anti-inflammatory effects and are recommended by the GINA guidelines[136], and this current asthma therapy has also been found to be effective in preventing oxidative stress. After treatment with inhaled corticosteroids, asthma scores were significantly improved, and Cys-LT and 8-isoprostane concentration in EBC were notably decreased in asthmatic children[137]. Inhaled corticosteroid treatment causes significantly lower expression of CYBB mRNA in the NADPH oxidase system[59]. Montelukast is a leukotriene receptor antagonist, and plasma total thiol was lower in asthmatic patients that were not given montelukast therapy in comparison to montelukast therapy patients and healthy controls[138], although other studies have demonstrated that montelukast therapy resulted in no significant improvement in TOS, TAS and DNA damage parameters[139]. Currently, the mechanism of montelukast antioxidative stress remains unclear. Treatment with procaterol, a long-acting β2 agonist, enhances human bronchial epithelial cell viability, while decreasing the percentage of apoptotic cells and reducing MDA and ROS in a dose-dependent manner[140].

Ambroxol is used to increase mucociliary clearance and regulate surfactant levels. Clinical studies have reported that ambroxol decreases the levels of protein carbonyls (an oxidative biomarker), increases the level of Th1 cytokines - such as IL-10, IFN-γ, and IL-12-from lung mononuclear cells and alveolar macrophages, but had no effect on Th2 cell cytokines[141]. 5-Aminosalicylic acid significantly inhibits the expression of Th2 cytokines, while also decreasing MDA and MPO levels in BALF of mice[142].

Sitagliptin and Cinnarizine also reduce proinflammatory cytokine release and inflammatory infiltration, while also restoring GSH and SOD, thus playing a role in reducing airway inflammation and remodeling *via* antioxidative stress[143,144].

CONCLUSION

Asthma is one of atopic diseases which including

allergic rhinitis and atopic dermatitis. Low respiratory tract is affected in asthma while other atopic diseases involve other lesions. Airway oxidative stress is a complex condition with important physiological and pathophysiological implications in asthma. Imbalance of the oxidative and antioxidative systems is caused by exogenous and endogenous ROS. Some elevated substances can serve as oxidative or antioxidative biomarkers. Different kinds of treatments have demonstrated antioxidative roles, including diet, vitamin and food supplements, natural extracts, magnetic fields and lasers treatments. However, some of these methods have been unsuccessful due to unforeseen side effects, thus no antioxidants have been applied as first-line therapy in asthma treatment. The choice of antioxidants must be made in regard to individual and environmental factors. More research is required, especially large and well-designed clinical trials, to clarify the clinical value of antioxidant therapy.

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CASE REPORT

Synchronous lung and breast cancer

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Abstract

Synchronous tumors are an uncommon finding. We present a case of metastatic carcinoma of right breast and a left lung adenocarcinoma in a patient with previous history of left breast cancer diagnosed twelve years ago. She was then treated with chemotherapy, radiotherapy and hormone therapy. Initially, the greatest diagnostic challenge was which of them had spread or if both had. Or even if, any of these lesions resulted from the primary left breast cancer. So, specimens of different metastatic lesions were crucial to answer this query and to decide the best therapeutic approach. Sequencing the treatment options in managing two synchronous secondary malignancies, where one of them is metastatic and the other one is potentially curable, was a demanding clinical decision.

Key words: Breast cancer; Non-small cell lung cancer; Synchronous tumors

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Core tip: This case presents a patient with previous left breast cancer in the past that now has a diagnosis of right breast cancer. The staging exams revealed metastatic lesions on bone and left adrenal gland and a suspicious lesion on the left lung. Histology of those lesions allowed us to conclude there were a metastatic breast cancer and a localized lung cancer.

de Macedo JE. Synchronous lung and breast cancer. *World J Respirol* 2017; 7(1): 29-34 Available from: URL: http://www.wjgnet.com/2218-6255/full/v7/i1/29.htm DOI: http://dx.doi.org/10.5320/wjr.v7.i1.29

INTRODUCTION

Synchronous tumors are defined as distinct tumors

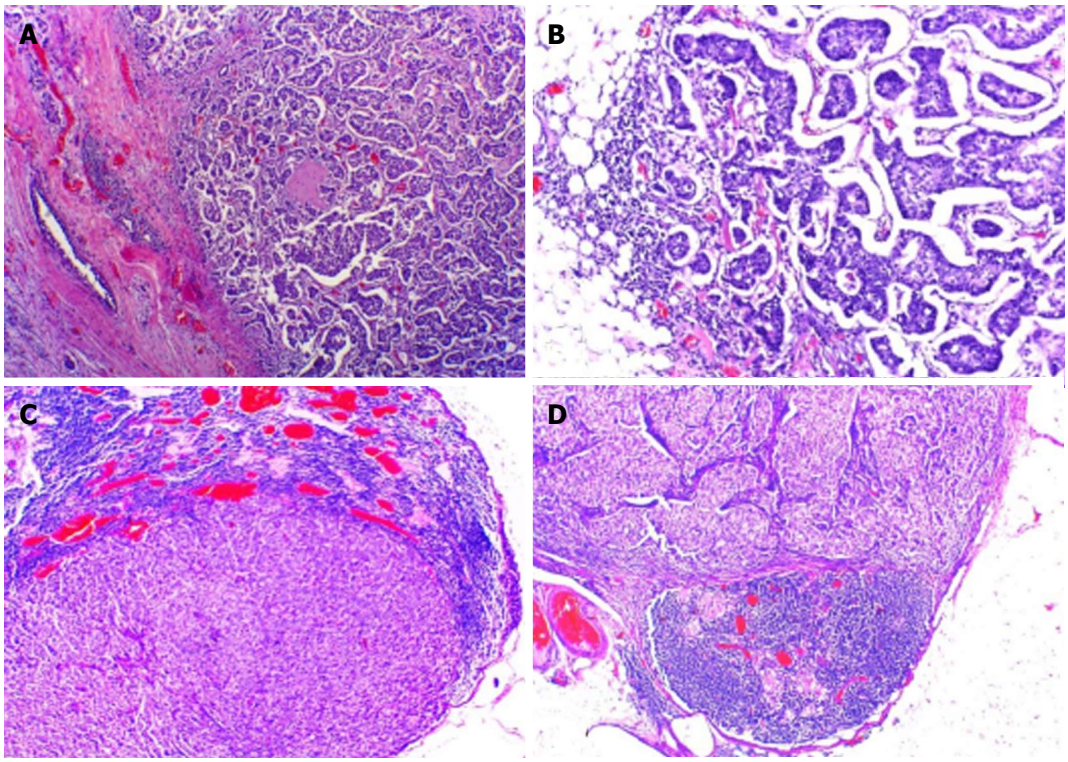

Figure 1 Invasive mammary carcinoma non-special type (A and B) (hematoxylin and eosin × 40-× 110), axillary lymph nodes metastases from mammary carcinoma (C and D) (hematoxylin and eosin × 40).

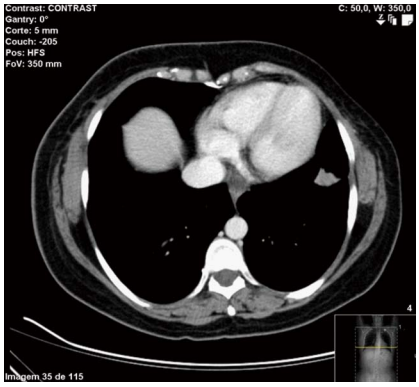

Figure 2 Spiculated nodular lesion with 18 mm localized in the left lower pulmonary lobe.

arising simultaneously or diagnosed in a range of less than 6 mo apart[1]. The incidence of multiple primary malignancies is 0.73%-11.7%[2,3] and it is believed that the incidence has been increasing due to increased life expectancy and progressive advances in diagnostic techniques. It remains in debate the contribution of genetic, immunological and environmental mechanisms[4]. In this clinical case, there is an increased risk of second malignancies related to previous breast cancer history and respective treatments performed.

