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Determination of lung capillary blood volume and membrane diffusing capacity in patients with COLD using the NO-CO method

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ABSTRACT: Lung capillary blood volume (Qc) and the membrane diffusing capacity (Dm) can both be determined from the combined measurement of nitric oxide (NO) and carbon monoxide (CO) transfers using the single-breath method. In ten healthy subjects, no difference was observed between the values of transfer factor of the lungs for carbon monoxide (TLCO) recovered after a 3 s or 9 s breath-holding time (tBH). The NO-CO method could thus be used with a short tBH and a low fraction of inspired nitric oxide (FINO) (8 ppm). However, in ten patients with chronic obstructive lung disease (COLD), the values of both transfer factor of the lungs for nitric oxide (TLNO) and TLCO were underestimated by around 20% at a short tBH (3 s). In COLD patients, the NO-CO method therefore requires a longer tBH and a higher inspired fraction of NO (30 ppm) than in healthy subjects. Similar values of Dm and Qc were obtained using the NO-CO method and the two-step conventional method, at two levels of the oxygenation. The former method gave less scatter. Furthermore, TLNO is independent of the fraction of inspired oxygen (FIO_2) and directly proportional to carbon monoxide membrane diffusing capacity (Dmco).

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In healthy subjects, lung capillary blood volume (Qc) and the membrane diffusing capacity (Dm) can be accurately measured by the nitric oxide-carbon monoxide (NO-CO) method [1, 2]. Dm and Qc are calculated by measuring the transfer capacities of two gases, nitric oxide (NO) and carbon monoxide (CO), with the single-breath method (SB). In previous experiments, we calculated both parameters using a short true breath-holding duration (tBH=3 s), and a low inspired fraction of NO (FINO=8 ppm) [1]. Borland and Higenbottam [2] employed a higher inspired fraction (40 ppm), allowing a greater duration of tBH (about 6 s). As the transfer factor of the lungs for NO (TLNO) is about five times greater than the transfer factor of the lungs for CO (TLCO), the alveolar concentration of NO (FANO), at the end of the breath-hold period is lower than that of FACO. The longer the tBH, the smaller the FANO and the less accurate the measurement. A compromise between FINO and tBH needs to be found for both healthy subjects and those with chronic obstructive lung disease (COLD). This study was designed to find out whether the NO-CO method could be applied to patients with COLD and, if so, under what conditions of tBH and FINO.

Methods

Experiments were performed in three groups of subjects: two groups of ten healthy subjects and ten patients with COLD.

Measurements of TLCO and TLNO were performed with the SB method. Measurements of gas volumes were made in a bag-in-box system connected to a dry spirometer. A rotating system of electronically controlled valves (Morgan, UK) connected to the subject enabled all measurements to be carried out in one manoeuvre. Helium (He) was used as the insoluble gas. He and CO were measured with Hewlett Packard analysers (USA), oxygen (O_2) with a Sensormedics OM-11 and NO by chemiluminescence (Thermoelectron, USA). The sensitivity of the NO analyser was 1% in the 10 ppm range. Calibration was performed prior to the experiment with a mixture containing 10 ppm NO in nitrogen (N_2) and air without NO. Linearity was checked before the first experiments were carried out by diluting the calibration gas with nitrogen using a Wösthoff pump.

After a full exhalation, the subject inspired the chosen mixture up to a volume close to his predetermined vital capacity, then held his breath for a predetermined time (tBH) and finally performed a rapid expiration. During this expiration a 900 ml "alveolar" sample was withdrawn after the wash-out of the first 900 ml. Inspired volume, inspired and alveolar fractions of CO and/or NO, He and O_2 were then measured. The delay between the preparation of the inspired mixture and the analysis of the alveolar gas was about 60 s. As we estimated the disappearance of NO in air or pure O_2 to 0.5 ppm and 1.5 ppm per h, respectively, the transformation of NO in other forms was not taken into account. The total breath holding time (tTOT) was taken as the sum of the inspired time, the true breath-holding time (tBH) and the expired time up to the start of the sampling period. TL and Dm were expressed in ml standard temperature and pressure dry (STPD)·min^{-1}·torr^{-1}, and Qc in ml. The following formulae [1] were used for calculating Dm and Qc:

$$Dm_{CO} = T_{LNO}/a$$

$$1/Qc = \theta_{CO}(1/T_{LCO} - a/T_{LNO})$$

where "a" is a coefficient taking into account the solubilities and the molecular weights of NO and CO.

