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Multiple inert gases elimination technique (MIGET) and gas exchange in obstructive respiratory diseases.

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Index

Summary

Among the four traditional causes of arterial hypoxemia (table 1) and hypercapnia (alveolar hypoventilation and V_A/Q heterogeneity) V_A/Q inequality is the most important mechanism of respiratory gas exchange impairment. In the mild 1970s a new technique has been developed able to study ventilation-perfusion ratios distributions named “multiple inert gases elimination technique”, *MIGET*.

The technique is based on the quantitative relation of the blood-gas partition coefficient (λ) of a gas - the V_A/Q ratio - and the capacity of an alveolar unit to exchange that gas (Fahri, 1967). By using 6 different inert gases in trace concentrations, covering a wide spectrum of partition coefficients from 0.005 (SF6) to 300 (acetone), it is possible to characterize the distribution of the V_A/Q ratios within the whole lung. By using a lung model of 50 compartments, retentions and excretions of six gases give an estimation of a continuous distribution of the pulmonary blood flow and alveolar ventilation, respectively, against V_A/Q ratios on a logarithmic scale.

A complete V_A/Q study also analyses extra pulmonary factors determining gas exchange as cardiac output, total ventilation, oxygen consumption, FIO_2, type and concentration of Hb, pH and body temperature.

The two *major advantages* of this technique are:

- It gives an estimation of alveolar ventilation and pulmonary blood flow without disturbing either vascular or bronchomotor tone;

Summary.

- It facilitates the understanding of the complex interplay of intra and extra pulmonary factors determining pulmonary gas exchange.

MIGET has been used to study the mechanisms of gas exchange impairment in main respiratory diseases, their eventual relationships with structural alterations, the effects of O_2 breathing and the effects of some of the drugs commonly used.

The first studies conducted between 70s and 80s on *asthmatic patients* described a variable degree of V_A/Q inequalities (increased blood flow dispersion) beside a normal or mildly reduced PaO_2.

Patients with stable chronic severe asthma showed modest V_A/Q abnormalities maintaining a near normal PaO_2, despite a severe degree of airway obstruction, likely because of less inflammatory changes at a peripheral level or because of a more active hypoxic pulmonary vasoconstriction. These data contrast with those of COPD patients showing, for a similar degree of bronchoconstriction, much greater V_A/Q inequalities.

In acute severe asthma gross V_A/Q abnormalities were observed, proportionally with asthma attack severity, with predominant bimodal blood flow distribution and a variable amount of cardiac output to areas with low V_A/Q ratios.

No correlation has been found between respiratory and inert gases exchange and airways bronchoconstriction indices. This finding suggests that spirometric abnormalities reflect predominantly bronchoconstriction in larger and medium sized airways, while gas exchange and V_A/Q abnormalities reflect more peripheral airways impairment.

Summary.

In *COPD* patterns and severity of V_A/Q inequalities differ among patients and change with the evolution of the disease and with the clinical state of the patient.

In all cases V_A/Q inequalities appear to be the main cause of hypoxemia but no O_2 diffusion limitation or true shunt have never been detected.

No correlation has been found between severity of airflow obstruction and V_A/Q inequalities as patients with mild to moderate COPD already show notable V_A/Q mismatch. However the degree of V_A/Q dispersion is usually higher in more severe patients.

Barberà and coworkers studied the mechanisms of gas exchange impairment during COPD exacerbation. They observed increases in Log SDQ, low V_A/Q ratio units, cardiac output and oxygen consumption. By using MIGET algorithm, they definitely demonstrated that gas exchange worsening during exacerbations is essentially due to V_A/Q mismatching and amplified by the decreased mixed venous PO_2, resulting from a greater VO_2.

The effects of bronchodilators in COPD have also been investigated since the beginning of 80s: an unexpected worsening of basal degree of V_A/Q mismatching was observed after β_2-agonists, likely as effect of hypoxic vasoconstriction release.

Summary.

"Adenosine 5'-monophosphate (AMP) challenge in mild asthma: cellular and gas exchange responses"

Abstract

BACKGROUND: It has been suggested in asthma that bronchial challenge to indirect agents, such as 5'-adenosine monophosphate (AMP), offers a better estimate of airway inflammation than direct agents. We explored the effects of AMP challenge on lung function, including ventilation-perfusion (V_A/Q) relationships as a marker of peripheral airway inflammation, and induced sputum, and compared with methacholine (MCh) bronchoprovocation. METHODS: A randomized, single-blinded, cross-over study using dose-response curves was designed, one week apart. Twelve non-smoking mild asthmatics (age, 25±(SE)1 yr; FEV_1, 92±4% predicted) were studied at baseline and 5, 15 and 45 min after equivalent target responses to provoke a 30% fall in FEV_1 (AMP, by 35±2%; MCh, by 37±2%). Sputum was collected before and 4 h after each challenge. RESULTS: Five min after challenge, PaO_2 fell (AMP, by 4.1±0.4 kPa; MCh, by 4.3±0.4 kPa) due to V_A/Q mismatching as assessed by an increased overall index of V_A/Q heterogeneity (DISP R-E*; normal values, <3.0) (AMP, by 3.8±0.7; MCh, by 4.6±0.7), without differences between agents. Compared with MCh, there were increases in cardiac output (by 20±8%) and oxygen consumption (by 10±4%) ($p<0.05$ each) after AMP possibly related to an inotropic effect. Four h after challenge, sputum neutrophils increased after AMP (from 48±7% to 62±6%, $p<0.05$) without associated changes in eosinophils. The number of neutrophils in the baseline sputum showed a significant correlation with the

Summary.

concentration of IL-8 in the supernatant (r, 0.767; $p<0.01$) and with the increases in respiratory system resistances (Rrs; r, 0.803; $p<0.01$) and alveolar-arterial PO_2 difference ($AaPO_2$; r, 0.657; $p<0.05$) after AMP challenge. CONCLUSIONS: AMP challenge provoked an intense bronchoconstriction of similar magnitude to that of gas exchange abnormalities along with a neutrophilic response. MCh caused similar lung function changes without any sputum cellular effect.

"Effects of nebulized salbutamol on pulmonary gas exchange during COPD exacerbations and in stable conditions".

Abstract

Short-acting β_2-agonists are commonly used in COPD, although their effects on pulmonary gas exchange are not fully understood.

We investigated the effects of nebulized salbutamol (5 mg) on ventilation-perfusion (V_A/Q) inequalities in COPD patients during exacerbations (phase E) and in stable clinical condition (phase S). We studied 20 patients (1F:19M; 5 smokers, 15 ex-smokers; 67±2[SEM] yrs; FEV_1, 37±4% pred.) before and at 30 and 90 min after salbutamol. Nine patients completed both phases E and S (seven with V_A/Q measurements in both phases), 11 only phase E.

In phase E salbutamol significantly improved FEV_1 and inspiratory capacity (by 14±3% and 10±3%; $p<0.01$), increased cardiac output (Q_T, by 13±3%, $p<0.01$) and decreased mean arterial pressure (MAP; by 8±2%, $p<0.01$), while PaO_2 showed a mild, not significant, decrease only at 30 min (PaO_2 from 61.1±2.0 to 59.1±2.0) due to a mild increase in the dispersion of

Summary.

pulmonary blood flow (Log SDQ; from 1.17±0.07 to 1.23±0.06, $p<0.01$). Patients of phase S, in comparison with phase E (paired analysis), showed a similar spirometric response to salbutamol but more marked and prolonged negative effects on gas exchange, as PaO_2 passed from 70.7±4.4 to 63.6±3.5 (30 min) and to 64.5±3.2 mmHg (90 min) and LOG SDQ passed from 0.96±0.09 to 1.12±0.13 and to 1.15±0.13 (two-ways ANOVA, $p<0.01$; $p<0.02$). Q_T and MAP responses to salbutamol in phase S did not significantly differ from phase E.

The bronchodilator effect of salbutamol in COPD is associated with worsening of gas exchange and V_A/Q mismatching due to pulmonary vasodilatation. This effect is more evident in stable condition as pulmonary vasculature tone is more relaxed and liable to vasodilatation than during exacerbations (hypoxic pulmonary vasoconstriction).

In conclusion, the first study on AMP and MCh challenges in asthma was aimed at the investigation on the old hypothesis of dissociation between large and medium sized airways, accounting for the obstructive spirometric abnormalities of asthma, and small airways, where inflammatory and remodelling processes can affect gas exchange efficacy (PaO_2, $AaPO_2$, V_A/Q balance) independently by the bronchomotor tone. Unfortunately we could not find any differences in terms of V_A/Q imbalance between two challenges, as probably the impact of such an intense bronchoconstriction (PD30) is too high to let visible gas exchange differences specifically related to peripheral airways involvement. However the neutrofilic response we observed after

Summary.

AMP is a confirmation that this model of airways challenge is more representative of natural broncho-constrictive and inflammatory processes of real asthma attacks.

By continuing on this line of investigation on small airways we intend to integrate the information deriving from MIGET with old and newer other techniques (closing volume, exhaled NO, ventilatory scan, etc.) looking for parameters providing some functional and morphological information on pathological processes of small airways. We aim to evaluate the presence of V_A/Q and gas exchange abnormalities, which remain commonly unidentified, beside mild spirometric abnormalities and to correlate them with the other parameters considered, hoping to achieve a new definition of small airways diseases in asthma.

The second work focuses on COPD by comparing the effects of inhaled salbutamol on gas exchange during exacerbations and under stable conditions.

In particular, this clinical research experience has provided a better knowledge of β_2-agonists vasoactive and inotropic effects, potentially influencing the general hemodynamic balance, and has underlined, therefore, the complexity of the interaction among intra pulmonary and extra pulmonary factors determining pulmonary gas exchange in COPD.

Summary.

Introduction

Principles of physiology and assumptions.

The principal function of the lungs is to provide oxygen uptake (VO_2) and carbon dioxide elimination (VCO_2) adequate to satisfy the whole body metabolic request. Pulmonary gas exchange requires adequate levels of ventilation and perfusion in the alveoli. The old compartmental model of Fenn et al. and Riley and Cournard describes three ideal lung zones of ventilation-perfusion matching: 1. the *ideal lung*, where ventilation and blood flow are appropriately apportioned; 2. the *shunt fraction*, in which perfusion reaches unventilated areas; the *physiological dead space*, where the compartment is ventilated but not perfused [1-2]. Unfortunately, this historical model is not sufficient to explain V_A/Q relations inequalities that have been demonstrated to be the main cause of gas exchange abnormalities. Among the four traditional causes of arterial hypoxemia (table 1) and hypercapnia (alveolar hypoventilation and V_A/Q heterogeneity) V_A/Q inequality is the most important mechanism of respiratory gas exchange impairment [3-5].

Table 1. Factors determining arterial hypoxemia.

Intrapulmonary	Extrapulmonary
Main factors	
• Alveolar hypoventilation	• ↓Minute Ventilation
• V_A/Q mismatching	• ↓Cardiac Output
• Shunt	• ↓Inspired PO_2
• Alveolar-end capillary O_2 diffusion limitation	• ↑O_2 uptake
Secondary factors	
	• ↓P_{50}
	• ↓ [Hb]
	• ↑pH

P_{50}= PO_2 that corresponds to 50% oxyhaemoglobin saturation

Consequently, the most complete approach to investigate the pathophysiology of gas exchange impairment in respiratory diseases is the study of V_A/Q inequality.

In the mild 1970s a new technique has been developed able to study ventilation-perfusion ratios distributions named “multiple inert gases elimination technique”, *MIGET*.