CASE REPORT

A 43-year-old non-smoker women, asymptomatic, with a previous history of cancer on the left breast diagnosed and treated in 2003. The patient was treated with left modified radical mastectomy, adjuvant chemotherapy with 5-fluorouracil 780 mg, epirubicin 117 mg and cyclophosphamide 780 mg, 21-21 d for six cycles. Adjuvant radiotherapy was performed (50Gy left chest wall, supraclavicular region at 50 Gy and 45 Gy in internal mammary lymph node chain). Hormone therapy with tamoxifen and LHRH analog was delivered for 5 years. After seven years of follow-up, she was referred to her personal physician.

Asymptomatic until June 2015, when she was referenced to our Institution for evaluation of two lumps in her right breast (31 mm nodule in inferior lateral quadrant and another in the transition of the external quadrants with 11 mm) detected in her annual mammography. A palpable right axillary lymph node, painless and with a hard consistence was diagnosed on physical examination.

A fine needle aspiration biopsy was performed of the larger breast nodule and the palpable lymphadenopathy revealed an invasive ductal carcinoma of breast, RE 100% PR < 10%, negative HER2, Ki67 60%-75%. The patient underwent a right modified radical mastectomy and axillary lymph node dissection in July of 2015. Histology confirmed a breast carcinoma and showed an extensive metastasis of ipsilateral axillary lymph nodes (21 positive lymph nodes in 25), pT2N2aMx (Figure 1). Thoracic and abdominal computed tomography (CT) scan revealed a 17 mm suspicious lung nodule

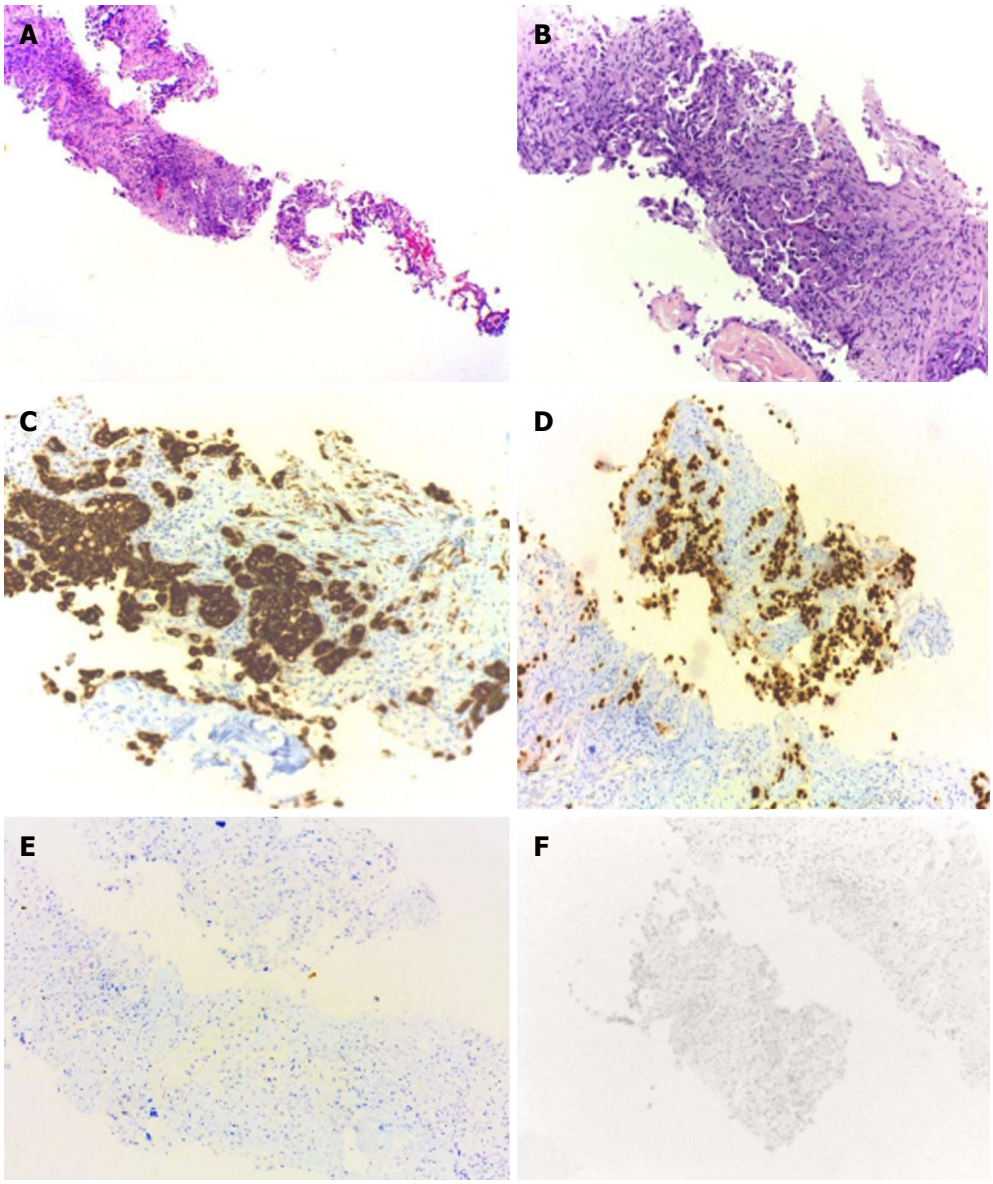

Figure 3 Transtoracic pulmonary biopsy. Invasive adenocarcinoma (A and B) (HE × 40-× 100), Keratin 7 (C) and TTF1 (D) were positives. GATA 3 (E) and mammaglobin (F) were negatives. ER were negative (not shown). HE: Hematoxylin and eosin; ER: Estrogen receptor.

in the left lower lobe and two lytic lesions in dorso-lumbar column (Figure 2). The whole spine magnetic resonance imaging confirmed infiltrative lesions of secondary nature in D8, D11, L3 and S1. The biopsy of the lung lesion showed features of moderately differentiated adenocarcinoma, positive for CK7 and TTF1 and negative for estrogen receptor, pT1aN0Mx (Figure 3). The positron emission tomography-CT scan showed uptake in the right infra-clavicle and axillary lymph nodes, lump in the basal segment of the lower lobe of the left lung, left adrenal gland and D8, D11, L3 vertebral bodies and left iliac wing.

In the presence of synchronous secondary malignancies of the breast and lung with potential for bone and adrenal metastases, it was necessary to clarify the origin of these lesions to guide the best therapeutic approach. So, the patient underwent bone and adrenal gland biopsies. Both histology's confirmed metastasis of breast cancer (Figure 4). Palliative chemotherapy was delivered with docetaxel 120 mg and cyclophosphamide 960 mg, every three weeks, for a total of four cycles; zoledronic acid 4 mg *iv*, every four weeks and calcium and vitamin D supplementation. This particular regimen of chemotherapy now chosen was justified by the cumulative dose of anthracyclines already performed in the past.

Not neglecting a 43-year-old fit woman, imaging re-evaluation after chemotherapy will be performed, to consider radical treatment of lung adenocarcinoma and initiate hormone therapy.

