

Clinical Study

Combined Lung Transfer of NO and CO in Patients Receiving Methotrexate or Bleomycin Therapy Compared to Normal Subjects

Chantal Viart-Ferber,^{1,2} Sébastien Couraud,^{3,4} Frédéric Gormand,^{1,3} and Yves Pacheco^{3,4}

¹ Pulmonary Function Testing Department, Lyon University Hospital, 165 Chemin du Grand-Revoyet, 69 495 Pierre-Bénite Cedex, France

² UCBL1 FRE CNRS 3310, 7 Passage du Vercors, 69367 Lyon Cedex, France

³ Respiratory Diseases Department, Lyon University Hospital, 165 Chemin du Grand-Revoyet, 69495 Pierre-Bénite Cedex, France

⁴ Lyon Sud-Charles Mérieux Medical Faculty, Claude Bernard Lyon 1 University, 69600 Oullins, France

Correspondence should be addressed to Chantal Viart-Ferber; chantal.viart-ferber@chu-lyon.fr

Received 11 October 2012; Revised 26 November 2012; Accepted 24 December 2012

Academic Editor: Vincent Pialoux

Copyright © 2013 Chantal Viart-Ferber et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The first aim of the study is to determine whether combined lung diffusing capacities of nitric oxide (TLNO) and of carbon monoxide (TLCO) are accurate in the followup of patients receiving either methotrexate (MTX) or bleomycin (BLM). The second objective is to determine whether TLCO, TLNO, KCO, and TLCO/VI% (inspiratory volume expressed as percentage of predicted value) correlate better with the diffusing capacity of the membrane (Dm) and/or capillary lung volume (Vc). TLNO and TLCO were measured in three groups: 22 “normal” subjects (N group), 17 patients receiving MTX, and 12 patients treated with BLM. TLCO, TLNO, Dm, and Vc were much lower in the MTX and BLM groups compared to those of the N one. The ratio TLNO/TLCO was higher in the BLM group compared to that of the N group and compared to that of the MTX group. KCO correlated neither with Dc nor with Vc, whereas TLCO/VI% correlated significantly with both Dm and Vc. Combined measurement of TLCO and TLNO seems to be useful in the followup of patients receiving agents inducing lung toxicity and gives a good idea of the alveolar membrane and the capillary volume.

1. Introduction

Methotrexate (MTX), a folic acid antagonist, is widely used for the treatment of many autoimmune diseases such as rheumatoid arthritis (RA), psoriasis, or lupus erythematosus (LE). This agent acts as a folic acid antagonist and is known to be an effective anti-inflammatory agent. However, it is also known for its pulmonary toxicity which is independent of the dose delivered [1–7]. MTX-induced pulmonary toxicity is an unpredictable, unusual, and mostly reversible event. Nevertheless, it is a serious adverse effect, since it may be fatal, particularly in patients with psoriatic arthritis [1]. Its incidence ranges from 1% to 5% [2, 3], and its prognosis is usually favorable [4].

Bleomycin (BLM) is another agent known for its pulmonary toxicity [8–13]. This cytotoxic agent is successfully

used in the treatment of several malignancies such as germ cell tumors, lymphomas, and some squamous cell carcinomas. The most frequent adverse effects of BLM are interstitial pneumonitis (ILD), followed by pulmonary fibrosis. BLM-induced pneumonitis occurs in around 46% of patients treated with a BLM-based chemotherapy. This lung toxicity usually appears during treatment [13] but can also appear up to 10 years after the incriminated treatment. Mulder et al. studied a cohort of childhood cancer survivor who received a BLM-based chemotherapy. After a followup of 18 years, 44% of patients presented with a pulmonary function impairment including 39.9% with a decreased carbon monoxide diffusing capacity (DLCO) [8]. Both MTX and BLM increase the risk to develop a pulmonary interstitial disease. Two components of the pulmonary alveolar membrane are concerned: the alveolar membrane (Dm) and the pulmonary capillary volume

(Vc). Consequently, a strict pulmonary followup including pulmonary functional tests should absolutely be set up when MTX or BLM has been used in order to diagnose lung toxicity as early as possible [14].

Measurement of the gases transfers through the lung is one of the few tests enabling physicians to investigate alveolar function.