Experiments

Healthy subjects

Two groups of ten subjects were studied.

First group. All were members of the staff with ages ranging from 24–43 yrs (5 men, 5 women). Two of them were light smokers and were asked to refrain from smoking on the day of the experiment. They performed three protocols successively: A, B, C. For a given subject each measurement was done on separate days whatever the protocol.

A) As a tBH over 3 s (tTOT about 5 s) would lead to a small fraction of NO in the alveolar sample (less than 10% of F_{INO}), this experiment was designed to check the effect of a reduction in tBH on the recovered values of T_{LCO}. T_{LCO} was measured twice for tBH of 9 s and 3 s in all ten subjects. The inspired mixture used for the SB test contained 21% O_2, 10% He, 2,800 ppm CO in N_2 (standard mixture).

B) The aim of the second experiment was to compute Dm and Qc with the NO-CO method using a short tBH (3 s). NO and CO transfers were determined simultaneously (one-step method). NO (8 ppm) was added to the standard mixture described above. Five measurements were performed on each subject.

C) In this experiment, O_2 and NO were added to the standard mixture in order: 1) to compute T_{LCO} at a high oxygenation level and calculate Dm_{CO} and Qc from the results of T_{LCO} in air (experiment A), and with the hyperoxygenated mixture (two-step method); 2) to assess the sensitivity of T_{LNO} to alterations in the level of oxygenation. The inspired mixture used for the SB test contained 60% O_2, 5% He, 1,400 ppm CO, 8 ppm NO in N_2. Five measurements were performed on each subject at a tBH of 3 s.

Second group. The aim of these experiments was to check the validity of the total breath-holding time chosen. The ten subjects (6 women and 4 men, aged 22–60 yrs) were asked to inspire at different rates and to hold their breath for different times. Each subject performed the experiment five times. tBH ranged from 0.6–5.0 s and tTOT from 1.8–8.6 s. The coefficient of variation of the recovered T_{LNO} values was calculated for each subject. These experiments were performed prior to all others (protocol D).

Patients

Ten patients, nine men and one woman, with a history of COLD and with permanent hypoxaemia (arterial oxygen tension Pa_{O_2} 44.3–70.5 torr) carried out three types of experiment; two with tBH of 8 s, one with the one-step NO-CO method with 30 ppm NO in the inspired mixture, the other without NO but with 60% O_2 along with CO and He. These two experiments enabled calculation of Qc and Dm using both the one-step NO-CO method and the two-step conventional method from the same parameters. The third experiment was performed using the NO-CO method with 8 ppm F_{INO} and a short tBH (3 s). The three experiments were carried out within a half day. A quarter of an hour was left between two measurements. As the carboxyhaemoglobin (Hbco) levels measured prior to the first experiment were low, the back pressure of CO was not taken into account to calculate T_{LCO}. Qc values were corrected according to a 150 g·l^{-1} haemoglobin (Hb) concentration, making the assumption that θ_{CO} is proportional to Hb concentration.

Statistical analysis

Means and their coefficient of variation, *i.e.* the ratio of standard deviation (SD) to the mean, were calculated. The data were compared using Student's t-test for paired samples.

Results

Healthy subjects

Protocol A. Reduction of the breath-holding duration did not affect T_{LCO} (t=0.451, p>0.5) or carbon monoxide transfer coefficient (Kco) (t=0.853, p>0.3). Individual values are shown in table 1.

Protocols B and C. Dm and Qc calculated from the two-step method or the NO-CO method were not significantly different (t=1.23, p>0.2 for Dm, t=1.35, p>0.2 for Qc) (table 2). However, the scatter in the calculated values of Dm and Qc was lower with the NO-CO method (differences in the coefficients of variations were 5.3±4.5

Table 1. – Effect of the breath-holding time on TLco and Kco in ten healthy subjects performing the SB method with either a 3 s or a 9 s true breath-hold

	tBH	TLco ml·min⁻¹·torr⁻¹ 3 s	9 s	d	Kco ml·min⁻¹·torr⁻¹·l⁻¹ 3 s	9 s	d
Name							
JM		32.9	29.2	3.7	6.89	5.96	0.93
MF		25.8	25.4	0.4	6.36	5.94	0.42
AML		21.3	21.6	-0.3	4.59	4.77	-0.18
HG		28.0	30.0	-2.0	5.73	6.24	-0.51
FP		24.7	25.4	-0.7	5.76	5.86	-0.10
MM		22.5	22.9	-0.4	5.02	5.03	-0.01
EA		23.1	22.5	0.6	6.17	5.72	0.45
EL		30.4	30.6	-0.2	6.57	6.52	0.05
CK		31.1	33.3	-2.2	6.09	6.59	-0.50
GM		35.0	30.9	4.1	7.69	6.90	0.79
mean				0.3			0.134
SD				2.1			0.499