DISCUSSION

Despite of breast and lung cancers being the most

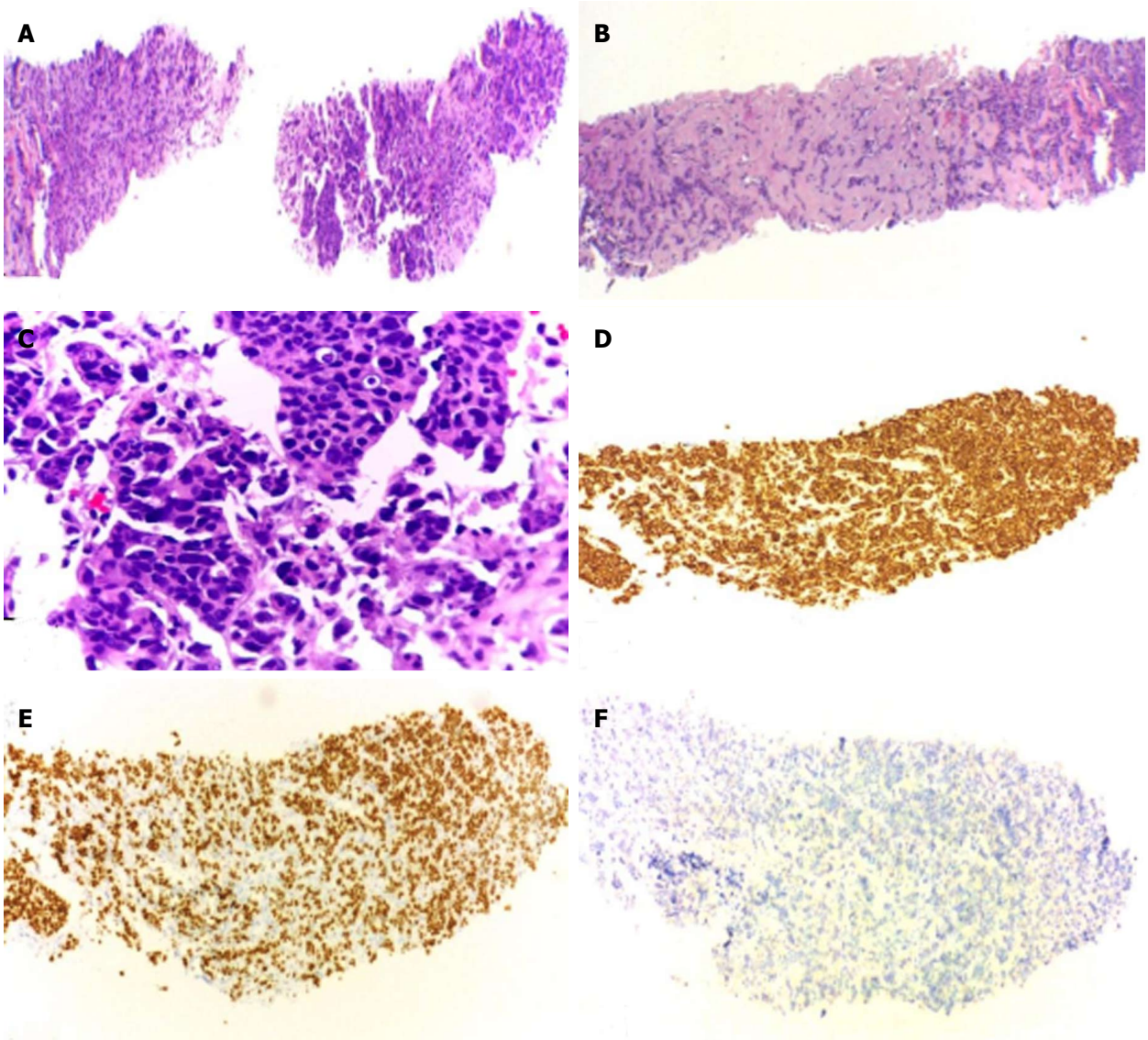

Figure 4 Left adrenal biopsy. Solid-pattern carcinoma (A-C) (HE 40 ×-400 ×), Keratin 7 (D), GATA 3 (E) were positives. TTF1 was negative (F). ER positive and negative mammaglobin not shown. HE: Hematoxylin and eosin; ER: Estrogen receptor.

common tumors in females, the diagnosis of synchronous cancer is nevertheless an uncommon finding. Multiple primary malignancies in a single patient were first described in 1870 by Billroth[6]. The increase incidence of multiple primary cancers may be due to long life expectancy, progressive advances in diagnostic techniques, regular follow-up and genetic predisposition to cancer[5]. It remains in debate the contribution of genetic, immunological and environmental mechanisms[4]. In this particular case, previous exposure to chemotherapy and endocrine therapy may have protected the patient form tumor relapse and metachronous breast tumors. On the other hand, a slightly increased risk of lung cancer after radiotherapy for breast cancer, on the same side, has been previously reported. The current criteria for the diagnosis of multiple primary malignancies, which were established by Warren *et al*[6], are as follows: First, each of the lesions must be malignant; secondly, each of the lesions must exhibit distinctively different pathologies and thirdly, metastases from the prior malignancies must be excluded. Synchronous carcinomas are defined as those tumors diagnosed either simultaneously or within an interval of six months, after the diagnosis of the first one[1].

A pulmonary left nodule in women with history of left breast cancer could be a metastatic disease, primary lung cancer or a benign lesion. In 55% of the cases, it represents a primary lung cancer and in 37% a metastatic breast cancer[7,8]. The major diagnostic challenge in this case report, is whether which of the cancers (breast carcinoma or adenocarcinoma of the lung) had spread, if both or only one. Secondly, where to: Bone, adrenal gland or both. Nowadays, it is mandatory to perform biopsies of the different meta-

stases and immunohistochemistry staining. Specimen of different metastatic sites is crucial in order to discriminate the oncogenic pathway of proliferation, and define the primary origin of those secondary lesions.

Nowadays, intraoperative frozen sections make false-positive diagnosis that mistakes benign for malignant, but there are 1.1% to 4.0% of false-negative diagnoses[9]. However, in this case report multiple frozen biopsies could have been performed, if possible, at same surgical timing in different locations (bone, left adrenal gland and left lung nodule biopsy). Nevertheless, nowadays liquid biopsies and circulating free DNA are gaining evidence with statistical sensitivity and sensibility in a non-invasive and less expensive procedure, with a more accurate molecular characterization[10].

The treatment of such cases depends on the individual primary location, tumor's aggressiveness, staging, genetic profile, co-morbidities, performance status and toxicities of previous treatments performed. All of these details should be discussed in a multidisciplinary team, with the patient's medical oncologist and most importantly, with the patient in order to preserve his quality of life.

This case illustrates the challenge in the diagnosis and characterization of metastatic lesions, but also, the difficulty in managing two synchronous secondary malignancies, where one of them is metastatic and the other one is potentially curable.

COMMENTS

Case characteristics

A 43-year-old non-smoker women, asymptomatic, with a previous history of cancer on the left breast, presented with two lumps in her right breast in an annual mammography, and a painless, with a hard consistence, palpable right axillary lymph node on her physical examination.

Clinical diagnosis

Two metachronous cancers: A right breast cancers and a left lung nodule.

Differential diagnosis

Diagnostic challenge in this case report, is whether which of the cancers (right breast carcinoma or adenocarcinoma of the left lung) had spread, if both or only one, and secondly, where to: Bone, adrenal gland or both. A third option is if any of the metastases could even be result from dissemination of the left breast tumor twelve years ago.

Laboratory diagnosis

Perform biopsies of the different metastases and immunohistochemistry staining is mandatory.

Imaging diagnosis

Thoracic and abdominal computed tomography (CT) scan, spine magnetic resonance imaging and positron emission tomography-fluorodeoxyglucose CT scan is essential to evaluate the extent of the disease and its characterization.

Pathological diagnosis

Specimen of different metastatic sites is crucial in order to discriminate the primary origin of those secondary lesions.

Treatment

The treatment of such cases depends on the individual primary location, tumor's aggressiveness, staging, genetic profile, co-morbidities, performance status and toxicities of previous treatments performed, namely accumulated dose from the anthracyclines performed earlier.

Related reports

A pulmonary left nodule in women with history of left breast cancer could be a metastatic disease, primary lung cancer or a benign lesion. In 55% of the cases, it represents a primary lung cancer and in 37% a metastatic breast cancer.

Term explanation

Further molecular profiling for selecting the specific target therapy, is of upmost importance for defining the molecular signature of these tumors.

Experiences and lessons

Tumor characterization by clinical, imagological and histological means is still vital, but molecular profiling will allow us to treat the patients more efficiently, with better quality of life and lower toxicity profile.

Peer-review

This case report based on the reality of practice will draw attention from wide-ranged repirology specialist. It is a very attractive topic.

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FRONTIER

Is the determination of ctDNA a scientific "spy" that foresees cancer?

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Abstract

Since 1948, circulating tumour DNA (ctDNA) was first identified in human blood. ctDNA is in fact DNA shed by tumour cells from all metastatic tumour locations throughout the whole body, and is thrown into the bloodstream and can then be isolated by a standard blood draw. Using this technique scientists can obtain a wide view of tumour heterogeneity, identify different mechanisms of drug resistance, what is its predominance and the clinical rational of precision cancer medicine become a part of our daily practice. Secondly, early detection of cancer may also contribute to global decrease in cancer mortality.

Key words: Tumour biopsies; Liquid biopsies; Circulating tumour DNA; Precision cancer medicine

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Core tip: With the increase development of molecular medicine we may further change our clinical rational to a precise cancer medicine rational way. Consequently, we may improve the quality of life of our patients, with less toxicity, more cost-effectiveness decisions and above all improve response rate and survival. Defining the complete genomic "picture" of all cancerous lesions, in the near future as a standard of care, will require all genetic information concerning each individual cancer.