The single-breath carbon monoxide diffusing capacity (TLCO) is a well-known and helpful parameter used in many respiratory fields. [15–20]. TLCO is the product of two distinct but concurrent measurements during breath: the rate constant for carbon monoxide uptake from alveolar gas (KCO) and the alveolar volume (VA) [21]. Recently, by using Roughton and Forster's equations [21, 22], the combined measurement of TLCO and TLNO (nitric oxide diffusing capacity) allowed the measurement of two other parameters: the alveolarcapillary membrane conductance (Dm) and the pulmonary capillary blood volume (Vc) [23, 24]. Actually, in this model, carbon monoxide switches through the alveolar and capillary structures and is split into two resistances. The first one is for the alveolar membrane (Dm). The other one is for the blood reacting with the gas ($1/\theta\text{COVc}$, in which θCOVc is the red cell conductance at a particular concentration and Vc the capillary volume). The resulting equation is $1/\text{DLCO} = (1/\text{Dm}) + (1/\theta\text{COVc})$. Guenard et al. [24] reported measurements of Dm and Vc using TLNO and TLCO and considering θNO as an infinite value resulting in $\text{TLNO} = \text{DmNO}$. Therefore, the measurement of DLNO leads to the calculation of DmCO and finally of Vc.

Considering that the VA value depends on the performance of inspiratory muscles and of chest-lung mechanisms, the interpretation of $\text{KCO} = \text{TLCO}/\text{VA}$ should, therefore, be taken into account with caution, as suggested by many authors [21, 25, 26]. VA is measured during a single breath, from a volume diluted with helium and after subtracting an “estimated” anatomic space from the inspired volume (VI) [21]. VI is usually defined as the volume comprised between residual volume and maximal inflation. It is measured by the device also used for TLNO and TLCO measurements. Finally, a high TLNO/TLCO ratio has been suggested to be a reflection of a decrease in the thickness of the capillary membrane [27].

The aims of this study were to determine whether MTX and/or BLM have a specific toxicity either for Dm or for Vc compared to subjects not treated with such agents, and to determine whether KCO and TLCO/VI% (VI% = % of predicted VI) correlate better with Dm and/or Vc than KCO.

2. Methods

2.1. Studied Population. The patients treated in our hospital and who underwent functional pulmonary tests as part of their regular followup were included in this study. Three groups were studied: a “normal” control group ($n = 22$), a group of patient treated with MTX ($n = 17$), and a last a group of patients treated with BLM ($n = 12$). All subjects knew the aim of the study and gave their informed consent. The study was conducted in accordance with the declaration of Helsinki (1964) and was approved by the Internal Review

Board of the University of Lyon [11–20]. The control group was composed of patients who underwent pulmonary tests as part as their routine medical followup, and whom results were $100\% \pm 10\%$ of the normal predicted values. The MTX group included 17 patients: two with dermatomyositis, one with lupus erythematosus, three with rheumatoid arthritis, three with cutaneous psoriasis, two with psoriatic arthritis, one with scleroderma, one with inflammatory rheumatism, three with ankylosing spondylitis arthritis, and one with a chronic lymphoid leukemia before undergoing a bone marrow autograft. Each patient received MTX for at least one year and was treated for a mean of 1.94 ± 0.28 years. The BLM group included 12 patients: six suffering from a testis cancer, five from Hodgkin's lymphoma, and one who presented with an acute lymphoid leukemia (before bone marrow autograft). A mean of $5.8 (\pm 1.7)$ cycles of bleomycin-based chemotherapy were administered to these patients (min: 2–max: 9). Hemoglobin in these 12 patients was 106, 111, 146, 126, 87, 103, 151, 86, 11.5, 143, and 131 g/L.

2.2. Pulmonary Function Tests. Spirometry and plethysmography were performed using Bodybox 5 500 from Mediso cardiorespiratory instrumentation (Dinant, Belgium). In the whole cohort, the following parameters were measured: forced vital capacity (FVC), forced expiratory volume in one second (FEV1), FEV1/FCV ratio, and functional residual capacity FRC. TLCO and TLNO were measured simultaneously, as appropriate [25]. Patients were seated and wore a nose clip. A gas mixture containing 0.28% CO, 14% helium (He), and 21% oxygen (O_2) balanced with nitrogen (N_2) was mixed with a NO/ N_2 mixture (450 ppm NO in N_2 ; Air Liquide Santé, Vénissieux, France). The final concentrations of NO and O_2 in inspired gas were 40 ppm and 19.1%, respectively. The device was calibrated for gas fractions using automated procedures as appropriate (dilution of the NO mixture with the CO/He/ O_2 mixture in order to check the NO-analyzer linearity). The pneumotachograph was calibrated daily using a 2 L syringe as specified by the manufacturer. Patients breathed through a mouth bit committed to a single-use filter and connected to the pneumotachograph. At the right time, patients were requested to inspire the gas mixture in a rapid and deep inspiration followed by a brief (4 seconds) and full expiration. The first 0.8 L of expired gas was rejected and the following 0.6 L was sampled in a bag and then automatically analyzed for NO, CO, and He concentrations. This sample was analyzed for 35 seconds. VA during apnea was calculated using the He-dilution method. TLCO and TLNO measurements enabled physicians to determine Dm and Vc, according to Roughton and Forster's equations [21, 22]. TLCO/VA and VI were automatically calculated by the device. DLCO/VI% was determined from these two measures. The reference values for TLCO were those suggested by the European Coal and Steel Community (ECSC) in 1993. The values were then reviewed according to hemoglobin. The reference values for TLNO were those suggested by Aguilani et al. [25].

