

# EVALUATION OF THE INTRA-BREATH METHOD FOR THE MEASUREMENT OF CARBONMONOXIDE TRANSFER FACTOR IN ASTHMA†

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## SUMMARY :

**Purpose:** Singlebreath (SB) transfer factor ( $TF_{SB}$ ) may be normal or increased in asthma but the mechanism of the elevated TF is unclear. Both technical and physiological explanations have been proposed. On the other hand, previously described intrabreath (IB) method, based on absorption of CO gas during a constant exhalation is applied to determine TF even in patients with airflow limitation. The aim of this study is to compare the IB technique with SB in asthma. **Methods :** The study population consisted of 70 patients with asthma and 45 healthy individuals. All subjects were nonsmokers. TF (ml/min/mm Hg) and TF/VA values were measured by both SB and IB methods in all subjects. To assess whether the accuracy of the IB method changed over the expected clinical range of FEV<sub>1</sub> predicted, the agreement between measured variables was tested over four ranges. **Results :** When measured with the IB method, the group with asthma showed significantly lower TF ( $p<0.001$ ) and TF/VA ( $p<0.001$ ) values than the normal subjects (expressed as a percent of predicted value). When measured with the SB, TF and TF/VA values were similar in asthmatics and normal subjects ( $p>0.05$ ). On the other hand in the IB tests, significantly lower TF and TF/VA values were observed in the asthma group than in the tests with SB method ( $p<0.05$ ). In contrast, TF and TF/VA values measured with IB and SB methods were not significantly different in healthy subjects ( $p>0.05$ ). When we compared two methods we found higher bias and precision value and wider limits of agreement in patients with asthma than in the reference group. The relationship between the two methods was not uniform across the tested range. Stratification of the TF data according to FEV<sub>1</sub>% affected the bias, precision, and limits of agreement. **Conclusion:** These results suggest that IB methods underestimate TF and TF/VA values in patients with asthma but not in normal subjects.

**Key Words:** Asthma, Transfer Factor, Intrabreath, Singlebreath.

## INTRODUCTION

There are several methods for clinical measurement of transfer factor of the lung for

carbonmonoxide, but the single-breath transfer factor is usually used in clinical practice. Studies of the TF in bronchial asthma have yielded conflicting results: decreased, normal, or increased values

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have all been reported (1-3) Furthermore, measurement with steady state, rebreathing and single breath techniques have led to discordant results.

Both technical and physiological factors have been invoked to explain this discordance. Some of the methodological explanations put forward have related to the effects of prolonged expiration and include errors in the estimation of diffusing time, timing and volume of alveolar gas sampling and measurement of alveolar volume (VA). These factors become important when there is significant inspiratory and/ or expiratory airflow limitation and ineffective gas mixing. Physiological explanations of the elevated TF in asthma include increased perfusion of the apices, increased pulmonary capillary blood volume (as a result of inspiration through obstructed airways) and increased membrane diffusing capacity, resulting from hyperinflation.

The steady state carbonmonoxide diffusing capacity is generally low in asthma, reflecting the known abnormalities in the distribution of ventilation and perfusion in these patients. In contrast, the single breath transfer factor is often elevated in asthma. The common factor causing the increased TF in these patients may be the degree of obstruction of their airways. Studies dealing with single breath technique in asthma demonstrated that, TF measured by the SB technique was normal or greater than normal when limitation to airflow was greatest and decreased toward normal as airflow improved (1).

Intrabreath is another measurement technique of TF and is not new (4-6). After development of rapid infrared gas analyser IB has been the more widely applied method today. Since this technique is based solely upon exhalation, anomalies associated with inspiration and breath holding do not affect results. Additionally, because prolonged breath holding is not required; measurements can readily be made in dyspnoeic patients. Regardless of these advantages, this method is greatly influenced by changes in the distribution of the inspired gas and the sequence of lung emptying (7, 8). For this reason the use of this method is not recommended in patients with airflow limitation. In contrast, recent studies demonstrated that, IB method can safely be used in this patient population (9, 10).

The present study is designed to compare IB and SB methods, by measuring the transfer factor in patients with asthma and healthy controls.

## MATERIALS AND METHODS

### *Study population:*

Forty-five healthy individuals and seventy asthmatic patients were studied. All asthmatics met the diagnostic criteria of NHLBI (11). None of the patients and control subjects had other disease. None were smoker or exsmoker. Great majority of the asthmatics was in stable period of their disease. To assess the impact of the airflow limitation on  $TF_{IB}$  measurements, patients with asthma were divided into four groups according to  $FEV_1\%$  predicted: mild (70-100%), moderate (50-69%), severe (<50%), and normal (>100%)(12).