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INTRODUCTION

Cancer mortality has decreased globally in the United

States and Europe, as well as the individual risk of dying from cancer, due to recent reliable data[1]. Although analysis confirms that decrease in cancer mortality is across all income levels, a difference has also been reported in low and middle income countries in which this decline still needs to be clarified. Preventive strategies in cancer control, specifically addressing risk factors, have been one of the measures that lead to these results. Nevertheless, early detection and intervention in high risk groups, with a non-invasive, accurate, sensitive and specific method such as analysis of circulating tumour DNA (ctDNA), is still the most effective measure to reduce cancer mortality[2].

Since 1948, ctDNA was first identified in human blood[3]. At that time scientist were innocent of the real meaning and consequences of this procedure. The discovery of tumour DNA in blood samples, also called "liquid biopsy", may have bypassed the need for traditional invasive measures. The first challenge was how to discriminate ctDNA from normal cell free DNA. The sensitivity of polymerase chain reaction (PCR) based on digital techniques improved along time with the addition of next generation sequencing (NGS). ctDNA have a median half-life round about two hours, and changes in ctDNA levels can be detected for weeks, before changes in imaging or in protein biomarkers. The blood stream is in fact the reservoir of ctDNA of all sites of metastases[4,5].

ctDNA is in fact DNA shed by tumour cells from all metastatic tumour locations throughout the whole body, and is thrown into the bloodstream and can then be isolated by a standard blood draw. Using this technique scientist can obtain a wide view of tumour heterogeneity and identify different mechanisms of drug resistance and what is it's predominance in a global view. This open view may be missed by a single lesion tumour biopsy, and only a small perspective of the whole disease may be captured. In other words, additional resistance alterations may not be found[5].

DISCUSSION

What evidence do we have that justify the benefit of identifying early ctDNA?

The answer is just because the test exists doesn't mean we all have to do it. There is evidence that cancers that may rapidly lead to resistance such as lung, melanoma and colorectal cancer, should be mandatory to be monitored by genetic analysis of ctDNA[6]. Secondly, is there a prognostic and predictive factor in early stage surgical lung cancers patients? As Dr. Karachaliou mentioned at ASCO 2016, in 25% of lung cancer cases, the tissue from small biopsies is insufficient to execute the genotyping sequencing in order to offer a personalizing cancer therapy[7].

The idea that liquid biopsy matches tissue biopsy has been around for several years now. In ASCO 2016, a study funded by Guardian Health Inc., obtained liquid biopsies form 15000 cancer patients (lung cancer 37%, breast cancer 14%, colorectal cancer 10% and other cancers 38%). From 386 of these patients, tumour biopsies were also available. When compared tissue samples with ctDNA, by sequencing method, the results reported showed, an overall accuracy of 87% (336/386 patients)[6,7].

There has also been established a correlation between quantification of ctDNA with stage and tumour burden in colon, breast and lung cancer. Some investigation has also proven a statistical significance between detection also of ctDNA tumour relapse and resistance to target therapies[8].

Considering surgical stages, the presence of ctDNA in localized disease at the time of sample collection among different types of cancer, levels of ctDNA were detectable in 55%[8]. This percentage was lower than in metastatic stages.

In patients submitted to surgical resection of their localized tumour, but before chemotherapy, identification of ctDNA may indicate residual disease. Its absence may identify subgroup of patients at low risk of recurrence, who could be spared of adjuvant treatment and all its consequences: Risk, cost and discomfort. Nevertheless, regular measurements of ctDNA could monitor total systemic tumour burden, as it should decrease after complete surgical resection. As a monitoring tool, it should increase before new radiological lesions become apparent. It has also been reported that micrometastatic lesions, smaller than a few millimetres may also be detected by increase in ctDNA (86% to 100%) and not yet detected by imaging[8].

ctDNA levels can also predict early relapse and early identification of resistance to treatment, namely detect sensitive and resistant EGFR mutations in lung cancer, such as T790M. Concerning EGFR mutation several studies have reported a wide range of concordance rates between ctDNA and tissue samples[9-12]. T790M genetic aberrations in EGFR have also been referenced as acquired resistance to target therapies[13,14]. For example, as stated by Naygaard *et al*[15] in plasma using the ARMS-qPCR technique KRAS mutations have also been reported in NSCLC. Concerning ALK mutations such as, C1156Y and L1196M have also been identified as acquired resistance to target therapy with crizotinib[16]. Understanding the mechanisms of acquired resistance to target agents at molecular levels can allow science to use selective targeted treatments focusing in a modern precision medical care[17].

How should then ctDNA sequences be checked for early detection, evaluate relapse and how often? First it requires detection of a specific mutation or mutations in the tumour tissue to then look for same ones in the ctDNA after surgery and during follow-up. Concerning detection of residual disease after curative surgery, 6 to 8 wk ctDNA should be measured. They were measured in this study during two to five years after surgery. ctDNA can in fact detect upfront specific genetic alterations months before clinical biomarkers or imaging studies[18].

Table 1 Pros and Cons: Liquid *vs* tumour biopsy[20]

Liquid biopsy	Tumour biopsy
Non-invasive	Invasive
Better compliance	Difficult to tolerate
Several withdrawals	Serial biopsies
Easily performed	Difficult to biopsy
Independent	Dependent
Less expensive	Expensive
Early detection of cancer	
Clonal heterogeneity	Minor sub-clone
Evaluate response to treatment	
Evaluate residual disease	
Evaluate relapse	Non-prognostic
Evaluate therapy resistance	Non-predictive

What are the pros and cons in performing liquid biopsies?

The advantages in performing liquid biopsies are many. First of all, it's a harmless, non-invasive and easily executed technique. Blood samples are easily obtained and patients tolerate well several blood draws and when explained, there is a greater compliance. Secondly, we become less dependent on the original tumour site, since either primary tumour or metastatic lesions, both release DNA into the bloodstream. ctDNA is representative for all sites of metastases. Concerning cost-effectiveness it's a cheap method with a good sensitivity and specificity as demonstrated in several studies[19]. As scientists, we may bypass clinical signs and symptoms and various diagnostic invasive and more expensive diagnostic methods by introducing liquid biopsies in our daily clinical practice. Early detection by sequential analysis, in absence of detectable primary tumours or metastases, response or failure to therapy, detection of residual disease after primary surgery and development of resistance during therapy are the fundamental advantages of detection of ctDNA.

The main disadvantages of tumour biopsies, besides being the gold standard for diagnosis, are the facts that it's an invasive procedure, requires an experience technician to target the specific tumour site. On the other hand, being cancer a heterogeneous disease, the biopsy may be limited to a section of the tumour biopsied and analysed. Different sites of the disease have distinct mutational profiles. Some lung locations are difficult to target, even if well localised *via* imaging exams. If the tumour was resected it can no longer be biopsied, only in metastatic locations (stage Ⅳ). Serial lung or mediastinal biopsies are not very friendly to be performed. Obviously, we have a problem with surgically treated patients, non-metastatic if further genetically analysis or disease monitoring wants to be performed, unless liquid biopsies can be done as a monitoring tool (Table 1)[20,21].

FUTURE PERSPECTIVE OF LIQUID BIOPSIES

The possibility of identifying neoplastic ctDNA is a silent inoffensive tool, that acts as a cancer spy and can trigger appropriate reactions even before the disease (the enemy) or the doctors (the fighters), could have the minimal idea of its manifestation, besides all experience and scientific knowledge of time to progression or acquired resistance to target therapies[22].

A good spy (ctDNA) is the one which triggers the alarm discretely but with high sensitivity and specificity, to produce a good defence and an unexpected combat. It should be performed at the appropriate time (fit patient) permitting to use the best armament (target therapies). This is what all clinicians, molecular scientists and patients should wish for: An excellent precision cancer medicine in a silent mode (minimal side effects), in order to preserve and recover benign territory, before further one is damaged or definitely lost.

The turnaround timer is quicker on liquid biopsies than tissue biopsy, which means that the molecular personalized information of this type of cancer is available more rapidly. In other words, liquid biopsies may be useful at early diagnosis, real-time monitoring disease and at estimating risk of relapse (prognostic information) in order to define therapy selection, therapy resistance (predictive information) and secondary therapy selection[23-25].