This technique is based on the historical work of Fahri of the mid 1960s that demonstrated the quantitative relation of the blood-gas partition coefficient (λ) of a gas - the V_A/Q ratio - and the capacity of an alveolar unit to exchange that gas [6].

The basic equation for a single lung unit gas exchange was established:

$$P_cP_A=P_v\cdot\lambda/(\lambda+V_A/Q) \qquad \text{Equation 1}$$

This equation expresses mass balance during steady state elimination of the inert gas (gas not participating in metabolic processes) and indicates that both end capillary (P_c) and alveolar (P_A) partial pressures of an inert gas (assumed to be equal in a single lung unit) depend on the partial pressure of this gas in the venous side of the capillary (P_v) and on the solubility of that gas, expressed as partition coefficient (λ) and the V_A/Q ratio of the lung unit.

As P_c/P_v and P_A/P_v represent retention in the blood and excretion of the gas, respectively, the above equation can also be expressed as following:

$$R = E = \lambda/(\lambda+V_A/Q). \qquad \text{Equation 2}$$

The retention and excretion of an inert gas depend on its partition coefficient and on V_A/Q ratio of that unit. By using 6 different inert gases in trace

concentrations, covering a wide spectrum of partition coefficients from 0.005 (SF6) to 300 (acetone), it is possible to characterize the distribution of the V_A/Q ratios within the whole lung within the widest possible range (from 0.05 to 100). The six gases generally used, in increasing order of λ, are: SF6, ethane, cyclopropane, enflurane or halothane, diethilether and acetone. By using a lung model of 50 compartments, the retentions of six gases give an estimation of a continuous distribution of the pulmonary blood flow against V_A/Q ratios on a logarithmic scale (fig 1). Analogously, the excretions of six inert gases provide an estimation of the distribution of the alveolar ventilation against V_A/Q ratios.

This technique, applicable both in health and disease, despite the classical division of lung in three functional department [1-2] permits to separate areas with low but finite V_A/Q ratios (V_A/Q ratio<0.1) from areas whose V_A/Q ratios is zero (shunt), and regions with high V_A/Q ratios (V_A/Q ratio>10) from regions that are unperfused (dead space). After reaching a steady state, the concentrations of each gas are measured in the mixed arterial blood and mixed expiratory gas. The curve relating arterial concentration and solubility is transformed into a virtually continuous distribution of blood flow against V_A/Q, using techniques of numerical analysis. The relation of between expired concentration and solubility is similarly converted into the distribution of ventilation.

Introduction.

Some important *assumptions* are at the base of MIGET:

a. The existence of steady state conditions in all V_A/Q units that means the constancy of excretion and retention during the whole period of measurements.
b. Diffusion equilibration between alveolar gas and end capillary blood for each gas.
c. A lung model consisting in a number of homogenous compartments in parallel, each with constant and continuous ventilation and perfusion.
d. All diffusive processes affecting pulmonary gas movements are sufficiently rapid not to affect gas exchange.

MIGET is able to provide then, a quantitative picture of lung units with particular V_A/Q ratios in a good graphical representation and computes the amount of blood flow and ventilation associated with these lung units (Figure 1). This image is useful in giving an overview of the distributions and suggesting patterns of distribution as unimodal (normal or broad), bimodal or trimodal.

The quantification of V_A/Q inequalities is better expressed through some numerical indices:

- *Mean V, Mean Q*, mean values of V_A/Q ratios of ventilation and perfusion curves.
- *Log SDV, Log SDQ*, the standard deviation of mean values in a logarithmic scale.

Introduction.

- Low V_A/Q, the percentage of blood flow in units whose V_A/Q ratio is lower than 0.01.
- *High V_A/Q*, the percentage of blood flow in units whose V_A/Q ratio is greater than 10.
- *Shunt*, blood flow percentage to lung units with V_A/Q ratio<0.05. No post-pulmonary shunt is detectable by MIGET.
- *Dead space*, ventilation in units of V_A/Q ratio>100.
- *DISP R-E*,* the root mean square value of retention minus excretion, an overall index of V_A/Q heterogeneity.

As the MIGET algorithm does not consider diffusion limitations, the expected value of PaO_2 through this mathematical model does not always correspond to the real PaO_2 measured in the blood; the positive *difference between expected and measured PaO_2* can likely be ascribed to an oxygen diffusion limitation.

Since the first works made in the 70s on the mathematical analysis of O_2 and CO_2 behaviour in blood, it was clear that variables derived from physiological gases (PaO_2, $PaCO_2$, alveolar-arterial PO_2 difference [$AaPO_2$], venous admixture and physiological dead space) vary with V_A/Q matching, but also with changes in minute ventilation, cardiac output, inspired PO_2,etc. (table 1) [7].

A complete V_A/Q study also analyses extra pulmonary factors determining gas exchange as cardiac output, that can directly be measured (if inert gases levels are known in mixed venous blood, arterial blood and mixed expired gas) or using indocyanine green, total ventilation (V_E), oxygen

Introduction.

consumption (VO_2), FIO_2, type and concentration of Hb, pH and body temperature. MIGET permits to estimate the influence of each of extra pulmonary factors by introducing the observed values (singly or in any combination) of each factor in the algorithm that calculates V_A/Q inequality and the expected PaO_2.

This estimation is useful to understand the qualitative and quantitative role of each intra pulmonary and extra pulmonary factors in determining gas exchange.

The two *major advantages* of this technique are:

- It gives an estimation of alveolar ventilation and pulmonary blood flow without disturbing either vascular or bronchomotor tone;
- It facilitates the understanding of the complex interplay of intra and extra pulmonary factors determining pulmonary gas exchange.

Practical aspects

Originally the technique was performed using inert gases levels determined from three different sites: mixed venous blood, arterial blood and mixed expiratory gas. The modality permits a direct measurement of all variables of equation 2 and a direct calculation of cardiac output. It implies the placement of a catheter in the pulmonary artery.

The version of MIGET most commonly used only requires mixed expiratory and peripheral venous sampling. In this case cardiac output is measured by indocyanine green (dye dilution) (Figure 2).

Introduction.

To inscribe the dye curve, 5 mg of dye in 1 ml of water are rapidly flushed into the venous catheter and arterial blood is withdrawn at a constant rate of 20 ml·min^{-1}. Adequate curves are obtained prior to recirculation for the conventional Stewart-Hamilton analysis with a cardiac output computer (DC-410 Waters Instruments Inc. Rochester, MN).

The following equation is at the base of cardiac output measurement:

$$Q_T \cdot \lambda \cdot P_v = Q_T \cdot \lambda \cdot Pa + V_E \cdot P_E \qquad \text{Equation 3}$$

Where P_E is mixed expiratory gas and P_v can be computed from mass balance:

$$P_v = P_a + (V_E \cdot P_E / \lambda \cdot Q_T). \qquad \text{Equation 4}$$

Finally, a third modality of MIGET requiring only mixed expiratory and peripheral venous sampling is also available. This is obtained from a distally oriented canula inserted into a peripheral vein (usually in the forearm opposite to the side of the infusion of the inert gas solution). As inert gases are not metabolized by tissues after 90 min of infusion in a resting position virtual equilibration between blood and tissues is achieved and the peripheral venous blood reflects the inert gas concentration of the inflowing arterial blood.

Introduction.

Instrumentation

Some fundamental instruments are necessary in MIGET:

- *Exhaled air mixing box* (heated), for the mixed expiratory gas sampling: an adapted circuit takes the expired air to a mixing box (10 L) that is heated at a temperature of about 40-45 ℃ , in order to avoid inert gases deposition on tube internal walls. Mixed expiratory samples are collected from the mixing box to measure inert and respiratory gases.
- *Respirometer*, Ventilatory recordings (minute ventilation, respiratory rate, tidal volume) are taken during the study. A Wright respirometer is commonly used.
- *Inert and respiratory gases analyser*. Inert gases detection is commonly made by a chromatograph accurately set-up to describe all gases peaks. Chromatograph is provided with a flame ionisation detector (FID) able to measure all gases excluding SF6 that are hydrocarbons and with an electron capture detector (ECD) to measure SF6 [8-9].
- *A cardiac output computer*, if a pulmonary catheterisation is not performed.

MIGET in healthy subjects

The typical V_A/Q distribution of normal subjects aged less than 30 years at rest, breathing room air, is characterised by narrow perfusion and ventilation curves centred around 1 V_A/Q ratio in the abscissa (figure 1).

Mean values of the second moment of the distribution (Log SDQ and Log SDV) range from 0.35 to 0.43 [10]. The upper 95% confidence limit for Log SDQ is 0.60 and for Log SDV is 0.65 [11-14].

No perfusion in lung units with V_A/Q ratios <0.005 (shunt) is present [15]. The amount of ventilation to lung units with V_A/Q ratios >100 (dead space) is approximately 30%, including anatomical, physiological and instrumental dead space.

Neither perfusion to lung units with V_A/Q ratio <0.10 (low V_A/Q) is observed, nor ventilation to lung units with V_A/Q ratio>10 (high V_A/Q).

Cardus et al. investigated changes of V_A/Q inequality in healthy subjects with aging [16]. By studying 64 individuals aged between 18 to 71 years they observed only a slight increase in Log SDQ and Log SDV in parallel with PaO_2 mild decrease (by 6 mmHg). The upper 95% confidence limits in subjects aged 70 yrs were 0.70 for Log SDQ and 0.75 for Log SDV. They demonstrated that the observed decrease in arterial oxygenation (increased alveolar-arterial O_2 gradient, $AaPO_2$) with age is due to V_A/Q inequality.

Introduction.

MIGET in respiratory diseases

In the last three decades enormous progress has been made in the study of pathophysiology of acute and chronic respiratory diseases with the contribution of MIGET.

After a wide investigation on MIGET in normal subjects under different conditions of exercise, altitude, O_2 inspiratory fractions (FIO_2) and age [10-16], MIGET has been used to study the mechanisms of gas exchange impairment in main respiratory diseases, their eventual relationships with structural alterations, the effects of O_2 breathing and the effects of some of the drugs commonly used [17-18].

The milestone in the literature on the contribution of MGET to pulmonary medicine is the series published on Thorax between 1994 and 1995 composed of six articles [19-24].

Introduction.

Bronchial asthma

The first description of gas exchange impairment (mild to moderate hypoxemia) in asthmatic patients belongs to two Australian chest physicians in the 1967 [25]. McFadden et al confirmed the presence of hypoxemia and, in more severe cases, of hypercapnia in 101 patients suffering an acute exacerbation of asthma [26]. They postulated that obstructive changes in peripheral airways could explain the refractoriness to standard bronchodilators and relapses. Since MIGET was introduced in respiratory medicine in the 70s, a big amount of information has been collected on the mechanisms of gas exchange impairment in the different clinical forms of asthma.

The first study of Wagner on asymptomatic asthmatics in the 1978 showed that beside normal values of PaO_2 (all but one patient had normal or slightly reduced PaO_2) a bimodal blood flow distribution (increased Log SDQ) was described, with areas with low V_A/Q ratio [27]. Subsequent studies showed lower prevalence of V_A/Q inequalities, probably as a consequence of differences in patient selection, clinical management and treatment [28-31].

In patients with stable chronic severe asthma the V_A/Q abnormalities were quite modest (broad unimodal distribution, mildly increased Log SDQ and Log SDV) [32-33]. Likely, these patients maintain a near normal PaO_2 despite a severe degree of airway obstruction because of less inflammatory changes at a peripheral level or because of a more active hypoxic pulmonary vasoconstriction. This findings contrast with those of COPD patients that,

with a similar degree of bronchoconstriction, show much greater V_A/Q inequalities and gas exchange impairment.