### *Measurements:*

All forced expiratory manoeuvre parameters, lung volume and TF measurements were performed with an integrated computerised infrared analyser (model 2200/SensorMedics, Bilthoven, Netherlands). The criteria established by ATS were met both in performance of the breath holding manoeuvre and for computation (13). Alveolar volume was obtained simultaneously by using methane as an inert tracer gas. The analyser had a 0-90% response time of less than 300 ms (including transit delay time) and could analyse samples with volumes as small as 10 ml. The sample flow of the analyser was 500 ml/min, and the dead space of the system was 80 ml, which had been corrected mathematically in the calculation of VA.

Volume and flow were measured by a heated wire device and calibrated daily using a 3 litre precision syringe. Before each test the analyser was zeroed against room air and calibrated using a test gas containing 3300 ppm carbonmonoxide, methane, and acetylene. The single breath TF tests were analysed using the visually adjusted method, where a washout volume adequate to clear the anatomic and apparatus deadspace of carbonmonoxide, as well as methane and a sample volume of 0.75 L (or 0.5 L if the patient's vital capacity was < 2.0 L) were set under visual control. This was accomplished by moving a cursor into the horizontal plateau of the graphical display of the gas concentrations on the monitor. Breath holding



time was set according to the Meade-Jones method including two thirds of the time of inspiration and the time of expiration up to halfway through the period of sample collection. Other characteristics of the instrument have been described elsewhere (14) Reference values were taken from the TF standards of the European Respiratory Society (ERS) for the calculation of TF predicted and TF/VA predicted (15). SB method was performed as defined by ATS (13). In the IB method the subject was asked to exhale to residual volume and this was followed by a rapid inhalation of dilute (0.3 percent each) CO, C<sub>2</sub>H<sub>2</sub>, and CH<sub>4</sub> to total lung capacity. After a brief breath hold (1 to 2 s), the subject then performed a slow exhalation at a relatively constant flow rate (0.3 to 0.6 L/s). Maintenance of a relatively constant expiratory flow was assisted by having the subject follow on-screen flow indicator. Gas concentrations were measured continuously during the exhalation period. TF was calculated using the regression method of Martonen and Wilson (16).

*Protocol :*

Tests were carried out on subjects in a relaxed seated posture maintained for 10 min. Priority of IB and SB measurements was determined randomly in all subjects. Between consecutive measurements there was an interval of at least 4 min. Hemoglobin correction was performed in all study populations

(17). TF<sub>IB</sub> was adjusted for maldistribution of ventilation using phase 3 slope of the CH<sub>4</sub>

*Statistical Analysis :*

Paired TF measurements were analysed using tests of bias, precision, and 95% limits of agreement (bias 2SD)(18). To assess whether the accuracy of the IB method changed over the expected clinical range of FEV<sub>1</sub>% pred, the agreement between measured variables were tested over four ranges. The individual ranges for FEV<sub>1</sub> were less than 50%(n= 5), 50- 69%(n=15), 70-100%(n=28), and more than 100%(n=22). The agreement of the values within a range was assessed and tested for significance. In addition to determine which parameters affect the IB measurements we analysed correlation coefficients(r) between FVC, FEV<sub>1</sub>, FEF<sub>25-75</sub>, FEV<sub>1</sub>/FVC, TF, TF/VA, TLC, RV/TLC values. Comparison of the TF, TF/VA, VA, IVC values measured by means of both methods in asthmatics and normal controls was performed using unpaired t test. Comparison of the two methods in the same group (asthma and controls) was made by paired t test.