Is the cost-benefit increment of liquid biopsies as a routine practice, an affordable procedure?

If we think what a normal tissue sample biopsy implies we can enumerate. First of all, the interventional radiologist and all occupational time of the computed tomography (CT) scan or other technique chosen; secondly the cost of the pathologist and all that it implies in time and material to extract DNA, and finally it's analysis. Whilst ctDNA relies on a blood sample withdrawal, happening every day, and the final procedure is the same. Indeed we are removing steps and costs, in favour of a more modern, comfortable and updated technique and also, a more accurate and precise diagnostic method. Based on two articles, both sate that tissue biopsies increase cost for patient care and an uncomfortable invasive procedure[20,21].

Unfortunately, it is not yet a standard of care because only, this May 2017, were the guidelines for NGS suggestions for liquid biopsies validated by the College of American Pathologists, by the Association of Molecular Pathology as well as the European Society of Pathology. For these reasons, although they have been added to the testing palette for NSCLC by NCCN, only now has validation occurred and therefore, it is not yet a standard of care. Nevertheless many clinical studies are attempting to empower the utility and benefit of liquid biopsies as a whole, and not only ctDNA[26].

CONCLUSION

With the increase development of molecular medicine and expanding fields in translational cancer research, we may further change our clinical rational to a new era of precision cancer medicine[20]. Consequently, we

may improve the quality of life of our patients, with less toxicity, more cost-effectiveness decisions and above all, improve response rate and survival. Defining the whole genetic "picture" or genomic landscape of each patient, in the near future as a standard of care, will require all genetic information concerning each individual cancer, in order to offer personalized medicine by customizing healthcare by molecular analyses.

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MINIREVIEWS

Lung microbiome in healthy and diseased individuals

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Abstract

The data on quantitative and qualitative microbial composition of the respiratory tract of healthy individuals revealed significant differences when compared with the microbiota of patients suffering from respiratory diseases. Possible etiological role of microbiota in pulmonary diseases as well as drug resistance development is of profound interest nowadays. Numerous studies have provided evidence confirming the relationship between gut microbiome and those of lungs. This relationship could explain how changes in the microbial communities in one organ may lead to pathological changes in the other. Till date, some progress has been made in the study of the biological properties of probiotic bacteria, considering their modulating effect on inflammatory immune response. The use of probiotics which exhibits an immunomodulatory potential looks promising.

Key words: Microbiome; Respiratory diseases; Probiotics; Prebiotics; Gut-lung axis; Synbiotics

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Core tip: The role of the respiratory tract microbiota in a healthy state and in respiratory diseases is broadly discussed nowadays. There is also a big amount of data regarding contribution of gastrointestinal microbiota changes in respiratory diseases development. A gut-lung axis conception is of great interest. Perspective of prebiotics and probiotics application in lung diseases treatment looks very promising. Huge number of researches has been done on topics mentioned above. Our objective is to consolidate the current literature to summarize the most recent and most important data concerning this subject.

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INTRODUCTION

Until recently, microbial structure of a human body remained poorly understood. Nowadays large-scale research conducted within the framework of the "Human Microbiome Project" (HMP 2007) provide us with novel knowledge on the diversity of human microflora. Previously lower respiratory tract supposed to be sterile, except when infection affects it. This concept existed due to limited experimental access to the respiratory tract of healthy individuals, and limitations of classical methods of culturing. Therefore, the study of lungs was initially not included in the original HMP. However later, molecular-genetic identification methods showed that native microbiome exists in lungs as well. Significant progress in the study of microbial ecosystems was associated with genomic analysis of 16S ribosomal RNA (16S rRNA). Currently more than 16000 sequences of bacterial 16S rRNA gene have been described. Preliminary data showed differences in the composition of respiratory tract microbiome in patients suffering from various respiratory diseases and relatively healthy volunteers. In comparison with the gastrointestinal tract (GIT) maintenance of the natural microbiome composition in the respiratory tract appears to be an important factor for protection against bronchopulmonary diseases.

Pulmonary microbiome

One of the earliest researches was focused on quantitative and qualitative analysis of the upper and lower respiratory tracts microbiome in relatively healthy volunteers, refuting the hypothesis of lung sterility[1-3]. The lower respiratory tract was found to contain bacterial 16S rRNA sequences. Thus, constant presence of unique symbiotic microbiota in a healthy lung was confirmed. The composition of the microorganisms in the lower respiratory tract was generally indistinguishable from those in the upper respiratory tract which explained their origin[2-4]. The respiratory tract was shown to contain 2000 bacterial genomes per sm[2]. Bacteroidetes (*Pseudomonas*, *Streptococcus*, *Prevotella*, *Fusobacteria* and *Veillonella*), particularly *Prevotella* spp. predominate in healthy individuals[5]. Despite the whole genome sequencing techniques do not provide firm conclusion of live microorganisms presence in the lower respiratory tract, there are some indirect evidences supporting the existence of an active, viable lung microbiome. For instance, a considerable variation was shown in both quantitative and qualitative composition of the microorganisms in the different regions of the respiratory tract of the same individual, suggesting growth inhibition of one type and active reproduction of another type of bacteria to be possible mechanism, depending on various local environmental conditions (temperature, pH, oxygen saturation, *etc.*)[6].

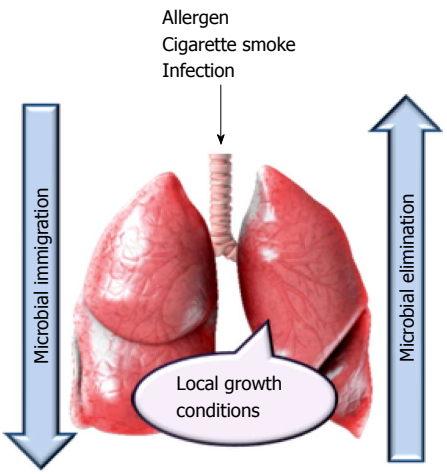

Figure 1 Ecological determinants of the lung microbiome. The respiratory microbiome is determined by three factors: Microbial immigration, microbial elimination (mainly in health individuals) and regional growth conditions (mainly in advanced lung disease) (adapted from Dickson RP 2015).

The composition of lung microbiome is determined by the balance of three factors: Microbial immigration, microbial elimination and local growth conditions (Figure 1)[4]. The respiratory microbiome is determined by three factors: Microbial immigration, microbial elimination (mainly in healthy individuals) and local growth conditions (mainly in advanced lung disease) (adapted from Dickson RP 2015). Therefore, any modifications in microbiome in pathogenic conditions occur due to changes in these factors. Potential sources of microbial immigration are: Air inhalation (contains 10^4-10^6 bacterial cells/mm^3), microaspiration (found in healthy individuals) and direct dispersion through the respiratory tract mucosa[4]. Thus high affinity of lung and oral cavity microbiome compared to air microbiome supports microaspiration and direct dispersion to be major microbial sources[7,8]. Clinical studies confirmed that the microbiome of lungs and oral cavity resemble each other more than the microbiome of the nasal mucosa[4,9]. Microbial elimination is determined by mucociliary clearance, cough and antimicrobial mechanisms - innate and adaptive immunity[4]. pO_2, pH, blood perfusion, alveolar ventilation, temperature, lung epithelium, mucociliary clearance and activity of inflammatory cells are major components of microbial local growth conditions[6]. Surfactant that covers distal alveoli has bacteriostatic activity affecting the reproduction of bacterial communities[10].