In acute severe asthma gross V_A/Q abnormalities were observed, proportionally with asthma attack severity, with predominant bimodal blood flow distribution and a variable amount of cardiac output to areas with low V_A/Q ratios [34-36]. Neither dead space nor high V_A/Q ratios were described. The increased concentration of inspired O_2 and the administration of bronchodilators appeared to enhance the perfusion of low V_A/Q ratios.

All these studies have led to some general conclusions:

- Usually PaO_2 is relatively preserved; this data could be explained with the contribution of increased ventilation and cardiac output (extra pulmonary factors);
- No correlation has been found between respiratory and inert gases exchange and airways bronchoconstriction indices [37-38]. This suggests that spirometric abnormalities reflect predominantly bronchoconstriction in larger and medium sized airways, while gas exchange and V_A/Q abnormalities reflect more peripheral airways impairment.

COPD

Chronic obstructive pulmonary diseases are characterised by a wide spectrum of gas exchange abnormalities, ranging from mild hypoxemia to severe respiratory failure requiring ventilatory support. In all cases V_A/Q inequalities appear to be the main cause of hypoxemia [39]. By converse, no O_2 diffusion limitation or true shunt have been demonstrated in COPD patients.

The patterns and the severity of V_A/Q inequalities differ among COPD patients and change with the evolution of the disease and with the clinical state of the patient.

An historical work of Wagner and coworkers described tow different patterns of V_A/Q mismatch in a group of severe COPD patients [39]. Some patients showed an increased number of high V_A/Q ratio units (*high pattern*); other patients showed numerous areas with low V_A/Q ratios (*low pattern*) (Figure 3). While the *high pattern* was more frequent among emphysematous patients, no other consistent association between V_A/Q pattern and clinical pictures was found.

Other studies followed this one but no correlation was found between severity of airflow obstruction and V_A/Q inequalities as patients with mild to moderate COPD already showed notable V_A/Q mismatch [40-41]. However the degree of V_A/Q dispersion of these patients was lower than in more severe patients.

During exacerbations it has been observed worsening of V_A/Q distributions that are probably related to reversible functional abnormalities (mucus

impaction, bronchospasm, bronchial wall oedema) [42-43]. Barberà and coworkers studied the mechanisms of gas exchange impairment during COPD exacerbation [43]. Thirteen patients were studied during hospitalization and approximately 5 weeks after discharge. During exacerbations they observed increases in Log SDQ, due to a greater perfusion of low V_A/Q ratio units, in cardiac output and oxygen consumption (VO_2). By analysing the specific effect of each intra and extra pulmonary factor determining arterial oxygenation through MIGET logarithm it was demonstrated that gas exchange worsening during exacerbations is essentially due to V_A/Q mismatching and amplified by the decreased mixed venous PO_2, resulting from a greater VO_2.

The effects of bronchodilators in COPD have been investigated since the beginning of 80s, as they have known spirometric and clinical effects but not so clear effects on gas exchange. The bronchodilation induced by these drugs was unexpectedly accompanied by a worsening of basal degree of V_A/Q mismatching, likely as effect of a possible interaction with the mechanism of hypoxic vasoconstriction [44-46]. This hypothesis is sustained by the work of Melot and coworkers that described a fall in pulmonary vascular resistance and PaO_2 after the administration of nifedipine, a calcium channel blocker, able to suppress the beneficial effect of hypoxic vasoconstriction [47].

Other bronchodilators, like ipratropium bromure or aminophylline, acting through different mechanisms of action, did not change V_A/Q balance [45,48].

Introduction.

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Introduction.

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Introduction.

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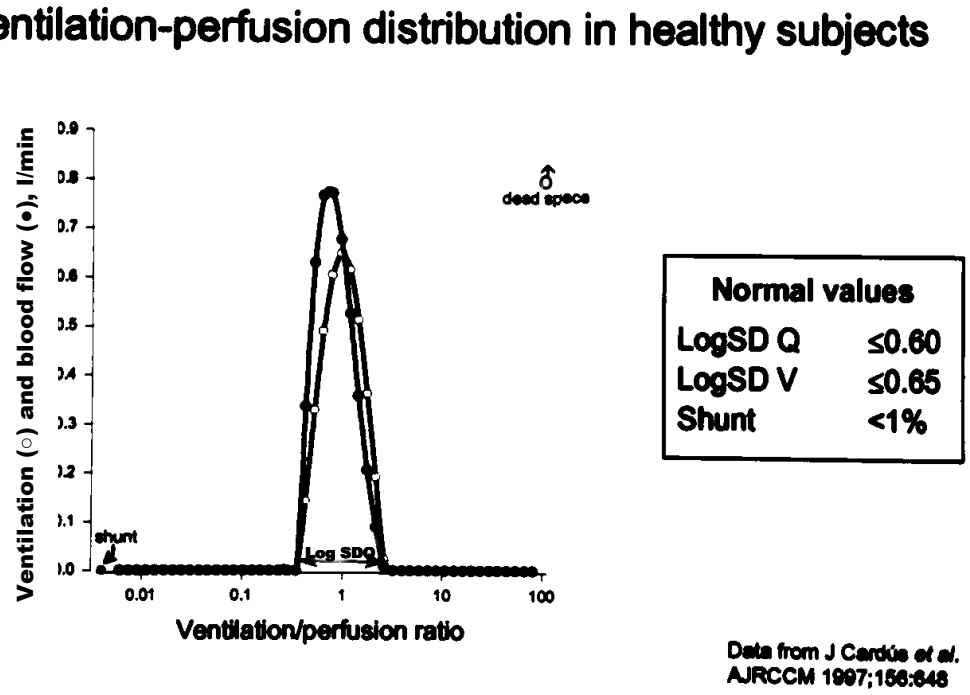

Figure 1.

Ventilation-perfusion distributions. Ventilation (○) and perfusion (●) are plotted against V_A/Q ratio on a logarithmic scale in a resting young healthy subject breathing room air. Both curves are centred (first moment) around a V_A/Q ratio of 1 and they are narrow (second moment). No perfusion to low V_A/Q units (V_A/Q ratio<0.1) nor ventilation to high V_A/Q units (V_A/Q ratio>10) are observed. Note also the absence of shunt. Each individual data point represents a particular amount of blood flow (●) or alveolar ventilation (○) to the corresponding pulmonary compartment (V_A/Q ratio). Total cardiac output corresponds to the sum of the 50 blood flow points and total alveolar ventilation is the sum of the 50 ventilation points.

Introduction.

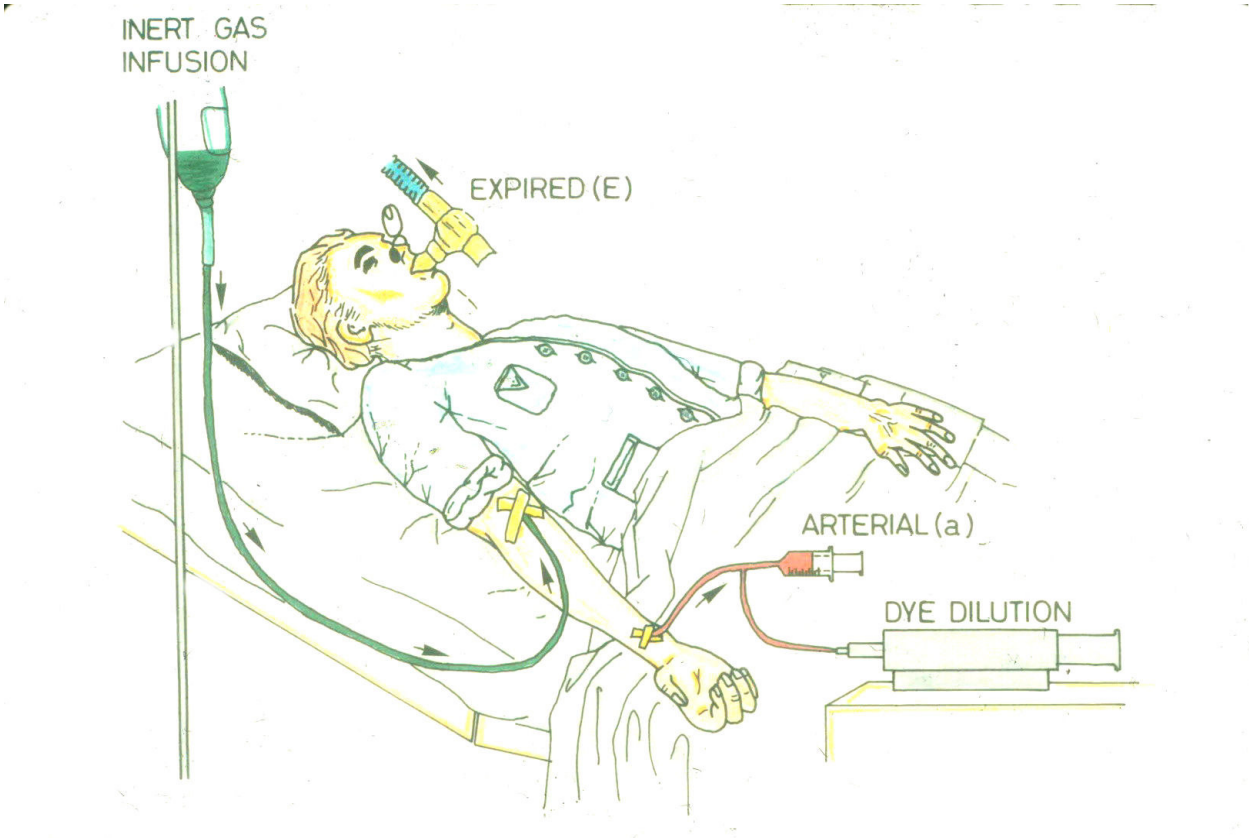

Figure 2.

With this MIGET modality mixed expiratory gas and arterial blood samples are collected; the inert gases solution is infused into a peripheral superficial vein. Through the arterial catheter the dye dilution is detected after indocyanine green bolus (5 mg of dye in 1 ml of water) is rapidly flushed into the venous catheter and arterial blood is withdrawn at a constant rate of 20 $ml \cdot min^{-1}$.

Introduction.

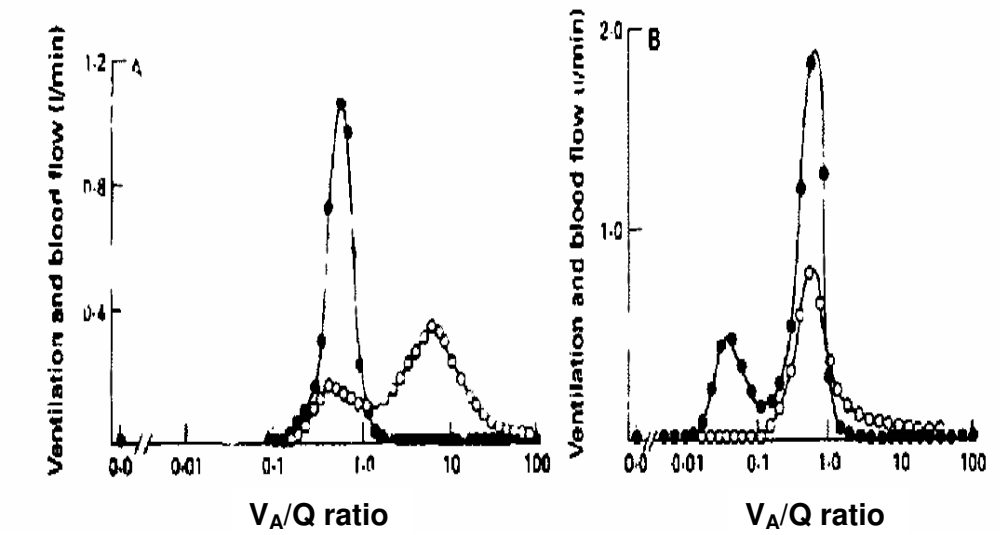

Figure 3.