**RESULTS**

The patients were instructed both before and during the tests, and most had no difficulty in achieving two reproducible measurements with

	ASTHMA(n= 70)		NORMAL(n=45)	
	absolute	predicted%	absolute	predicted%
F/M	23/47		15/30	
Age	32.33(111.52)		30.22(10.18)	
Height, cm	165.7(10.23)		168.09(8.51)	
Weight, kg	70.31 (11.92)		64.64(9.89)	
FEV <sub>1</sub> (% pred)	2.49(1.02) ;	85.34(22.81)	3.69(0.81) ;	102.08(12.15)
FVC(% pred)	3.56(1.22) ;	104.59(19.86)	4.64(0.87) ;	109.93(11.93)
FEV <sub>1</sub> /FVC	67.97(12.04)		79.53(9.36)	
PEF(%pred)	5.46(2.07) ;	77.04(20.64)	6.82(2.13) ;	80.44(20.26)
TLC(%pred)	5.47(1.22) ;	108.54(15.17)	6.38(1.26) ;	106.95(14.33)
FRC(%pred)	3.03(0.79) ;	110.45(23.02)	3.47(0.94) ;	114.81(25.80)
RV(% pred)	1.93(0.72) ;	121.97(41.04)	1.74(0.70) ;	109.44(41.63)
FEF <sub>25-75</sub> (%pred)	1.88(1.26) ;	47.97(27.83)	3.73(1.44) ;	84.38(27.19)
N2 delta	17.26(8.22)			

Table 1 : Patients' demographics and PFT results [mean (SD)].

	Asthma n= 70	Normals n= 45	95% CI
TF <sub>IB</sub> (% pred)	80.79 (21.33)	94.96 (13.31)	(-21.22 ; -7.11)
absolute	21.28 (7.53)	28.90 (5.64)	
TF/VA(%pred)	72.04 (15.35)	81.67 (10.75)	(-14.84 ; -4.42)
absolute	4.43 (0.88)	5.10(0.73)	
VA	4.72 (1.49)	5.71 (1.14)	(-1.51 ; -0.48)
IVC	3.23 (1.14)	4.23 (0.87)	(-1.39 ; -0.60)

Table 2 : Comparison of the TF, TF/VA, VA and IVC values in patients with asthma and normal individuals measured by IB method. [mean (SD)].

both techniques. Patient demographics and PFT results were shown in table 1.

The comparison of the means between the asthmatics and the controls showed a significant difference with lower values in the obstructive group for TF (95% CI: -21.22; -7.11) and TF/VA (95% CI: -14.84; -4.42) when measured with the IB method (expressed as a percent of predicted) (Table 2). The other tests, including those obtained with the SB method (percent of predicted), showed no significant difference between the normal individuals and asthmatics (Table 3).

(p< 0.001).

The mean of the differences between methods (bias)  $\pm$  the limits of agreement are shown in Table 4 and figure 1. The relationship between the two techniques was not uniform across the tested range. Stratification of the TF data according to FEV<sub>1</sub> % affected the bias, precision and limits of agreement values.

The slope of the single breath nitrogen exhalation curve was recorded in 19 patients. There was no correlation between single breath nitrogen and the difference between TF<sub>IB</sub> and TF<sub>SB</sub>

	Asthma (n=70)	Normals (n=45)	95 CI%
TF <sub>SB</sub> (% pred)	91.14(21.65)	92.16(15.45)	(-8.39 ; 6.36)
absolute	23.76(6.97)	27.96(5.85)	
TF/VA(% pred)	86.89(18.21)	84.49 (12.33)	(-3.73 ; 8.52)
absolute	5.37(1.03)	5.27 (0.79)	
VA	4.44(1.35)	5.34(1.01)	(-1.37 ; -0.44)
IVC	3.23(1.18)	4.27(0.85)	(-1.43 ; -0.62)

Table 3. Comparison of the TF, TF/VA, VA and IVC values in patients with asthma and normal individuals measured by SB method. Mean (SD).

#### Asthma Group :

In the IB tests significantly lower TF (95% CI: -4.91; -0.06) and TF/VA (95% CI: -1.27; -0.63) values were observed in the asthma group than in the tests in the SB method (pred %). The VA and IVC values showed no significant differences between the two methods in asthmatics.

On analysing the correlation coefficients, a strong linear relationship was found between the IB and SB methods for all indexes measured. TF(r=0.85), TF/VA(r= 0.70), IVC(r=0.99), VA(r= 0.94),

Agreement Bias limits	
Normals	0.94 $\pm$ 3.78
Total asthmatics	-2.49 $\pm$ 4.02
<b>Stratified sample</b>	
<b>FEV<sub>1</sub> %</b>	
FEV <sub>1</sub> > 100% (n= 22)	1.69 $\pm$ 4.49
70-100% (n= 28)	2.51 $\pm$ 3.93
50-69% (n=15)	3.29 $\pm$ 4.00
<50% (n=5)	3.45 $\pm$ 2.47

Table 4 : Agreement for ranges of FEV<sub>1</sub> in patients with asthma.