Lung pathology leads to both structural and microbial changes. For example, in destructive pulmonary diseases (emphysema, idiopathic pulmonary fibrosis) inner surface area of lungs is significantly reduced (up to 90%)[11]. Gastroesophageal reflux disease (GERD), which is often found in patients with progressive pulmonary diseases, increases microbial immigration and acts as an additional source of bacteria[12]. Chronic pulmonary diseases (cystic fibrosis, bronchiectasis, chronic bronchitis) lead to impairment of the mucociliary

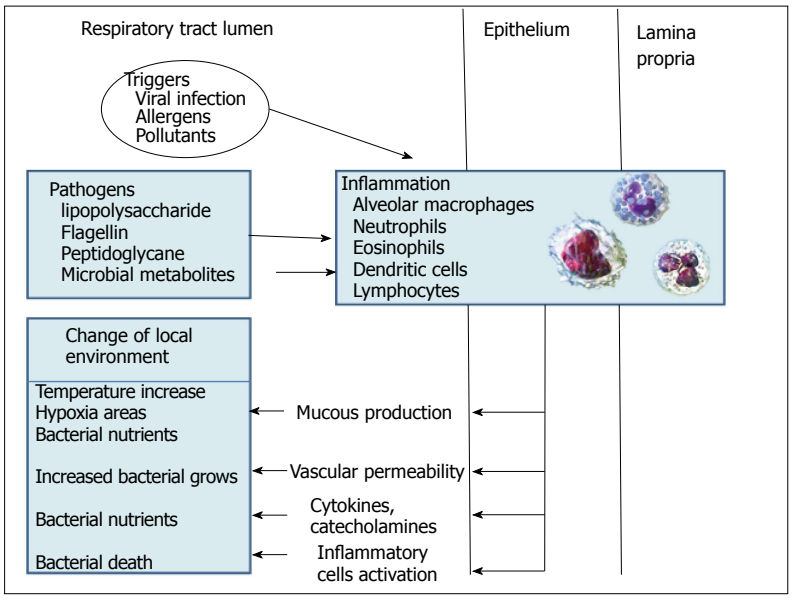

Figure 2 Lung microbiome disturbances following respiratory diseases exacerbations (adapted from Dickson RP 2014). The triggers like virus, allergens, pollutants initiate airway inflammation with activation of alveolar macrophages, neutrophils, eosinophils, dendritic cells, lymphocytes, which alters growth conditions of airway microbiota. Altered growth conditions result in a disturbed microbiome, which promotes further airway inflammation *via* pathogen-associated molecular patterns and pattern recognition receptor interactions.

clearance, which in turn affects the microbial elimination. In addition, such conditions associated with increased mucus production contribute to bacterial growth and promotes formation of zones with low oxygen concentration and high temperature[13]. Exacerbation of chronic diseases results in microbiome alteration due to following mechanisms: Hyperventilation, cough, bronchoconstriction, overproduction of proinflammatory cytokines, catecholamines, glucose and reactive oxygen species, increased vascular permeability and mucus production[13-15]. The model of lung microbiome disturbances following respiratory diseases exacerbations has been proposed (Figure 2)[6].

The triggers like viruses, allergens, pollutants initiate airway inflammation with activation of alveolar macrophages, neutrophils, eosinophils, dendritic cells, lymphocytes, which alters growth conditions of airway microbiota. Altered growth conditions result in a disturbed microbiome, which promotes further airway inflammation *via* pathogen-associated molecular patterns and pattern recognition receptor interactions.

Triggers (viral, bacterial infection, allergens, pollutants) upregulate a cascade of inflammatory reactions involving alveolar macrophages, neutrophils, eosinophils, dendritic cells and lymphocytes significantly affects microbial growth. Thus, for example, excessive production of proinflammatory cytokines [tumor necrosis factor alpha (TNF-α), interleukin (IL)-1, 6, 8] directly activates growth of *P. aeruginosa*, *S. aureus, S. pneumonia*, *Burkholderia cepacia* and others[14,16,17]. Remarkably some microorganisms modify virulent factors making them more aggressive and hence increase immunogenicity. These factors promote further inflammation by increasing expression of pathogen-associated molecular patterns (lipopolysaccharide, flagellin), which in turn activate pathogens recognizing receptors [*e.g.*, toll-like receptors (TLR)][18].

Gut-lung axis

GIT appears to be the most bacterial populated organ of our body [up to 10^{14} colony-forming units (CFU)/mL in colon][5]. Microbiota plays an important role in health maintenance. In GIT it promotes formation of local and systemic immunity, induces intestinal angiogenesis and is supposed to be an important factor for normal digestion. In healthy individuals Bacteroidetes represent the most abundant phylum, followed by Firmicutes. The lower respiratory tract has a much lower level of contamination, but Firmicutes and Bacteroidetes are predominants in the lung microbiome of healthy individuals as well as in the gut while Actinobacteria, Proteobacteria and Fusobacteria are presented in rather small numbers[19].

Gut-lung axis is of particular interest. While the gut and lungs are both mucosa-lined luminal organs with a shared embryological origin, their gross and micro-anatomical features are different. In the absence of emesis or gastroesophageal reflux, migration of microbes in GIT is unidirectional (from the mouth to the anus), and is serially interrupted by chemical and physical barriers. In contrast, the movement of air, microbes and mucus in the lung, is bidirectional, with no physical barriers between the larynx and the most distal alveoli. Consequently, the lung microbiome is more dynamic than the lower GIT[4]. The differences in the composition of lung and intestine microbiome are also associated with oxygen distribution and temperature, which represents a gradient from ambient temperature at the point of inhalation to core body temperature in the alveoli[4]. Trachea and bronchi like intestine are lined with glycosylated proteins of secreted mucus; the vast majority of the lung's surface area is lined with lipid-rich surfactant, which has bacteriostatic effects against selected bacterial species[18]. Intestinal microbiome greatly contributes to the regulation of the immune response, in particular directly in the lung (Figure 3)[20].

Commensal bacteria with their metabolic products interact with TLR inducing Tregs and dendritic cells activation; chemokines and cytokines production and expression of transcription factors therefore regulate

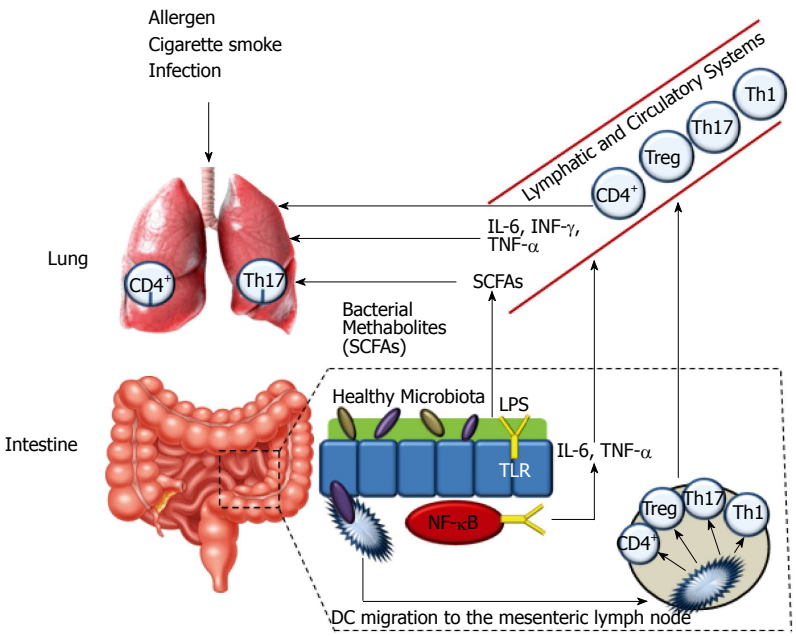

Figure 3 Model of intestinal microbiome effects on lung immunology (adapted from Samuelson DR 2015). Microbes in the intestine is sampled by dendritic cells (DCs) either directly from the lumen or following translocation through M-cells to the gut-associated lymphoid tissue. A combination of signals from the microbes results in phenotypic changes in the DCs. DCs promote activation of various T-cell subsets within the mesenteric lymph nodes (MLN) and production of regulatory cytokines. Following the immune challenge in the airways T-cells activated in the gastrointestinal associated lymphoid tissue (GALT) and MLN move to the respiratory mucosa where they promote protective and anti-inflammatory responses. Production of various bacterial metabolites (*e.g.*, SCFAs) also affects the gut-lung axis, as these products get to the lung, where they can alter the levels of inflammation. SCFA: Short chain fatty acid; IL: Interleukin; TNF: Tumor necrosis factor.

immune response[20-22].