A) Ventilation-perfusion distribution in patient with emphysema-type COPD. Note the bimodal pattern of ventilation distribution (○) with areas with high V_A/Q ratio. B) Ventilation-perfusion ratio distribution in a patient with bronchitis-type COPD. The blood flow distribution (●) is bimodally shaped due to the presence of alveolar units with low V/Q ratio.

Introduction.

ADENOSINE 5'-MONOPHOSPHATE (AMP) CHALLENGE IN MILD ASTHMA: CELLULAR AND GAS EXCHANGE RESPONSES

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Running head: *AMP challenge and asthma*

Key Words: Bronchial Challenge, Induced Sputum, Multiple Inert Gas Elimination Technique, Obstructive Airway Diseases.

INTRODUCTION

Adenosine 5'-monophosphate (AMP) is a natural nucleoside and mediator of airway inflammation increasingly used in inhalational challenge tests even though its pathophysiology remains insufficiently understood. It is a potent indirect agent inducing bronchoprovocation through the activation of inflammatory pathways at the level of bronchial surface and through local or central neuronal reflexes.[1-3]

It has been suggested that AMP is a more potent inflammatory mediator than methacholine (MCh), a well-known bronchoconstrictive agent directly acting on airway tone. Compared to direct agents such as MCh and histamine, an AMP challenge could reproduce the lung function abnormalities spontaneously occurring during asthma attacks and better detect the inflammatory events of the disease at both central and peripheral airways.[4-8]

Accordingly, we hypothesized that AMP challenge in asthmatic patients could evoke greater gas exchange abnormalities and a different cellular response to that induced by MCh. There is evidence for a partial dissociation between expiratory airflow obstruction and gas exchange disturbances in acute asthma, since spirometric abnormalities, that mainly reflect narrowing in large and middle-sized airways, do not run in parallel to gas exchange abnormalities, better correlated to more peripheral airways dysfunction. [9-11]

On this basis we used the multiple inert gas elimination technique (MIGET) to comprehensively assess pulmonary gas exchange and induced sputum cellular composition after AMP and MCh challenges producing an equivalent degree of bronchoconstriction. The comparison of the results obtained by the two challenges could facilitate a better insight into the pathophysiology of AMP and asthma.

MATERIAL AND METHODS

Study population. Twelve non-smokers patients (5 females) with stable mild asthma were recruited for the study, as approved by the Ethical Research Committee at Hospital Clínic of Barcelona (age, 25±1 yr; FEV_1, 3.7±0.2 L [92±4% predicted], FEV_1/FVC, 80±3%; PD_{20}, 0.4±0.2 μmol). All subjects gave informed written consent after the purpose, risks and potential benefits of the study were explained to them. Inclusion criteria were: age between 18 and 45 yr; diagnosis of bronchial asthma according to GINA criteria [12]; FEV_1>70% predicted (>1.5 L); a positive MCh (PD_{20}<1.9 μmol) bronchial challenge on their first visit; absence of respiratory co-morbidities or any systemic or cardiopulmonary disease other than asthma; no asthma exacerbation within the last 6 weeks before the study; no treatment with systemic glucocorticosteroids in the previous 3 months. One patient received regular inhaled glucorticosteroids (budesonide, 400 μg b.i.d.) and two patients fixed inhaled combination therapy (budesonide/formoterol, 320/9 μg b.i.d.). All patients referred use of rescue short-acting β_2-agonists.

Study design. A randomized, double-blinded, cross-over design was used. After a first visit to evaluate the clinical and functional status, a sample of induced sputum was collected in a second visit, one week later. Then, after another week, the patients were challenged with AMP and MCh (Sigma-Aldrich Química SA, Madrid, Spain), using a nebuliser attached to a breath-activated dosimeter (Spira Elektro2, Respiratory Care Center, Finlandia), one

week apart. The nebuliser was set to nebulise for 0.6 s at a pressure of 200 kPa with a flow of 0.6 L/s. During the challenges the patients breathed room air and were seated in an armchair. All asthma medication was withheld for 48 h before arrival to the laboratory and patients were asked to refrain from caffeine-containing beverages/foods for at least 12 h before testing. After ensuring steady-state conditions, a set of duplicate measurements of all variables was obtained (baseline). Maintenance of steady-state conditions was demonstrated by stability (±5%) of both ventilatory and haemodynamic variables, and by the close agreement between duplicate measurements of mixed expired and arterial O_2 and CO_2 (within ±5%). The patients were then challenged with AMP/MCh following the recommended standardized procedures. [13-15] Doubling concentrations of AMP/MCh (from 0.39 to 400 mg/dL) were administered by 5 consecutive dosimeter inhalations until the 30% fall in FEV_1 was achieved; at this point, resistance of respiratory system (Rrs) was also measured. Duplicate measurements were repeated after 5, 15, and 45 min. All sets consisted in the following steps in sequence: FEV_1, Rrs and ventilatory recordings; inert and respiratory gas sampling; and, haemodynamic recordings. Four hours after the beginning of each AMP/MCh challenge, induced sputum was collected. No patient needed rescue medication with short-acting bronchodilators during or immediately after the end of the study.

Measurements. On the day of the study, forced spirometry (CPF-S; Medical Graphics Corporation, St. Paul, MN USA) was performed according

to ATS recommendations, using our own predicted equations.[16][17] Rrs, PaO_2, $PaCO_2$ and pH, the alveolar-arterial PO_2 difference ($AaPO_2$), oxygen uptake (VO_2) and carbon dioxide production (VCO_2), minute ventilation (V_E) and respiratory rate (RR) were measured, or calculated, as previously described. [18] MIGET was used to estimate the distributions of V_A/Q ratios without sampling mixed venous inert gases. Cardiac output was directly measured by dye dilution technique (indocyanine green), in the customary manner.[18-19] A three-lead electrocardiogram, heart rate (HR) and systemic arterial pressure (Ps) and arterial oxygen saturation (SaO_2) through a pulseoximeter (HP M1020A, Hewlett-Packard, Boblingen, Germany) were continuously recorded throughout the whole study (HP 1046A Monitor, Hewlett-Packard, Waltham, MA, USA).

Induced sputum. We performed the inhalation for the same period (7 min) of increasing concentrations of hypertonic saline (3%, 5%, 7%) following the technique described by Pizzichini et al.[20] The procedure was stopped when an adequate sputum sample was collected (approximately 1 g of plugs), or if troublesome symptoms were present. The sputum was processed within 30 and 120 min from the collection of the more viscid proportions of the sputum (plugs). The reproducibility of measurements was calculated by the intraclass correlation coefficient. In two patients, sputum data were not available due to their poor viability (<50%). Interleukin-8 (IL-8) in sputum supernatants was also assessed.

Statistical analysis. Results are expressed as mean±SE and 95% confidence interval (CI). The effects of AMP and MCh challenges on the different end-point variables were assessed by a two-way repeated analysis of variance (ANOVA). Whenever there was a significant difference, *post hoc* comparisons at each time point were performed using paired t-test. Sputum cell differences were assessed using Paired t-test. Pearson's rank test was used for correlations. All analyses were performed with SPSS version 10.1 (SPSS Inc, Chicago, IL, USA). Statistical significance was set at $p<0.05$ in all instances.

RESULTS

Baseline conditions. There were no clinical or functional differences between MCh and AMP study days, all patients having normal V_A/Q distributions (Table 1).[21]

Lung Function after AMP/MCh challenges. Along with a comparable degree of bronchoconstriction (FEV_1 fall: AMP, 35±2%; MCh, 37±2%; Rrs increase: AMP, 114±18%; MCh, 92±13%) both AMP and MCh challenges (cumulative doses, 6.67±13.33 mg and 0.15±0.13 mg) produced, at 5 min, similar gas exchange abnormalities, as shown by a decreased PaO_2 (by 31±3% and 32±3%) and an increased $AaPO_2$, respectively (Table 2). The arterial oxygenation defects observed after both challenges were essentially caused by mild-to-moderate V_A/Q imbalance, as reflected by increases in the dispersion of blood flow (Log SDQ, normal values, ≤0.60 [21]) and DISP R-E* (normal values, ≤3.0) (Table 2).[22] Broadened unimodal V_A/Q patterns were observed in each participant. All these abnormalities showed at 15 and 45 min a similar trend to recover after both challenges, except for a mild, residual, increased Rrs and $AaPO_2$ observed at the last time point. By contrast, compared with MCh, AMP induced at 5 min increases in VO_2 (by 10±4%) and in Q_T (by 20±8%) ($p<0.05$ each); VO_2 also resulted increased at 45 min and Q_T at 15 min (Table 2).

Induced sputum. Four hours after AMP challenge neutrophils increased from 48±7% to 62±6%, ($p<0.05$) without other associated cellular changes (Table 3; Figure 1). By contrast, the cellular composition of induced sputum collected after MCh did not vary from baseline. The number of neutrophils in the baseline sputum showed a significant correlation with the concentration of IL-8 in the supernatant (r, 0.769; $p<0.01$) and with the increases in Rrs (r, 0.803; $p<0.01$) and $AaPO_2$ (r, 0.657; $p<0.05$) observed after AMP challenge (Figure 2). A significant correlation was also shown between the baseline concentration of IL-8 in the sputum supernatant and the increase in Rrs at 5 min on AMP study day (r, 0.726; $p<0.01$).

DISCUSSION

There were two major novel findings in our study. Firstly, we demonstrated that AMP bronchoconstriction provoked, in patients with mild asthma, identical gas exchange abnormalities and V_A/Q imbalance than MCh while undergoing an equivalent degree of airflow limitation. Secondly, the AMP challenge caused mild neutrophilia in induced sputum 4 hours after challenge. In addition, we confirmed that AMP is an effective and clinically well-tolerated bronchoconstrictive agent that associates haemodynamic and metabolic effects.[6]

There is strong evidence in the literature that bronchial responsiveness to AMP correlates well with airways inflammatory markers (blood and sputum eosinophils) and that reflects acute changes in airways inflammation, as seen by therapeutical (corticosteroids) and/or environmental (allergens avoidance) interventions.[4][7][8] On this basis, we postulated that AMP challenge, by acting through inflammatory mechanisms, could be more influential in small, peripheral airways, thereby inducing greater gas exchange and V_A/Q abnormalities than MCh, a more centrally acting agent. Notwithstanding, our study failed to confirm our hypothesis, in that after both challenges patients showed a similar degree of hypoxaemia and increased $AaPO_2$, due to identical V_A/Q mismatching. It is generally held that gas exchange impairment in asthma is mainly induced by small airways, but experimental studies have shown that the V_A/Q imbalance becomes much more disrupted with larger airways narrowing. It is highly likely that the intense level of

bronchoconstriction (~ PD_{30}) could hide any difference in V_A/Q mismatching due to the different bronchoconstrictive mechanisms of action of AMP and MCh.

Our study demonstrated that AMP is a well-tolerated effective bronchoconstrictive agent, with an average cumulative dose ratio to MCh of approximately 45. Furthermore, AMP challenge induced increases Q_T and VO_2 at different points in time, possibly as a consequence of the inotropic effects of AMP and its metabolite adenosine, via the activation of specific receptors (A_2).[23] These complementary effects need to be taken into account in the management of asthmatic patients with potential cardiovascular disturbances.