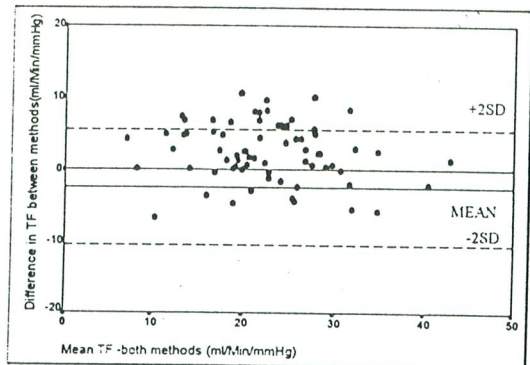


Fig - 1 : Difference between the methods plotted against their mean in asthma.

TF	FVC	FEV <sub>1</sub>	FEF <sub>25-75</sub>	PEF	N <sub>2</sub> delta	TLC	RV/TLC
IB	0.75*	0.69*	0.50*	0.69*	-0.06	0.63*	-0.55*
SB	0.67*	0.59*	0.44*	0.56	-0.09	0.57*	-0.46*

\* p< 0.0001

Table 5 : Correlation coefficients between the TF<sub>IB</sub>, TF<sub>SB</sub> and PFT variables in asthma.

(r=-0.02, p= 0.94). When we assessed the impact of airflow limitation or unequal ventilation on TF and TF/VA values; we found significant correlations between TF and FEV<sub>1</sub> (r=0.69, p<0.001), FEF<sub>25-75</sub> (r=0.50, p< 0.001), PEF(r=0.69,

p<0.001) but not between TF and N<sub>2</sub> delta. In addition there was a significant negative correlation between TF/VA and N<sub>2</sub> delta (r=-0.46, p<0.05) values (Table 5).

#### Control Group :

In the IB tests, higher TF and VA values were observed in the control subjects, but the difference was not significant. The IVC and VA values showed no significant differences between the two methods in normal individuals (Fig. 2).

On analysing the correlation coefficients, a strong linear relationship was found between the IB and SB methods for all indexes measured in this group. TF(r= 0.79), TF/VA(r= 0.64), IVC(r=0.99), VA(r= 0.91).

#### DISCUSSION

It is recognised for many years that the single-breath transfer factor of the lung may be normal or increased in asthmatic patients. On the other hand, Graham et al. demonstrated that conventional methods (Ogilvie, Jones and Meade and Ferris) overestimate TF<sub>SB</sub> when the expiratory flow rate is reduced and overestimation of TF<sub>SB</sub> increases as the amount of airway obstruction is augmented (1). For this reason, in this study we aimed to assess the IB method by comparing it against the widely used SB method in asthma. In contrast to previous reports, TF<sub>IB</sub> is found significantly lower in patients with asthma compared to healthy subjects, while TF<sub>SB</sub> gave comparable results in both groups. This finding supports the hypothesis that technical differences in the measurement of TF might markedly affect measurement results in asthma. Ohman et al demonstrated that the TF<sub>SB</sub> could be above normal at a time when the steady state TF was less than normal in asthma exacerbation (2). Another study compared

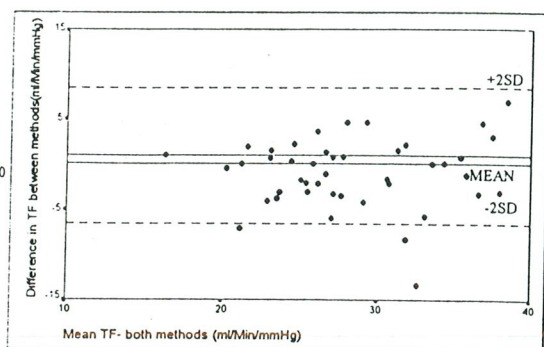


Fig - 2 : Difference between the methods plotted against their mean in reference group.

rebreathing method with SB to measure TF in unequal ventilation and found that the SB measurements overestimate TF with increasing unequal ventilation (19). In his study Haydu concluded that the steady state method of determining TF gave values which were higher than those obtained from the single breath method but was unable to demonstrate that TF was higher in asthma compared to healthy individuals (20). On the other hand, consistent with our findings, Kiss et al. recently reported that when measured with the IB method, the group with airway obstruction showed lower TF and TF/VA values than the group without obstruction (10). In contrast to these studies, Wilson et al. observed very comparable results with IB method in patients with and without airway obstruction (9).