Each value is the mean of two measurements. d: the differences between values of TLco or Kco for 3 s and 9 s tBH; TLco: transfer factor of the lungs for carbon monoxide; Kco: carbon monoxide transfer coefficient; SB: single-breath; tBH: true breath-holding time.

Table 2. – Dmco and Qc with the NO-CO method and the two-step method in ten healthy subjects

	NO-CO method Dmco m	SD/m	Qc m	SD/m	TLNO/TLco	Two-step method Dmco m	SD/m	Qc m	SD/m
Name									
JM	65.3	11.5	99.0	16.0	4.0	67.5	12.7	96.3	17.3
MF	64.1	3.5	75.8	3.2	4.6	59.8	8.1	80.0	14.9
AML	63.0	13.0	62.9	2.8	5.1	62.5	13.2	63.2	21.3
HG	63.9	9.8	88.4	10.2	4.2	75.7	19.2	78.0	13.0
FP	55.5	0.5	80.8	19.1	4.1	58.1	11.7	77.6	17.1
MM	51.7	6.5	64.7	11.8	4.5	49.7	5.1	66.9	14.2
EA	57.9	4.5	68.9	1.2	4.6	53.1	15.8	74.0	10.2
EL	64.2	2.3	100.6	15.2	3.9	62.9	7.9	102.5	5.3
CK	75.0	5.2	96.2	9.8	4.4	115.4	8.8	75.1	16.7
GM	69.4	6.3	117.6	6.1	3.8	77.6	13.4	105.2	18.3
mean	63.0	6.3	85.5	9.5	4.32	68.2	11.6	81.9	14.8
SD	6.7	4.0	17.0	6.1	0.397	18.8	4.2	14.5	4.5

Five measurements per subject per method were performed. Dmco: membrane diffusing capacity for carbon monoxide (ml·min⁻¹·torr⁻¹); Qc: lung capillary blood volume (ml); TLNO and TLco: transfer factor of the lungs for nitric oxide and carbon monoxide, respectively; SD: standard deviation.

and 5.3±8.2% for Dm, $p<0.01$ and Qc, $p=0.07$, respectively). As expected, the TLco values fell when the subjects inspired the mixture containing 60% O_2 (at 21% F_{IO_2}, TLco=28.7±4.3, at 60% F_{IO_2}, TLco=18.4±2.8). However, TLNO was essentially the same for both concentrations of O_2 in the inspired mixtures, 124.2±13.2 and 128.4±11.6 ml·min⁻¹·torr⁻¹ for 21% O_2 and 60% O_2, respectively ($t=2.0$, $p=0.07$).

Protocol D. No correlation was found between the total breath-holding time and the recovered value of TLNO in any subject. The coefficient of variations of TLNO ranged from 16–1.4% within subjects (mean 8%).

Patients

All of the patients were hypoxaemic and obstructive, some were hypercapnic and/or polycythaemic (table 3). Values of Dm and Qc obtained by the NO-CO and the two-step methods are listed in table 4. There were no differences between the parameters calculated by the two methods ($p=0.30$ and 0.20 for Dm and Qc, respectively). Table 5 gives the results obtained with the NO-CO method with either a long (8 s), or a short (3 s) tBH. Both TLNO and TLco were significantly different. The underestimations for TLco and TLNO with a short tBH were 21% and 19.3%, respectively. The percentage decreases were

Table 3. – Characteristics of the patients with chronic obstructive lung disease

	age yrs	height m	FEV_1/FVC %	Pa_{O_2} torr	Pa_{CO_2} torr	Hb $g \cdot l^{-1}$	Hbco %
Name							
LARL	46	1.76	68	52.5	47.3	177	0
LARC	70	1.65	43	60.7	38.3	175	1.8
GAR	74	1.70	51	63.0	42.8	159	1.5
DRU	65	1.70	61	51.8	46.5	166	2.2
RAV	56	1.60	67	44.3	58.5	196	1.5
BED	57	1.60	55	62.3	67.5	173	2.0
DUV	29	1.50	63	61.5	41.3	150	1.2
TOUJ	51	1.56	30	51.0	44.3	154	2.4
BID	64	1.65	69	59.3	46.5	160	1.1
DUM	50	1.53	53	70.5	46.5	163	1.2

FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; Pa_{O_2} and Pa_{CO_2}: arterial oxygen and carbon dioxide tension, respectively; Hb: haemoglobin; Hbco: carboxyhaemoglobin.