In conjunction with the functional abnormalities alluded to, there was a significant mild increase in neutrophils in induced sputum 4 hours after AMP inhalation. Although there is certain evidence of a correlation between cellular composition of the sputum and AMP responsiveness in asthmatic patients, there are few data regarding the effects of AMP challenge on induced sputum.[4] Van der Berge and coworkers showed an increase in eosinophils in the sputum collected 1 hour after AMP inhalation in patients with mild asthma, at variance with our finding.[24] Differences in patients' characteristics, inhalational procedures and, above all, in the time course of sputum collection after challenge might explain in part these discrepancies. Notwithstanding, it might be possible that our finding reflect a late, neutrophilic stage of the inflammatory process triggered by AMP challenge. By contrast, the study of Van der Berge may well reflect an earlier phase,

mainly characterised by eosinophilic involvement. [24] In a previous work with mild asthmatic patients, we observed an increase in neutrophils in the sputum 4 hours after challenge with platelet-activating factor (PAF), a potent inflammatory mediator possibly involved, but never sufficiently proven, in the pathogenesis of asthma.[25] This late, neutrophilic response to PAF may thus support our hypothesis about the inflammatory response to AMP challenge. A different study in asthmatic patients challenged with inhaled leukotriene D4 (LTD4), demonstrated an increase of eosinophils in the sputum 4 hours after challenge, also shown by our group at a shorter time point (75 min) after LTD4. [26][27] The activation of a different pathway of bronchoconstriction (direct mechanisms of action) could explain such different findings. A confirmation of the validity of our "neutrophilc model of bronchoconstriction" comes from the interesting observation that a greater number of neutrophils in the baseline sputum and higher concentrations of IL-8 in the sputum supernatant, a chemotactive factor of neutrophils, correlated closely with the mechanic (Rrs) and gas exchange ($AaPO_2$) abnormalities provoked by AMP. In summary, for the same degree of bronchoconstriction, AMP challenge did not induce more marked gas exchange disturbances than MCh, but caused mild neutrophilia in induced sputum at 4 hours after challenge.

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Table 1. Basal conditions on AMP and MCh days.

	AMP	MCh
FEV_1, L	3.2 ± 0.2	3.2 ± 0.2
FEV_1, % predicted	85 ± 6	83 ± 6
FEV_1/FVC %	81 ± 3	81 ± 2
Rrs, cm $H_2O \cdot L^{-1} \cdot s$	4.6 ± 0.3	4.6 ± 0.4
PaO_2, KPa	13.4 ± 0.3	13.5 ± 0.2
$AaPO_2$, KPa	0.52 ± 0.16	0.27 ± 0.12
VO_2, $mL \cdot min^{-1}$	197 ± 9	186 ± 12
Q_T, $L \cdot min^{-1}$	5.7 ± 0.3	5.6 ± 0.3
Log SDQ	0.47 ± 0.04	0.44 ± 0.02
DISP R-E*	3.15 ± 0.49	2.38 ± 0.26

Rrs: respiratory system resistance; PaO_2: arterial oxygen partial pressure; $AaPO_2$: alveolar-arterial oxygen partial pressure difference; VO_2: oxygen uptake; Q_T: cardiac output; Log SDQ: dispersion of pulmonary blood flow (dimensionless); DISP R-E*: dispersion of retention minus excretion inert gases corrected for dead space (dimensionless).

Table 2. Mean differences (and 95% IC) after AMP and MCh challenges.

		5 min	15 min	45 min	p value†
FEV_1, L	AMP	-1.1 (-1.3 to -1.0)	-0.6 (-0.8 to -0.5)	-0.3 (-0.4 to -0.1)	NS
	MCh	-1.2 (-1.4 to -1.0)	-0.7(-0.8 to -0.5)	-0.2 (-0.4 to -0.1)	
Rrs, cm $H_2O \cdot L^{-1} \cdot s$	AMP	4.9 (3.4 to 6.5)	3.7 (0.9 to 6.6)	1.6 (0.2 to 3.0)	NS
	MCh	4.3 (2.9 to 5.6)	2.7 (1.4 to 3.9)	1.9 (1.1 to 2.7)	
PaO_2, KPa	AMP	-4.1 (-4.9 to –3.2)	-2.1 (-3.1 to –1.3)	-0.4 (-1.2 to 0.5)	NS
	MCh	-4.3 (-5.3 to –3.3)	-2.7 (-3.9 to –1.5)	-0.9 (-1.9 to 0.1)	
$AaPO_2$, KPa	AMP	3.3 (2.7 to 4.0)	1.7 (0.9 to 2.5)	0.7 (0.4 to 1.6)	NS
	MCh	3.7 (2.8 to 4.5)	2.3 (1.6 to 3.1)	0.8 (0.3 to 1.3)	
VO_2, $mL \cdot min^{-1}$	AMP	21 (2 to 40)‡	10 (-6 to 26)	17 (0.0 to 34)‡	<0.05
	MCh	8 (-8 to 23)	1 (-12 to 15)	-3 (-22 to 16)	
Q_T, $L \cdot min^{-1}$	AMP	1.0 (0.2 to 1.8)‡	0.6 (0.1 to 1.1)‡	0 (-0.3 to 0.3)	<0.01
	MCh	0.2 (-0.2 to 0.5)	0 (-0.45 to 0.49)	-0.2 (-0.6 to 0.1)	
Log SDQ	AMP	0.27 (0.18 to 0.36)	0.13 (0.03 to 0.23)	0.05 (0.04 to 0.14)	NS
	MCh	0.38 (0.26 to 0.49)	0.27 (0.17 to 0.36)	0.12 (0.06 to 0.18)	
DISP R-E*	AMP	3.77 (2.25 to 5.28)	1.41 (0.22 to 2.60)	0.33 (-1.45 to 0.80)	NS
	MCh	4.59 (3.09 to 6.08)	3.26 (1.75 to 4.77)	1.65 (0.81 to 2.49)	

For abbreviations see Table 1.
† Significance of the interaction between the effects of the bronchoprovocative agent and time course (repeated measures ANOVA);
‡ $p<0.05$ compared with MCh (paired t-test).

Table 3. Induced sputum analysis before and after AMP and MCh challenges

	Baseline	AMP	MCh
Total Cell Count, $x10^4 \cdot g^{-1}$	1,124 ± 215	1,136 ± 278	985 ± 230
Cell viability, %	71± 3	71 ± 5	71 ± 3
Squamous cells, %	3 ± 1	4 ± 1	4 ± 1
Macrophages, %	36 ± 5	31 ± 5	43 ± 8
Lymphocytes, %	4 ± 0	2 ± 1	4 ± 1
Neutrophils, %	48 ± 7	62 ± 6*	47 ± 9
Eosinophils, %	10 ± 5	4 ± 1	5 ± 3
IL-8, pg/mL	2204 ± 702	1624 ± 522	1246 ± 206

Values are mean ± SE; * p: 0.02 compared with baseline (paired t-test).

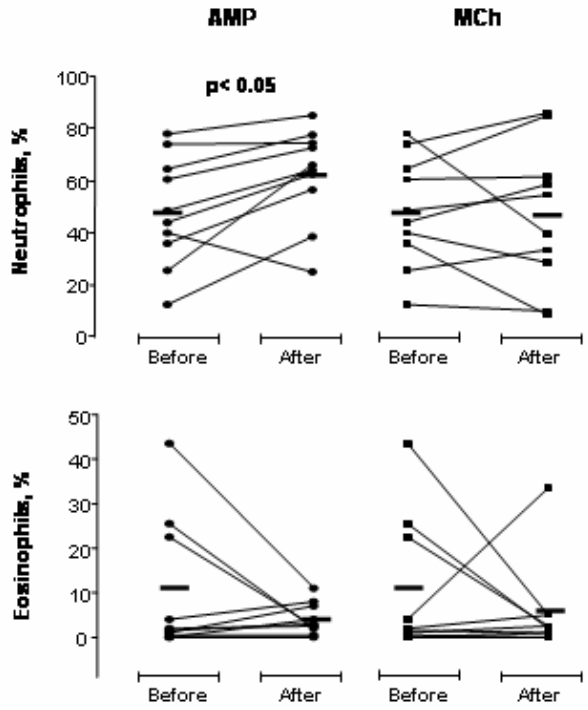

Figure 1.

Neutrophils and eosinophils in the induced sputum before and after each challenge, expressed as percentage of total cell count (TCC).

Polverino E et al. AMP and asthma.

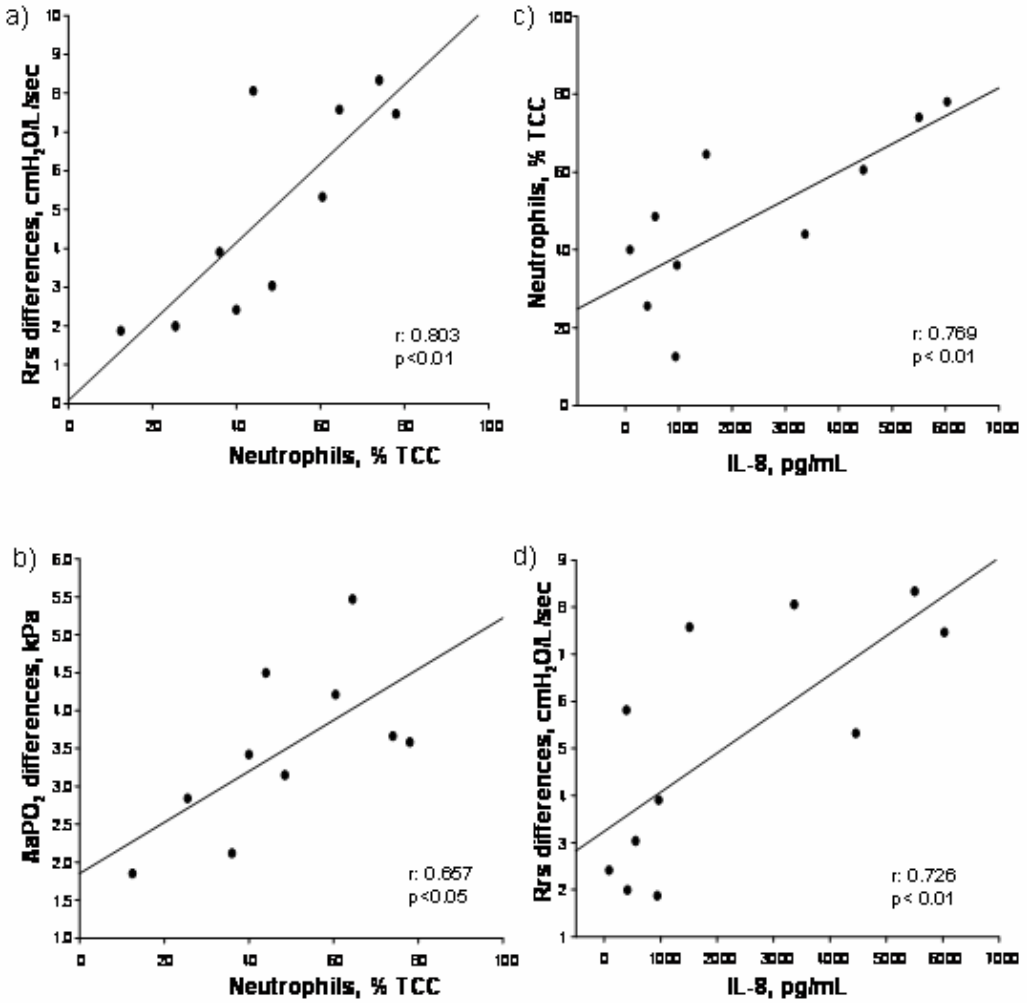

Figure 2.