It is a well-known that asthma, chronic cough, COPD, and idiopathic pulmonary fibrosis can be associated with GERD. Acid-suppression medications, including proton pump inhibitors (PPIs), are some of the most prescribed medications in patients with GERD. Rosen *et al*[23] investigated the impact of acid-suppression medication in children ages 1 to 18 years with chronic cough on gastric and lung microbiome. No significant differences in the prevalence of various bacterial genera or the median concentration of total bacteria in the lungs between treated and untreated patients were shown. There were positive correlations between proximal nonacid reflux burden and lung concentrations of Bacillus, Dermabacter, Lactobacillus, Peptostreptococcus, and Capnocytophagia. These results could be evidence of reflux influence on lung microbiome, but further studies are needed.

The effect of the bacterial metabolites, in particular short chain fatty acids (SCFAs) on modulation of the immune response is one of the most discussed topics. SCFAs act directly on the epithelial and immune cells, contributing to powerful anti-inflammatory effects[20-22,24]. SCFAs were shown to modulate the activity of NFκB, reduce TNF-α production and downregulate the PRRs stimulation (pattern recognition receptors)[21,22]. Postulated that the ability of SCFAs to interact with certain G-binding receptors of neutrophils depend on their profile which defined by bacterial composition. Stimulation of Ffar2 (GPR43) receptor was associated with decreased level of eosinophils and reduced bronchoconstriction compared with the Ffar3 (or GPR41) stimulation, which was associated with increased production of pro-inflammatory mediators[25]. SCFAs were also shown to downregulate expression of CD-markers on the surface of tissue specific DCs[26]. Depressed expression of costimulatory molecules CD80, CD86 and CD40 modify the DCs ability to interact with regulatory T-cells (T-regs).

It was found that mice fed with low-fiber diet had decreased levels of SCFAs and higher prevalence of allergic reactions in the respiratory tract[27]. The administration of probiotics was associated with IL-10 secretion by DCs, which promoted T-regs differentiation, causing shift to the Th1 response[26]. Bacterial colonization in sterile mice lead to stimulation of the secretory IgA and $CD4^+$ T-cells, reducing the IgE levels[22].

There is a strong correlation between the bacterial composition of the GIT in infancy and asthma phenotype in childhood[27,28]. Low total microbiome diversity of the colon during the first month of life was shown to be linked with bronchial asthma development at the age of 7 years. Also decrease in Bifidobacteria and an increase in the number of Clostridia in the colon at the early age were associated with the subsequent development of bronchial asthma[28]. In mice models it was found that the use of antibacterial drugs in the first 3 wk of life worsens the course of allergic respiratory inflammations in adulthood[29].

Microbiome and respiratory diseases

Currently, the role of lung microbiome in respiratory pathology is being discussed. Lung microbiome transformation, particularly reduction of probiotic species and potential increase of pathogenic bacteria appears to be the fundamental factor for susceptibility, chronization and progression of respiratory diseases. In recent study Bacteroidetes, mostly *Prevotella* spp., was significantly more common in healthy individuals, whereas Pseudomonas was frequently found in the lower respiratory tract of COPD patients[30]. A smaller diversity of bacteria in patients with COPD was also observed. Another study showed that Streptococcus, Prevotella, Fusobacterium and Veillonella were prevalent in individuals without COPD, while Pseudomonas and Haemophilus were dominated in COPD patients

microbiome[31]. International research revealed that in COPD patients Streptococcus sp. and Haemophilus sp. were associated with decreased pulmonary function, while low level of FEV1 was a predictor of bacterial diversity reduction[32]. COPD exacerbation increases the number of Proteobacteria (Moraxellaceae, Pasteurellaceae, Pseudomonadaceae, Enterobacteriaceae) and reduces the amount of Actinobacteria, Clostridia and Bacteroidia[33]. It is interesting that Actinobacteria produces metabolites with antimicrobial activity and classes IV and XIVa Clostridia known to be inducers of anti-inflammatory T-regs[34]. Treatment strategy was shown to modify lung microbiome in respiratory diseases. Thus antibacterial therapy in patients with COPD exacerbation results in reducing number of Proteobacteria. Administration of corticosteroids increases their number, as well as the number of Bacteroidetes and Firmicutes, especially Enterobacteriaceae (more than 16 times), Lachnospiraceae, Burkholderiaceae and Neisseriaceae. Combination therapy with corticosteroids and antibiotics leads to increase of Proteobacteria[33].

An attempt to prove the etiological role of the microbial composition of the respiratory tract in COPD development was made. In mice models, reduction in the microbial diversity significantly increases the number of Pseudomonas genera, Lactobacillus, Chryseobacterium and reduction Prevotella. Also there was a marked enhancement of the inflammatory response which included the formation of lymphoid follicles in the lung tissue, increased production of IL-17A, which level was positively correlated with limited airflow and COPD progress[35]. Finally broncho-alveolar lavage fluid (BALF) of such animals was intranasally translocated to sterile and antibiotic-treated mice, as a result an increase in the number of cells producing IL-17A in the lung tissue, particularly $CD4^+$ T cells in the recipients were noted[36].

One of the early studies confirmed the role of microbiome in the bronchial asthma development was done in Denmark. The presence of Moraxella catarrhalis, Haemophilus influenzae and Streptococcus pneumoniae in the oropharynx of children of 1-mo age significantly increased the risk of bronchial asthma development[37]. Pathologic role of mentioned bacteria in asthma development had been confirmed in more recent studies[38]. Asthmatic patients were found to have higher number of pathogenic proteobacteria (*e.g.*, Haemophilus) and significantly lesser Bacteroidetes, especially of genus Prevotella compared to healthy individuals[5]. The prevalence of families Comamonadaceae, Sphingomonadaceae, Oxatobacteraceae was shown to correlate with bronchial hyperresponsiveness. Interestingly, colonization with certain pathogenic bacteria is strictly associated with an immune response in newborns. Thus at high amounts of *M. catarrhalis* and *H. influenzae* production of IL-1, IL-17 increases. Also prevalence of S. aureus leads to overproduction of IL-17[39].

The microbiome composition varies in patients depending on the disease severity. Thus in patients with severe bronchial asthma when compared with those with mild to moderate severity, there is a significantly higher (7-8 times) number of Klebsiella[40]. In a recently published study healthy individuals when compared to patients with mild bronchial asthma were shown to have a decreased number of Bacteroidetes such as *Prevotella* spp.[41]. At the same time the number of pathogenic Proteobacteria, including Neisseria and Moraxella spp., were 2-times higher in patients with mild bronchial asthma. Bacteroidetes (OR = 0.62) and Fusobacteria (OR = 0.38) were decreased in patients with severe bronchial asthma, compared to the control group. Significant increase in Firmicutes, consisting mainly of streptococci in comparison with healthy individuals and patients with mild bronchial asthma (OR = 2.15 for both comparisons) was observed. Also there was a positive association between the severity of bronchial asthma and the level of Streptococcus (*Streptococcus* spp., *Streptococcus*_23 and *Streptococcus*_155) and negative with the level of *Prevotella* spp.

Imbalance in the oropharyngeal flora was found to decrease resistance, increase bacterial colonization and dissemination of the potential pathogen in the airway and pneumonia development. Oropharyngeal microbiome of healthy individuals and patients with pneumonia in two age groups: 18-59 years old and group 60 years and older were compared. Three microbial profiles associated with pneumonia in both age groups were revealed: prevalence of bacteria genus Streptococcus, Rothia and Lactobacillus. At the same time in healthy individuals, the microbiome was dominated by Veilonella, Prevotella, Leptotrichia and Gemellales. Moreover, the overall number of viruses in the microbiome of patients with pneumonia significantly increased. The composition of the microbiome was less diverse, while bacterial load was significantly higher which was also correlated with the disease severity. Furthermore the number of Anaerobes, Bacteroides decreases with age, while overgrowth of lactobacilli was noted[42].