Table 4. – Comparison of the results obtained with the NO-CO method and the two-step method in patients with COLD

	NO-CO method Dmco	NO-CO method Qc	Two-step method Dmco	Two-step method Qc
Name				
LARL	32.0	39.5	35.2	36.9
LARC	36.1	41.6	35.1	39.4
GAR	23.2	42.2	22.4	43.6
DRU	33.9	47.0	34.9	45.2
RAV	40.8	25.8	47.8	23.9
BED	28.2	31.4	32.0	28.6
DUV	20.4	42.4	22.6	35.9
TOUJ	17.4	26.5	15.2	29.1
BID	17.8	33.9	19.0	30.3
DUM	39.5	53.0	37.1	55.5
mean	28.9	38.3	30.1	36.8
SD	8.8	8.4	10.0	9.5

Dmco: membrane diffusing capacity for carbon monoxide; Qc: lung capillary blood volume; SD: standard deviation.

Table 5. – TLCO and TLNO values with a short (3 s) and a long (8 s) true breath-hold period in COLD patients

	tBH 3 s TLCO	tBH 3 s TLNO	tBH 8s TLCO	tBH 8s TLNO	TLNO/TLCO
Name					
LARL	10.0	47.6	15.3	63.2	4.1
LARC	12.6	57.7	16.5	71.1	4.3
GER	9.5	33.8	12.7	45.7	3.6
DRU	12.5	44.2	16.6	66.7	4.0
RAV	12.1	71.3	13.9	80.3	5.8
BED	11.0	50.0	12.5	55.5	4.4
DUV	9.5	35.0	11.4	40.2	3.5
TOUJ	5.5	22.8	8.5	54.2	6.4
BID	8.1	28.9	9.9	35.0	3.9
DUM	17.5	74.4	18.8	77.8	4.1
mean	10.8	46.6	13.6	57.0	4.43
SD	3.2	17.33	3.2	5.5	±0.927

tBH: true breath-holding time; TLCO and TLNO: transfer factor of the lungs for carbon monoxide and nitric oxide, respectively; SD: standard deviation.

similar for both transfer capacities. The scatter of the TLNO/TLCO ratio is greater in COLD patients (±0.927) than in healthy subjects (±0.397, table 2).

Discussion

The conventional two-step method for determination of Dm and Qc relies on the competition of the binding of O_2 and CO to haemoglobin. The values of Qc and Dm obtained in the present study using the NO-CO method were not significantly different from those obtained with the two-step method for both 8 s and 3 s tBH in healthy subjects. However, in the patients with COLD, there was only agreement at the 8 s tBH. The 20% underestimations in TLCO and TLNO with a 3 s tBH in the COLD patients was attributed to the airway obstruction in these patients [3]. With a 3 s tBH, tTOT is about 5 s, but with 8 s tBH, tTOT is around 10 s. In normal subjects, the decay in the alveolar concentration of CO is known to be mono-exponential in the first 30 s of the manoeuvre [2, 4]. In this condition, a short tBH can be used to calculate TLNO and TLCO with sufficient accuracy.

In patients with obstructive respiratory diseases, impairment in gaseous diffusion due to enlargement of the alveolar spaces is thought to slow down the transfer of CO to the alveolar capillary membrane [3]. A much longer tBH is therefore needed to obtain gas transfer values that are independent of breath-holding time. However, it has been reported that there is a difference between the values of TLCO recovered after 5 s and 10 s tBH in emphysematous patients [3]. Transfer measurements and, hence, calculation of Dm and Qc are thus not straightforward in these patients. The NO-CO method being a one-step method is likely to reduce the scatter of the lung transfer capacity values due to changes in the distribution of the inspired gas when two successive manoeuvres are needed to calculate Dm and Qc. Furthermore, NO and CO have very close molecular weights, therefore their distributions within the lungs should be the same. Nevertheless the NO-CO method, as the conventional one, remains sensitive to the distribution of alveolar ventilation perfusion rate ($\dot{V}_A/\dot{Q}$).