Plots between changes in Rrs (Fig. 2a) and $AaPO_2$ (Fig. 2b) after AMP with the number of neutrophils in the baseline sputum; plots between neutrophils in the baseline sputum (Fig. 2c) and changes in Rrs after AMP (Fig. 2d) with IL-8 concentration in sputum supernatant. For abbreviations see Table 1 and Figure 1.

EFFECTS OF NEBULIZED SALBUTAMOL ON PULMONARY GAS EXCHANGE DURING COPD EXACERBATIONS AND IN STABLE CONDITIONS

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Running head: *COPD and salbutamol*

Key Words: COPD exacerbations, Multiple Inert Gas Elimination Technique, nebulized salbutamol, Pulmonary gas exchange.

INTRODUCTION

Episodes of exacerbations are the most common complications in the natural history of chronic obstructive pulmonary diseases (COPD). These episodes are always characterized by a significant worsening in pulmonary gas exchange that results in severe hypoxemia with or without hypercapnia [1]. Both arterial hypoxemia and hypercapnia are modulated by a complex interplay between intrapulmonary (ventilation-perfusion [V_A/Q] mismatching) and extrapulmonary (minute ventilation [V_E], cardiac output [Q_T], and oxygen consumption [VO_2]) factors [2]. It has been demonstrated that the principal mechanism of arterial hypoxemia in patients with COPD is V_A/Q imbalance, both in stable conditions and during exacerbations [3].

Short-acting β_2-agonists [SABAs] are one of the mainstays of treatment of COPD exacerbations [4].

SABAs are potent bronchodilators but also vasoactive agents with inotropic and chronotropic effects, hence potentially influencing different aspects of cardiopulmonary function [5]. However, a comprehensive assessment of the effects of such bronchodilators on intrapulmonary and extrapulmonary determinants of gas exchange during COPD exacerbations remains unsettled. Accordingly, we investigated in patients with COPD exacerbations needing hospitalization the effects of a therapeutic dose of nebulized salbutamol (5.0 mg) on pulmonary gas exchange using the multiple inert gas elimination technique (MIGET).

The same patients were studied during stable conditions, 2-3 months after their episode of exacerbation in order to assess how the factors contributing to gas exchange abnormalities vary during recovery in response to nebulized salbutamol and to facilitate, therefore, a better insight into the pathophysiology of COPD.

MATERIAL AND METHODS

Study population. We studied 20 patients (1 female; 5 smokers, 15 ex-smokers, 58 ± 8 pack-years), mean (± SEM) age 67 ± 2 yrs who fulfilled the criteria for the diagnosis of COPD [6] and were admitted to hospital because of an acute exacerbation of their disease, according to the decision of the attending physicians. At the inclusion in the study ten patients reported a history of more than 10 yrs of COPD, 6 patients between 5 and 10 yrs, 4 patients less than 5 yrs. Pulmonary function tests performed during stable conditions (before or after 2-5 months the exacerbation episode) showed a severe airflow obstruction (post-bronchodilator FEV_1: 39 ± 4% of pred.; FVC: 2.8 ± 0.2 L; FEV_1/FVC ratio: 0.40 ± 0.03), a normal total lung capacity (TLC: 108% ± 4 of pred.) a severe lung hyperinflation (RV: 187 ± 13% of pred.; IC/TLC: 0.28 ± 0.03) and a moderate-to-severe reduction of carbon monoxide transfer factor (DL_{CO}: 53 ± 4% of pred.; K_{CO}: 65 ± 5% of pred.). Patients showed moderate hypoxemia and mild hypercapnia (PAO_2: 65.7 ± 2.7 mmHg; $PaCO_2$: 42.1 ± 1.5 mmHg). As two patients died for medical complications before completing the study (one for an acute intestinal occlusion, the other during an exacerbation of the respiratory disease) and other two were lost at follow-up (multiple re-exacerbations) data of lung volumes, DL_{CO} and arterial blood gases in stable conditions are available only from 16 patients. All patients were in GOLD stages III or IV. On admission to the emergency room, patients showed moderate hypoxemia (PaO_2 62.2 ± 2.2 mmHg) and hypercapnia ($PaCO_2$ 44.4 ± 3.0 mmHg) with a

normal arterial pH (7.42 ± 0.01) even considering that in 6 and 3 cases, respectively, the first measurement of arterial gases was performed with supplemental oxygen of 1 and 2 L/min. Patients with complications (pneumonia, pulmonary thromboembolism, pleural effusion) and with comorbidities, like bronchial asthma, lung cancer and cardiovascular diseases or requiring mechanical ventilation were excluded from the study. All patients received standard treatment with supplemental oxygen, inhaled bronchodilators (salbutamol and ipratropium bromure), intravenous corticosteroids and antibiotics. The mean length of hospital stay was of 7 ± 1 days.

Study design. Patients were studied within the first 4 days (median, 3) of hospitalization, when they were able to breathe through the breathing circuit and 2-3 months after hospital discharge, once they were in stable condition and in absence of any other re-exacerbation of the respiratory disease from the first study. Medication as decided by the attending physician was maintained unmodified. Only one patient received supplemental oxygen therapy at 1.5 L/min (16 hours daily) after the exacerbation. During the studies all patients were afebrile, breathed room air and were seated in an armchair. Measurements were performed before (at least 6 hrs after the last dose of inhaled bronchodilators and intravenous corticosteroids) and at 30 and 90 min after nebulization of 5mg of salbutamol. After ensuring steady-state conditions, a set of duplicate measurements of all variables was obtained at each time point. Maintenance of steady-state conditions was demonstrated by stability (±5%) of both ventilatory and

haemodynamic variables, and by the close agreement between duplicate measurements of mixed expired and arterial O_2 and CO_2 (within ±5%). All sets of measurements consisted in the following steps in sequence: FEV_1, forced vital capacity (FVC), inspiratory capacity (IC) and ventilatory recordings; inert and respiratory gas sampling; haemodynamic recordings (systemic arterial pressure; cardiac output, Q_T; heart rate, HR).

Spontaneous sputum was collected before and between 30 and 90 min after salbutamol.

Measurements. On the day of the study, forced spirometry and Inspiratory capacity (IC) (CPF-S; Medical Graphics Corporation, St. Paul, MN USA) were performed according to ATS/ERS recommendations, using our own predicted equations.[7][8] PaO_2, $PaCO_2$ and pH, alveolar-arterial PO_2 difference ($AaPO_2$), oxygen uptake (VO_2) and carbon dioxide production (VCO_2), minute ventilation (V_E) and respiratory rate (f) were measured, or calculated, as previously described. [9] MIGET was used to estimate the distributions of V_A/Q ratios without sampling mixed venous inert gases. Unfortunately, V_A/Q data were lost for technical problems in 4 studies of the exacerbation phase and in one study of stable phase. Q_T was directly measured by dye dilution technique (indocyanine green), in the customary manner.[9][10] A three-lead electrocardiogram, heart rate (HR) and systemic arterial pressure and arterial oxygen saturation (SaO_2) through a pulseoximeter (HP M1020A, Hewlett-Packard, Boblingen, Germany) were continuously recorded throughout the whole study (HP 1046A Monitor,

Hewlett-Packard, Waltham, MA, USA). Exhaled breath condensate (EBC) was also collected before and at 90 min after salbutamol using the EcoScreen (Jaeger, Germany), according to ATS/ERS recommendations. [11]

Statistical analysis. Results are expressed as mean ± SE. The effects of nebulized salbutamol on the different end-point variables in each phases of the study (exacerbation and stable condition) were assessed by a one-way and two-ways repeated analysis of variance (ANOVA). Whenever there was a significant difference, *post hoc* comparisons at each time point were performed using paired t-test. All analyses were performed with SPSS version 11.1 (SPSS Inc, Chicago, IL, USA). Statistical significance was set at $p<0.05$ in all instances.

RESULTS

Exacerbation.

At baseline, patients showed a severe degree of bronchoconstriction (FVC, 36 ± 2% of predicted; FEV_1, 24 ± 2% of predicted), with high values of V_E and f (Table 1). Mean PaO_2 was moderately to severely reduced (range, 45.7 - 76.0 mmHg) while $PaCO_2$ was moderately increased (range, 33.3 - 68.2 mmHg). Gas exchange abnormalities run in parallel with moderate-to-severe V_A/Q imbalance: the dispersions of both blood flow (Log SDQ, range: 0.60 - 1.75) and ventilation (Log SDV, range: 0.70 - 1.26) distributions and the overall index of V_A/Q heterogeneity (DISP R-E*, range: 8.7 - 20.7) were increased (Table 1). Shunt (percentage of blood flow to units with V_A/Q ratio<0.005) and areas with high (10 - 100) V_A/Q ratios were negligible, while areas with low (<0.1, excluding shunt) V_A/Q ratios and dead space (>100 V_A/Q ratios) were moderately increased. Patients showed increased Q_T and HR with normal values of mean arterial pressure (Ps) and O_2 uptake (VO_2). At 30 min, nebulized salbutamol significantly improved FEV_1 (by 14%) and IC (by 10%), while V_E and f remained stable. PaO_2 and $PaCO_2$ slightly decreased along further significant increases in $AaPO_2$ (by 2.0 mmHg), in Log SDQ (by 7%) and in areas with low V_A/Q ratios (by 27%). In addition, salbutamol increased Q_T (by 13%) and HR (by 11%) and decreased Ps (by 8 mmHg). At 90 min, in comparison with baseline, FEV_1, IC, were still increased; respiratory (PaO_2, $PaCO_2$, $AaPO_2$) and inert (Log SDQ, Low V_A/Q,

DISPR-E*) gas exchange indices showed a trend toward baseline. Hemodynamic changes (increased HR and decreased Ps) were still present at 90 min, except for Q_T that showed a trend toward baseline. VO_2 remained stable during all the study.

Stable conditions.

Nine patients of the initial sample were excluded from the second phase of the study for multiple re-exacerbations and two individuals, as previously described, died. The remaining nine patients repeated the study 14 ± 3 weeks after discharge, once they were in clinical stable conditions. At baseline, as compared to exacerbation, patients had lower V_E ($p<0.05$), a comparable f and a similar airflow obstruction but less hyperinflation, as shown by greater IC ($p<0.02$) (Table 2). Gas exchange impairment and V_A/Q inequality were significantly minor under stable condition as shown by PaO_2, $AaPO_2$ and Log SDQ ($p<0.05$ each). Cardiac Output and HR ($p<0.05$), as expected, were lower than during exacerbation. No significant differences were observed in Ps and VO_2. At 30 min, nebulized salbutamol induced significant increases in FEV_1 (by 17%) and IC (by 17%) without any change in the ventilatory pattern (V_E, f) (Table 3). We also observed significant decrease in PaO_2 (by 6.7 mmHg) and increase of $AaPO_2$ (by 5.3 mmHg), with an unchanged PCO_2. Arterial deoxygenation was associated with a significant worsening in V_A/Q imbalance as shown by increases in Log SDQ (by 15%) and DISP R-E* (by 33%). In addition, salbutamol induced

significant increases in Q_T (by 23%), HR (by 17%) and VO_2 (by 24 mL/min) and decreased Ps (by 6 mmHg). At 90 min FEV_1 and IC were still increased, the arterial deoxygenation (PaO_2, $AaPO_2$) was similar to 30 min, such as the increases in Log SDQ and DISPR-E* (Table 3). Also the hemodynamic changes (increased Q_T and HR, decreased Ps) were still present at 90 min, while VO_2 returned to baseline.