Several studies have shown protective role of the intestinal microbiota in the course of pneumonia. The role of normal gut microbiota in mice, particularly segmented filamentous bacteria (SFB) in the course of pneumonia caused by *S. aureus* was studied. It was shown that the number of CFU of *S. aureus* in the lungs and spleen were significantly higher in SFB-negative mice in comparison with SFB-positive mice and the clearance of pathogenic bacteria in SFB-negative mice was reduced. In addition, the bacterial load decreased in SFB-negative mice when they were co-housed with healthy mice and similarly after fecal transplantation from healthy mice. All SFB-negative infected mice died within 36 h, whereas the survival rate in mice with normal gut microbiota was 70%[43]. In another research the role of microbiota during the course of pneumococcal pneumonia was studied[44]. Microbiota-depleted mice were shown to have an increase in bacterial dissemination, inflammatory response, organ damage, higher mortality due to pneumonia, impaired phagocytic activity of alveolar macrophages, whereas after subsequent fecal transplantation from healthy mice, there

Table 1 Probiotics and synbiotics in respiratory diseases

Probiotics/synbiotics	Medical condition	Results	Source
Lactobacillus rhamnosus, Bifidobacterium breve	Smokers	Inhibition of nicotine -mediated IL-1β, IL-6, IL-10, TNF-α production, NF-KB, TLR4 and TLR9-induced expression of IL-8 activation	Mortaz *et al*[49], 2015
Lactobacillus rhamnosus, Bifidobacterium lactis, Bifidobacterium breve	Allergic asthma	Antigen-specific Tregs activation	Sagar *et al*[50], 2014 Jang *et al*[51], 2012
Lactobacillus reuteri ATCC 23272 Lactobacillus rhamnosus GG	Allergy	Significant reduction of inflammatory cells in BALF, increasing $CD4^+CD25^+Foxp3^+$ Treg in spleen and mediastinal lymph nodes	Forsythe *et al*[52], 2007
Lactobacillus rhamnosus GG Lactobacillus casei (Sirota and DN 114001)	Acute infectious respiratory diseases	Increasing of IgA- secreting cells in bronchial mucosa	Tapiovaara *et al*[53], 2016
Enterococcus faecalis FK-23	Asthma	Suppression of T-cells and cytokines production	Zhang *et al*[54], 2012
Bifidobacterium longum	Klebsiella-induced pneumoniae	Increased production of IL-10, decrease of TNF-α and IL-6 levels	Viera *et al*[55], 2016
Acidic oligosaccharides	*P. aeruginosa*-induced infection	Increase in IL-10 production, decrease in cytotoxic T lymphocyte production	Bernard *et al*[62], 2015
Bifidobacterium breve M-16V galacto-oligosaccharides fructo- oligosaccharides	Allergic asthma	Significant increase in peak expiratory flow rate and reduction of IL-5 production	van de Pol *et al*[63], 2011

IL: Interleukin; TNF: Tumor necrosis factor.

were cytokines normalization (TNF-α, IL-6 and IL-10) and an accelerated elimination of *Str. pneumoniae*.

Place of probiotics, prebiotics, and synbiotics in respiratory pathology treatment

Several studies confirm that antibiotic administration can result in gut microbiota dysbiosis. Broad-spectrum antibiotics can affect the bacterial abundance in the gut causing rapid and significant decrease in taxonomic richness and diversity. Thus Jernberg *et al*[45] documented a decline in the clonal diversity of Bacteroides isolates, insurgence of antibiotic-resistant strains, and upregulation of antibiotic resistance genes in healthy volunteers treated for 1 wk with clindamycin. These effects persisted up to 2 years after treatment[45]. In another study vancomycin has been shown to cause long-lasting susceptibility to secondary infections in humans and mice. Vancomycin markedly disrupted the microbiota, leading to prolonged loss of resistance to *C. difficile* infection and dense colonization by vancomycin-resistant *Enterococcus*, *K. pneumoniae*, and *E. coli*[46].

In mouse models antibiotic administration during the perinatal period changes the lung microbial composition towards Th2 (vancomycin) or Th17 immune responses (streptomycin)[47].

However, some antibiotics like azithromycin could reduce pulmonary inflammation and exacerbations in patients with COPD. In the recent randomized, double-blind, placebo-controlled trial of 20 smokers (current or ex-smokers) with emphysema and CORD, administration of azithromycin 250 mg daily for 8 wk compared with placebo led to reduce *in-vivo* levels of chemokine (C-X-C) ligand 1 (CXCL1), TNF-α, IL-13 and IL-12 p40 in BAL, but increase levels of bacterial metabolites such as glycolic acid, indol-3-acetate and linoleic acid. Azithromycin treatment altered both lung microbiota and metabolome, affecting anti-inflammatory bacterial metabolites that may contribute to its therapeutic effects[48].

The relationship between respiratory pathology and the changes in the microbiome composition predisposed the use of probiotics. Anti-inflammatory effects of Lactobacillus rhamnosus and Bifidobacterium breve in smokers were evaluated. Both probiotic strains significantly inhibited nicotine-mediated production of IL-1B, IL-6, IL-10, TNF-α, activation of the NF-κB as well as TLR4 and TLR9-induced expression of IL-8[49]. Use of Lactobacillus rhamnosus, Bifidobacterium lactis and Bifidobacterium breve in bronchial asthma resulted in reduction of allergic reactions[50,51]. Lactobacillus reuteri ATCC 23272 and Lactobacillus rhamnosus GG (LGG) administration leads to a significant reduction in the inflammatory cells of BALF[52]. The use of probiotic bacteria LGG and Lactobacillus casei (Sirota and DN 114 001 strain) showed high-potency for the prevention and treatment of both bacterial and viral infections of the respiratory tract[53]. The introduction of Enterococcus faecalis FK-23 in mice reduced the frequency of bronchial asthma exacerbations because of its ability to suppress T-lymphocytes and cytokine production[54].

Treatment of Klebsiella pneumoniae infected mice with Bifidobacterium longum, leads to more rapid resolution of inflammation, decrease in mortality, which has been associated with increased production of IL-10, lower levels of TNF-α and IL-6. Also in the group of mice, treated with probiotics, the ability of alveolar macrophages to produce reactive oxygen species was significantly higher when compared with the control group[55].

Probiotics are now used to treat and control a variety of gastrointestinal diseases including diarrhea, inflammatory bowel disease, irritable bowel syndrome, liver diseases. In rodent models, administration of probiotics prevents chronic stress-induced bacterial translocation[56], colorectal hypersensitivity[57], and

restored intestinal barrier dysfunction[58].

The overall effects of prebiotics are similar to those of probiotics. It was shown that prebiotics provide optimal facilities for functional capacity of resident microbiota, stimulate different biochemical reactions within intestinal microbiome, promoting proliferation and renewal of intestinal cells and therefore prebiotics appear to be an active and important part of gut-lung axis.

Prebiotics can ameliorate gut microbiota. Most prebiotics, including inulin and fructo-oligosaccharides are digested by Bifidobacteria and stimulate growth of their colonies[59]. These bacteria influence homeostasis of intestinal cells and inhibit the growth of pathogenic bacteria[60]. Moreover, SCFAs such as propionic acid, acetic acid, and butyric acid reduce the development of gastrointestinal disorders by inducing apoptosis[61]. Additionally SCFAs are important participants in macroorgsnism's immune system modulation as it was mentioned above[20-27].

As can be seen from the above prebiotics and probiotics are essentially different biological structures but their effects are mutually reinforcing.

The use of pre- and synbiotics in the treatment of the respiratory diseases was also studied. Effect of acidic oligosaccharides in the treatment of mice infected with P.aeruginosa was investigated. A significant reduction in mortality, an increase in IL-10 production was achieved. Diminished production of cytotoxic T-lymphocytes was revealed and as a result, reduction in the severity of inflammation and limitation of tissue damage was observed. In re-infected with Pseudomonas aeruginosa mice, the bacterial load in the lung tissue was lower when compared with the control group[62]. In the study on patients with allergic bronchial asthma treatment with synbiotic, containing Bifidobacterium breve M-16V showed a significant increase in peak expiratory flow rate and reduction in the production of IL-5 when compared with the placebo group[63]. Data summarized in Table 1.

CONCLUSION

The status of the lung microbiome is normally determined by the relationship between microbial immigration, elimination and local conditions of bacterial growth. The results of studies indicate changes both in the lung and the intestinal microbiome in patients with respiratory diseases, occurring due to imbalance between the factors mentioned above. The studies on human microbiome have aroused great interest in application of probiotics for the prevention and treatment of somatic diseases. However, there is a necessity of further studies to determine the appropriate dose, selecting an optimal bacterial strain, duration of treatment, as well as groups of patients that will provide desirable effect in the prevention and/or treatment of a particular disease. Studies on mice models have shown a positive effect of probiotics on the course of pneumonia, acute exacerbation of bronchial asthma and COPD, which dictates the need for its research on human population. It gives hope that the treatment of these diseases might be improved in the nearest future.

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