One of the possible explanations for the lower values of diffusing capacity with IB in this study is the significantly lower alveolar volume in the asthmatic subjects. In normal subjects, TF decreases and TF/VA increases when VA is decreased. Consequently, TF is lower and TF/VA is higher at lower lung volumes compared with reference values estimated at TLC. Thus, lower IB values in asthmatics compared to normal subjects may be due in part to the effects of lung volume on TF (21). In our study population, asthmatics had significantly lower IVC and VA values compared to normal individuals with both methods. This may adversely affect  $TF_{IB}$  because while  $TF_{SB}$  is measured at total lung capacity,  $TF_{IB}$  is measured in a range of volumes below total lung capacity (thus at a lower mean lung volume).

Theoretically, diffusion inhomogeneity causes a decrease in  $TF_{IB}$  with decreasing lung volume, but the slope of the disappearance of CO is also markedly affected by changes in the distribution of the inspired gas and the sequence of lung emptying. Consequently, we adjusted  $TF_{IB}$  for maldistribution of ventilation using phase 3 slope of the  $CH_4$  curve. Data from lung modelling and clinical patient testing have shown the unreliability of  $TF_{IB}$  in the presence of significant nonuniformity of ventilation and/or diffusion (22). Even with the simultaneous use of an insoluble gas such as helium or  $CH_4$  with CO the effects of ventilation and/or diffusion inhomogeneity may not be totally compensated (7, 23). Thus in patients

with significant obstructive lung diseases, the absolute values of  $TF_{IB}$  may be misleading. To investigate the mechanism of lower  $TF_{IB}$  values compared to  $TF_{SB}$  in asthma we analysed correlation coefficients between  $TF_{IB}$  and expiratory flow indices, lung volumes and  $N_2$  delta. Results showed that FVC,  $FEV_1$ , PEF,  $FEF_{25-75}$ , TLC and RV/TLC, values significantly and highly correlated with  $TF_{IB}$  than  $TF_{SB}$ . However the degree of unequal ventilation measured as  $N_2$  delta was not related to TF. These results suggested that  $TF_{IB}$  might have highly affected airflow limitation and lung volume. In their study Kiss et al reported that an index of gas mixing indicated a better distribution of the inspired air for the IB method than for the SB method and as expected, a better distribution was observed in the nonobstructive group compared to the obstructive group (10). But they did not analyse the relationship between distribution and TF. On the other hand Wilson et al. did not find any correlation between singlebreath nitrogen and the differences between,  $TF_{SB}$  and  $TF_{IB}$  (9).

Another possibility for the lower  $TF_{IB}$  values in asthma may be overestimation of the TF with SB. The mechanism for the increased  $TF_{SB}$  may, in part, be an artefact because conventional methods overestimate  $TF_{SB}$  when the obstruction in the airways is sufficient to lower the exhaled flow rate and prolong expiratory time (1). Another explanation is that intrinsic airflow obstruction during inspiration, which created much higher negative inspiratory pleural pressure, could raise  $TF_{SB}$  in asthma by pulmonary capillary recruitment. Alternatively, it has been suggested that the increase in  $TF_{SB}$  may be caused by pulmonary capillary recruitment in the apex of the lung in the seated position secondary to hypoxic vasoconstriction in poorly ventilated zones.

On the other hand assessment of difference between the two measurement techniques showed that, over the flow rates tested, IB was linear although tended to underestimate the TF in asthma. When assessing the agreement of the methods under study at different ranges of flows estimated by  $FEV_1$ , it was found that bias increased with



decreasing flow rates. This may be due either to increased overestimation of SB technique or to increased underestimation of IB technique, or both, with increasing airflow limitation. However, the limits of agreement between the two methods are wide (ie. the bias between them is not consistent) and the difference between their measurements for TF and TF/VA is significant in asthma. Thus, they can not be used interchangeably and results obtained using one can not be compared directly with those obtained by the other.

On the other hand, the present study shows good agreement between the TF, TF/VA, VA and IVC values as measured by IB and SB methods in the control group. The limits of agreement between the two methods are narrow and the difference between their measurements for TF, TF/VA, VA and IVC is not significant.

These findings lead us to conclude that one factor causing the difference between the two measurement methods is the airflow limitation in asthma. The magnitude of this effect will be variable depending on the severity of obstruction. This effect should be considered when interpreting the results of the test for TF in patients with asthma.

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