Comparison between exacerbation and stable COPD.

By comparing the effects of salbutamol observed during both phases of the study (9 patients, 7 with complete V_A/Q data) through a two-ways ANOVA analysis, we detected similar spirometric and hemodynamic responses, as shown by FEV_1, IC and Q_T time courses in Figure 1. By contrast, whereas arterial oxygenation showed only negligible changes during the exacerbation, PaO_2 in stable COPD decreased by 7 ± 2 mmHg at 30 min and by 6 ± 2 mmHg at 90 min and $AaPO_2$ increased by 5 ± 1 mmHg (30 min) and 6 ± 1 mmHg (90 min) ($p<0.01$ each). Similarly, V_A/Q inequality worsening after salbutamol resulted significantly greater and longer under stable conditions, as shown by DISP R-E* time course (Figure 1). In fact, during the exacerbation DISP R-E* increased by 6% at 30 min and returned to baseline at 90 min; by contrast under stable conditions we observed an increase of 31% up to 90 min ($p<0.05$).

DISCUSSION

During COPD exacerbations nebulized salbutamol induced significant bronchodilator (increases in FEV_1 and IC) and hemodynamic (increased Q_T and heart rate, decreased MAP) effects up to 90 min after nebulized salbutamol. We also observed a temporary, further, deterioration of V_A/Q distribution accompanied by a mild gas exchange worsening 30 min after salbutamol.

The same patients in stable convalescence showed a similar response to salbutamol in terms of spirometric and hemodynamic changes, even though at baseline they were in better clinical and functional conditions. By contrast, worsening in gas exchange and V_A/Q imbalance were much greater in stable conditions and lasted up to 90 min after salbutamol.

The strict relation between gas exchange and V_A/Q imbalance has been widely investigated. Barberá and coworkers by studying COPD exacerbated patients, estimated that 46% of hypoxemia is attributable to V_A/Q inequality, whether a 28% to the combined effect of increased VO_2 and Q_T[3]. In our study VO_2 did not appear to play a significant role in both phases of the study while Q_T was higher during the exacerbation. As Q_T increase is able to augment the oxygen concentration of mixed venous blood and potentially counterbalance the effects of V_A/Q imbalance on arterial oxygenation, it has often supposed an important contribution of Q_T in determining PaO_2 in COPD exacerbations.

By contrast, our study shows similar Q_T responses after salbutamol in both COPD exacerbation and stable convalescence, but a significant deoxygenation only in stable COPD, suggesting only a limited role of Q_T in determining PaO_2.

A worsening of arterial oxygenation and V_A/Q balance after β_2-agonists administration has been previously described and ascribed to the release of hypoxic pulmonary vasoconstriction [12-16]. Ringsted and coworkers, in comparison with our study, observed an analogous response of V_A/Q distributions after the administration of intravenous terbutaline in stable COPD patients with a similar degree of bronchoconstriction[14]. Viegas et al. also described similar data with the administration of nebulized fenoterol in stable COPD patients [16]. Our data confirm this hypothesis as PaO_2 fall in stable conditions runs in parallel with the increased dispersion of pulmonary blood flow (Log SDQ), suggesting pulmonary vasodilatation.

However, this phenomenon appears to be mild during COPD exacerbations as probably in presence of more hypoxemia, airflow obstruction, hyperinflation and higher hyperkinetic vascular state, the pulmonary vascular tone is more hypoxic and more constricted, hence less liable to vasodilatation. By contrast, under stable conditions gas exchange response to salbutamol is much greater as, in less hypoxemic conditions, the pulmonary vasculature is less constricted and more sensitive to vasodilatation i.e. hypoxic pulmonary vasoconstriction release and further V_A/Q worsening.

Similarly, the work of Ringsted showed minor V_A/Q and gas exchange abnormalities in those patients who presented very severe airways

obstruction, likely because pulmonary vascular tone and its flexibility are limited in more advanced stages of COPD such as during exacerbations [15]. A clinical implication of this finding is that particular attention is necessary when SABA are administered to COPD patients as arterial oxygenation can temporarily worsen notwithstanding the general improvement of dyspnoea that is likely more related, to the degree of hyperinflation than to PaO_2 [17].

In conclusion, this work by offering a complete and paired analysis of all parameters modulating respiratory gas exchange under different clinical conditions, clarifies effects and mechanisms of action of β_2-agonists in COPD and, contemporary, underlies the central role of hypoxic pulmonary vasoconstriction in regulating V_A/Q and gas exchange efficacy in COPD.

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Table 1. Acute effects of salbutamol during COPD exacerbations (n= 20).

	Baseline	30 min	90 min	p†
FEV_1, L	0.73 ± 0.06	0.83 ± 0.07**	0.81 ± 0.06**	<0.01
IC, L	1.62 ± 0.13	1.76 ± 0.13**	1.77 ± 0.13**	<0.01
V_E, L/min	8.6 ± 0.4	8.9 ± 0.4	8.6 ± 0.5	NS
f, min^{-1}	17 ± 1	18 ± 1	18 ± 1	NS
PaO_2, mmHg	61.1 ± 2.0	59.1 ± 2.0	60.7 ± 2.0	NS
$PaCO_2$, mmHg	46.2 ± 2.1	44.7 ± 1.9**	44.3 ± 1.6	<0.02
$AaPO_2$, mmHg	36.5 ± 2.5	38.5 ± 2.3**	35.2 ± 2.5	<0.01
HR, min^{-1}	90 ± 3	99 ± 4**	95 ± 3**	<0.01
Ps, mmHg.	95 ± 3	87 ± 2**	89 ± 2**	<0.01
Q_T, L/min	7.1 ± 0.4	8.0 ± 0.5**	7.3 ± 0.4	<0.01
VO_2, mL/min	242 ± 7	249 ± 8	257 ± 8	NS
Shunt, % of Q_T	1.8 ± 0.7	2.0 ± 0.8	1.7 ± 0.7	NS
Low V_A/Q, % Q_T	9.5 ± 2.4	12.6 ± 3.0**	8.7 ± 2.2	<0.05
Log SDQ	1.17 ± 0.07	1.23 ± 0.07**	1.13 ± 0.08	<0.01
Log SDV_E	1.00 ± 0.04	0.99 ± 0.04	0.98 ± 0.04	NS
High V_A/Q, % V_E	1.3 ± 0.7	1.4 ± 0.7	1.1 ± 0.4	NS
Dead space, % V_E	34.5 ± 2.0	35.8 ± 1.9	35.2 ± 2.0	NS
DISP R-E*	14.9 ± 0.8	15.9 ± 0.9	14.0 ± 1.0**	<0.01

† Repeated measures ANOVA; ** $p<0.05$, post-hoc analysis (paired t-test) compared with baseline. Definition of the abbreviations. f: respiratory rate; $AaPO_2$: alveolar-arterial oxygen difference; MAP: mean systemic arterial pressure; Shunt, % of Q_T: % of Q_T to lung units with V_A/Q ratios<0.05. Low V_A/Q, % Q_T: % of Q_T to lung units with V_A/Q ratio< 0.1, excluding shunt; Log SDQ: dispersion of blood flow distribution (normal, 0.30-0.65); Log SDV: dispersion of the alveolar ventilation distribution (normal, 0.30-0.60); High V_A/Q, % V_E: % of V_A to lung units with V_A/Q ratio>10, excluding dead space; dead space: % of V_A to lung units with V_A/Q ratio>

100; DISPR-E*: dispersion of retention minus excretion inert gases corrected for dead space (normal, ≤ 3).

Table 2. Spirometric, hemodynamic and gas exchange data during exacerbations and under stable conditions (n= 9).

	Exacerbation	Stable Conditions	p†
FEV_1, L	0.76 ± 0.08	0.97 ± 0.16	NS
FEV1, % pred.	24 ± 2	31 ± 4	NS
FVC, L	2.15 ± 0.18	2.42 ± 0.25	NS
FEV_1/FVC%	51 ± 3	54 ± 5	NS
IC, L	1.56 ± 0.15	1.99 ± 0.26	<0.02
V_E, L/min	8.9 ± 0.5	7.3 ± 0.5	<0.05
f, min^{-1}	17 ± 1	17 ± 2	NS
PaO_2, mmHg	63.5 ± 3.0	70.7 ± 4.4	<0.05
$PaCO_2$, mmHg	43.0 ± 1.9	43.4 ± 1.8	NS
$AaPO_2$, mmHg	36.9 ± 4.1	26.5 ± 3.1	<0.01
Q_T, L/min	6.5 ± 0.4	5.7 ± 0.5	NS
HR, min^{-1}	81 ± 4	73 ± 4	<0.05
Ps, mmHg	90 ± 4	96 ± 4	NS
VO_2, mL/min	246 ± 11	211 ± 12	NS
SHUNT % Q_T	0.5 ± 0.4	0.9 ± 0.5	NS
Low V_A/Q % Q_T	8.8 ± 3.6	4.5 ± 2.6	NS
Log SD Q	1.24 ± 0.11	0.98 ± 0.09	<0.05
Log SD V_E	0.93 ± 0.05	0.91 ± 0.07	NS
High V_A/Q % V_E	0.37 ± 0.23	1.63 ± 0.82	NS
Dead Space % V_E	35 ± 3	33 ± 4	NS
DISP R-E*	14.58 ± 1.40	11.72 ± 1.39	NS

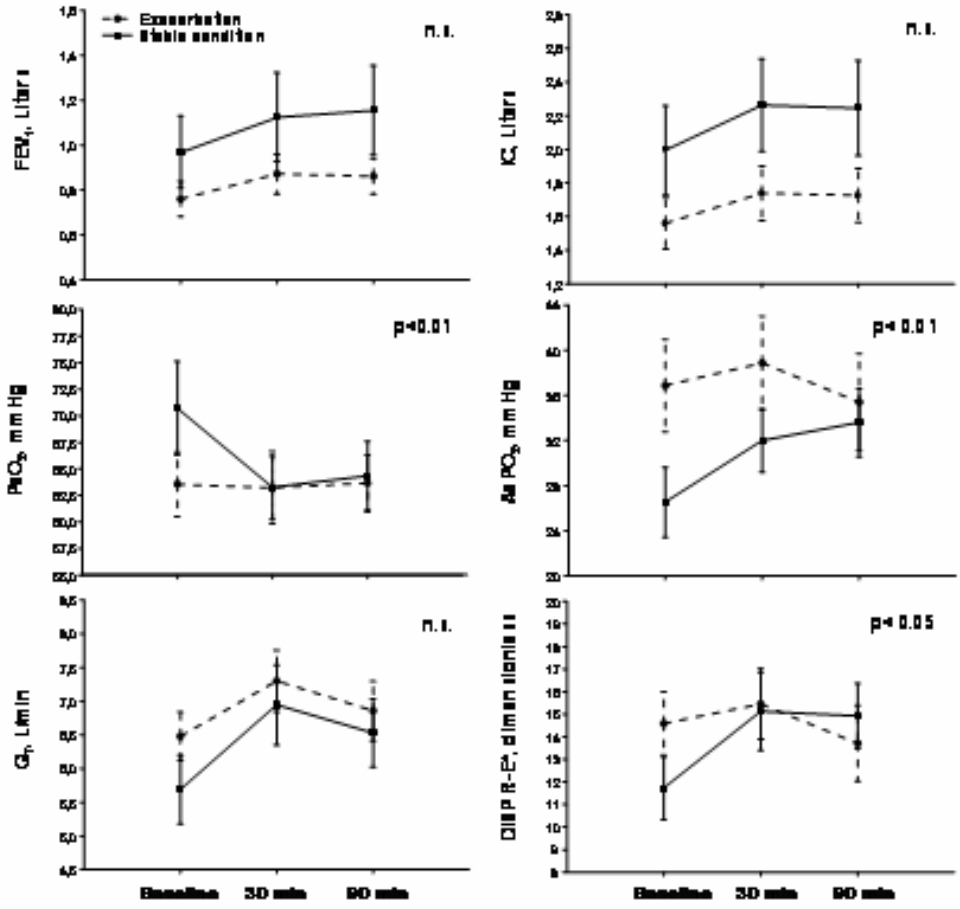
† Paired t-test analysis; (n= 7 for V_A/Q variables). Definition of the abbreviations: see Table 2.