TLNO is nearly equal to the product Dmco·a where "a" is a constant coefficient. Furthermore, in agreement with BORLAND and HIGENBOTTAM [2], we did not find any significant alteration in TLNO when the alveolar concentration in O_2 was changed. Therefore, TLNO, whatever the FIO_2, depends only on the membrane diffusing capacity. This shows that measurement of TLNO should be suitable for both experimental and clinical purposes. It may be surprising that TLNO only reflects DmNO. As gas exchange occurs in perfused area, DmNO and Qc should be related. However, DmNO depends on the thickness of the alveolar-capillary barrier as well as on its surface which are not both necessarily linked to Qc. Therefore, the ratio TLNO/TLCO should be more scattered in patients than in healthy subjects. We found, indeed, that the standard deviation of this ratio was 0.927 in patients and 0.397 in healthy subjects. However, the averages were not different, 4.43 and 4.32, respectively, suggesting that the reduction in Qc and Dm in COLD patients, as a whole, is similar.

There are several ways of measuring tTOT [5]. We chose the classic Ogilvie method on the basis of the absence of correlation between the total breath-holding time and the recovered value of TLNO in the normal subjects performing protocol D. As the coefficient of variation of TLNO can be as low as 1.4% using the Ogilvie method, it seems unlikely that another method could lead to better results.

Finally we should stress the complete absence of toxicity of NO under our experimental conditions. Short exposures to high inspired concentration (200–700 ppm) can lead to death [6], and longer exposures to 60 and 100 ppm can lead to irritation of mucous membrane or even acute respiratory distress [7]. The maximum allowed concentration for NO in the atmosphere of industrial buildings, 8 h a day for 5 days per week, is 25 ppm. VON NIEDING *et al.* [8] found no effect on gas exchange during 15 min exposure to 15 ppm NO. PaO_2, arterial carbon dioxide tension ($PaCO_2$), pH, $DaAO_2$, TLCO remained normal. The levels used in the present experiment were well below the threshold of toxicity. One vital capacity of 30 ppm NO is about a hundredth of the volume of NO given to the subjects in the study of Von Nieding *et al.*

In conclusion, the NO-CO method enables computation of both Dm and Qc in a one-step manoeuvre. It also reduces the scatter in the results. TLNO is directly proportional to Dmco and independent of FIO_2. These features of the method and its potential interest have recently been reviewed by MEYER and PIIPER [9]. In healthy subjects or patients without obstructive lung disease, the method can be used with a low NO concentration (8 ppm) and a low breath-holding time (3 s). In COLD patients, with an 8 s breath-holding time and 30 ppm NO in the inspired mixture, the NO-CO method, like the two-step method, gives higher values of Dm and Qc than those obtained with a shorter breath-holding time. In our experience, and in agreement with other published studies, the method appears completely innocuous and could, thus, be adopted clinically without exposing patients to unnecessary risks.

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Détermination du volume capillaire pulmonaire et de la capacitéde diffusion de la membrane chez les patients atteints de BPCO, par la méthode NO-CO. J. Moinard, H. Guenard.

RÉSUMÉ: Le volume sanguin capillaire pulmonaire (Qc) et le facteur de membrane (Dm) peuvent être déterminés tous les deux par la mesure combinée du transfert de NO et de CO, grâce à la méthode en respiration unique. Chez 10 sujets bien portants, l'on n'a observé aucune différence entre les valeurs de TLCO obtenues après 3 ou 6 secondes d'apnée (tBH). La méthode NO-CO pourrait donc être utilisée avec un tBH court et une faible fraction de NO inspiré (FINO) (8 ppm). Toutefois, chez 10 patients atteints de bronchopneumopathie chronique obstructive (BPCO), les valeurs, tant de TONO que de TLCO, sont sous-estimées d'environ 20% lorsque tBH est court (3 secondes). Chez les BPCO, la méthode NO-CO exige dès lors un tBH plus long et une fraction inspirée plus élevée de NO (30 ppm) que chez les sujets normaux. Des valeurs similaires de Dm et de Qc ont été obtenues avec la méthode NO-CO et avec la méthode conventionnelle en deux étapes, à deux niveaux d'oxygénation. La méthode ancienne donnait moins de dispersion. De plus, TLNO est indépendant de FIO_2 et directement proportionnel à Dmco.

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