Table 3. Effects of salbutamol during stable conditions (n= 9).

	Baseline	30 min	90 min	p†
FEV_1, L	0.97 ± 0.16	1.13 ± 0.20**	1.16 ± 0.20**	<0.01
FEV_1, % pred.	31 ± 4	36 ± 5**	36 ± 9**	<0.01
FVC, L	2.42 ± 0.25	2.57 ± 0.27**	2.62 ± 0.29**	<0.05
FEV_1/FVC%	40 ± 5	45 ± 5**	44 ± 5**	<0.05
IC, L	1.99 ± 0.26	2.26 ± 0.28**	2.24 ± 0.28**	<0.01
V_E, L/min	7.3 ± 0.5	7.9 ± 0.5	7.4 ± 0.4	NS
f, min^{-1}	16 ± 2	15 ± 1	16 ± 1	NS
PaO_2, mmHg	70.7 ± 4.4	63.2 ± 3.4**	64.3 ± 3.2**	<0.05
$PaCO_2$, mmHg	43.4 ± 1.8	43.0 ± 1.6	42.6 ± 1.7	NS
$AaPO_2$, mmHg	26.5 ± 3.1	32.0 ± 2.8**	33.6 ± 3.0**	<0.01
Q_T, L/min	5.7 ± 0.5	7.0 ± 0.6**	6.5 ± 0.5**	<0.01
VO_2, mL/min	211 ± 12	232 ± 11**	213 ± 14	<0.01
HR, min^{-1}	73 ± 4	85 ± 4**	82 ± 3**	<0.01
Ps, mmHg	96 ± 4	91 ± 4**	89 ± 4**	<0.05
SHUNT % Q_T	0.84 ± 0.44	1.36 ± 0.50**	1.32 ± 0.54**	<0.05
Low V_A/Q % Q_T	4.11 ± 2.43	8.06 ± 3.54	9.61 ± 3.92	NS
Log SDQ	0.96 ± 0.09	1.12 ± 0.13**	1.15 ± 0.13**	<0.05
Log SDV	0.91 ± 0.06	1.02 ± 0.07	0.96 ± 0.06	NS
High V_A/Q % V	1.39 ± 0.73	0.98 ± 0.71	0.89 ± 0.68	NS
Dead Space % V	32.80 ± 4.10	34.21 ± 3.63	35.56 ± 2.89	NS
DISP R-E*	11.35 ± 1.31	14.68 ± 1.48	14.14 ± 1.47	<0.01

† Repeated measures ANOVA; ** $p<0.05$, post-hoc analysis (paired t-test) compared with baseline; (n=8 for V_AQ data).
Definition of the abbreviations: see Table 1.

Figure 1. Compared effects of salbutamol during exacerbations and under stable conditions.



p-value, significance of two-ways ANOVA analysis

Conclusions and future perspectives

The main research work has been focused on the basic pathophysiology of gas exchange of two very common respiratory diseases, asthma and chronic obstructive pulmonary disease.

Particularly, research on bronchial *asthma* has been conducted through a model of bronchial challenge with adenosine 5'monophosphate that is considered today innovative as it can simulate a real asthma attack through the activation of indirect inflammatory and bronchoconstrictive patterns. In particular we wanted to investigate the old hypothesis of dissociation between large and medium sized airways, accounting for the obstructive spirometric abnormalities of asthma, and small airways, where inflammatory and remodelling processes can affect gas exchange efficacy (PaO_2, $AaPO_2$, V_A/Q balance) independently by the bronchomotor tone [1-2]. The existence of "a small airways disease" in asthma is an issue giving rise to an increasing interest of chest physicians. Unfortunately only MIGET studies of Wagner PD and Rodriguez Roisin R could give some scientific support to this hypothesis that remains difficult to detect as large airways tone influences the insight of pathological events affecting the more peripheral part of the lung. In addition, if we exclude few invasive methodologies analysing directly lung tissue (for instance, pulmonary biopsy), there are few possibilities to investigate pathophysiology of peripheral airways. A big contribution has been given by MIGET providing a picture of ventilation and distributions in functional lung units without interfering with broncho-motor and vascular tones. Even being a

difficult and not practical technique it gives, with the necessary experience and practice, a fundamental contribution to clinical research. Today we consider of great interest the investigation on small airways through the integration of MIGET with old and newer other techniques in order to obtain a better control and follow-up of the disease and to verify new therapeutical approaches. On this base a new research protocol on mild-to-moderate asthma has been proposed in order to study patients with intermittent symptoms, treated with combined inhaled therapy (β_2-agonists - corticosteroids), by using different techniques able to provide some functional and morphological information on pathological processes of small airways. In particular we plan to study:

1. *Flow-volume curves*, with particular attention at medium and low volumes (MEF and $FEF_{25-50-75}$];
2. *Closing volume*; an increased value of closing volume or closing capacity expresses the precocious collapse of lower parts of the lungs and seems to be a good marker of small airways obstruction even in presence of a normal FEV_1;
3. *Bronchial and alveolar production of NO*; exhaled NO is considered a good marker of airways inflammatory disorders and a recent mathematical model is able to discriminate the bronchial and alveolar production by applying different resistances at mouth.
4. *Respiratory gases and $AaPO_2$*, through the direct measurement of respiratory rate (VCO_2/VO_2);
5. *MIGET*;

6. *Pulmonary ventilation scan*. It analyses pulmonary ventilation distribution through the inhalation of TC_{99}-labelled micro-particles; the recent technique, developed in the Cardio-Thoracic Department of Pisa, avoids adequately spotting images due to large airways deposition of the particles and allows a good definition of more peripheral ventilated areas.

The aim is to evaluate the presence of V_A/Q and gas exchange abnormalities, which remain commonly unidentified, beside mild spirometric abnormalities and to correlate them with the other parameters considered, hoping to find a new definition of small airways diseases in asthma through the combination of new and old techniques and new parameters specific for small airways able to open to new follow-up and therapeutical approaches.

The other respiratory disease object of the present investigation with the contribution of MIGET is that heterogeneous group of clinical, functional and biological conditions called "*chronic obstructive pulmonary diseases*", including, basically, chronic bronchitis, emphysema and, according to some authors, chronic asthma when flow obstruction reversibility is lost.

Particularly, this clinical research experience has underlined the complexity of the interaction among intra pulmonary and extra pulmonary factors determining pulmonary gas exchange in COPD. Few methodologies can probably provide so complete information about pathophysiological mechanisms of a respiratory disease as MIGET. In addition, the model of study adopted in this work has two major advantages:

- it provides a better knowledge of β_2-agonists that are not only bronchodilators but also vasoactive and inotropic agents profoundly influencing, therefore, the general hemodynamic balance;

- it underlines the central role of pulmonary circulation and vascular tone regulation in pathophysiology of COPD, whereas the “vascular side” of respiratory diseases is often forgotten and airways abnormalities are uniquely considered.

On the base of this research experience on COPD a new research protocol is actually been considering, aimed at the investigation of COPD heterogeneity that remains, despite the enormous progresses made in this field, an unsettled issue.

Despite a universal definition of COPD, including all diseases characterized by poorly reversible airflow limitation [3], huge clinical, functional and radiological differences are described among individuals with COPD label and, more important, prognosis is diverse [4] and response to therapy may be different. COPD includes different specific phenotypes, chronic bronchitis (CB) pulmonary emphysema (PE) and, according to some authors, chronic asthma, when airways obstruction reversibility has disappeared. Major differences exist between asthma and COPD (CB/PE): clinical history, bronchial responsiveness, DLCO, biological markers and structural alterations of the lung usually permit to differentiate asthma from CB/PE, even for a similar degree of airway obstruction. A recent work of Fabbri et al. [5] highlights the importance of comparing asthma and chronic bronchitis patients of the same age class and with a comparable degree of airflow

obstruction. By using imaging (HRTC), functional (including salbutamol reversibility and methacholine responsiveness) and pathological techniques (exhaled NO, induced sputum, BAL, bronchial biopsy) the authors defined different phenotypes: patients with a history of asthma had more eosinophils in blood, sputum, BAL and airway mucosa, and a higher CD4/CD8 ratio of T cells infiltrating the airway mucosa. As expected, they also had less residual volume, higher DLCO, exhaled NO and reversibility to bronchodilator and steroids, and a lower HRTC-emphysema-score.

It is clear that a large variability exists also within the big group of patients with chronic bronchitis with or without emphysema, in symptoms (perception of dyspnoea, presence of cough and phlegm, etc.), time course, radiological aspects and systemic effects of the disease. Important functional differences are also observed in DLCO, residual volume and hyperinflation.

In addition, beyond the old definition of COPD subtypes of “blue brothers” and “pink puffer” that correspond to important pathological and functional differences, today it has become common to differentiate patients by systemic effects of COPD, on the basis of exercise capacity, body mass index and the involvement of peripheral muscles.

At the origin of such variability is probably the existence of different pathogenetic mechanisms, different factor risks and predisposing factors that are not easy to unravel. The literature on MIGET in COPD has put into evidence the existence of different patterns of V_A/Q distributions and has showed the intent to find some correlation between functional data and structure even with some difficulties but the combination of MIGET with more

modern radiological and biological techniques could give us much more information [6-8]

The coexistence of chronic inflammation and oxidative stress alterations at level of small airways, of obstructive bronchiolitis and of parenchymal destruction in a variable proportion, makes COPD pathology extremely heterogeneous [9-11]. On this line, a new project of investigation has been planned on COPD heterogeneity (functional, biological and radiological features) that integrates the information deriving from HRCT and functional tests with ventilation - perfusion study (MIGET), biological measurements and markers of systemic involvement.

The model of V_A/Q study before and after the administration of inhaled salbutamol, a bronchodilator and vasoactive agent, could help to investigate the interplay of intra-pulmonary and extra-pulmonary factors modulating gas exchange in different pathogenetic conditions.

The information deriving from the analysis of cell composition, inflammatory and oxidative stress markers in sputum and BAL (IL-8, TNF-a, NE, VEGF, MMP, NCA) and exhaled breath condensate (EBC) (8-isoprostane, MDA, LTB4, cys-LT) and from exhaled NO (bronchial and alveolar fractions) is fundamental to investigate the chronic inflammatory process causing airway remodelling, narrowing of small airways and loss of lung elastic recoil, and could have important therapeutical implications.

Three classes of COPD patients could be selected, similar for age (55 - 65 years) and airway obstruction (FEV_1: 50 - 70% of predicted), by clinical history and radiological aspects: chronic asthma, chronic bronchitis with and

without emphysema. Possible targets of investigation are: the effects of inhaled salbutamol on gas exchange and ventilation/perfusion relationships; differences among the three classes of patients by the analysis of sputum, BAL, exhaled air and blood samples. The correlation among basal measurements of pulmonary function, gas exchange, inflammatory and oxidative stress markers can also be evaluated. The peripheral involvement could be evaluated through 6 min walking test and the evaluation of free fat mass (bio-electric impedance).

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