2.3. Statistical Analysis. The statistical analysis was performed with the statistical package for social sciences for

TABLE 1: Characteristics of the three studied groups.

	N group (<i>n</i> = 22)	MTX group (<i>n</i> = 17)	BLM group (<i>n</i> = 12)	<i>P</i>
Males (<i>n</i>)	11	6	10	NS
Females (<i>n</i>)	11	11	2	<i>P</i> = 0.04: BLM versus MTX, BLM versus N
Smokers (<i>n</i>)	4	7	7	NS
Age (yrs)	43.2 ± 1.8	48.6 ± 4.1	35.5 ± 3.1	<i>P</i> = 0.007 BLM versus MTX
Height (cm)	169.5 ± 2.4	163.5 ± 1.4	172.2 ± 3.3	<i>P</i> = 0.02 BLM versus MTX
Weight (Kg)	71.2 ± 3.2	72.1 ± 4.1	67.2 ± 4.2	NS

Values are given by mean ± standard error. *P* is calculated by ANOVA and Chi-square tests. NS: not significant.

TABLE 2: Pulmonary functional tests' results in the three studied groups.

	N group	MTX group	BLM group	<i>P</i>
FVC (%PV)	114.4 ± 2.3	98.3 ± 3.7	102.5 ± 5.9	0.002 MTX versus N and 0.04 BLM versus N
FEV1 (%PV)	107.7 ± 2.8	89.8 ± 3.1	89.3 ± 4.6	0.0002 MTX versus N and 0.0005 BLM versus N
FEV1/FCV (%PV)	97.5 ± 1.8	94.5 ± 2.4	90.5 ± 2.4	0.03 BLM versus N
FRC (%PV)	111.3 ± 2.8	104.9 ± 3.4	112.0 ± 4.9	NS

Values are given by mean ± standard error. *P* is calculated by ANOVA. NS: not significant. %PV: percentage of predicted value.

TABLE 3: CO and NO lung transfers' results in the three studied groups.

	N Group	MTX group	BLM group	<i>P</i>
TLCO (%PV)	88.1 ± 4.5	70.6 ± 1.7	67.2 ± 4.9	0.002 MTX versus N and 0.001 BLM versus N
TLNO (%PV)	90.9 ± 1.8	66.3 ± 1.7	66.1 ± 4.2	<0.0001 MTX versus N and <0.0001 BLM versus N
VA (%PV)	97.9 ± 4.2	87.5 ± 3.2	92.9 ± 4.2	NS
KCO (%PV)	95.4 ± 1.9	87.0 ± 3.6	75.2 ± 4.7	0.02 BLM versus MTX, <0.0001 BLM versus N, and 0.05 MTX versus N
VI (%PV)	113 ± 2.7	95.9 ± 3.7	97.3 ± 5.3	0.05 MTX versus N, 0.02 BLM versus MTX, and <0.0001 BLM versus N
TLCO/VI (VI%PV)	0.27 ± 0.02	0.21 ± 0.02	0.25 ± 0.02	0.02 MTX versus N
Dm (%PV)	91.0 ± 1.8	66.3 ± 1.7	67.0 ± 4.0	<0.0001 MTX versus N and <0.0001 BLM versus N
Vc (%PV)	90.7 ± 2.1	70.4 ± 2.2	68.3 ± 5.1	<0.0001 MTX versus N and <0.0001 BLM versus N
TLNO/TLCO	4.60 ± 0.04	4.77 ± 0.07	5.14 ± 0.16	<0.0001 BLM versus N and 0.007 BLM versus MTX

Values are given by mean ± standard error. *P* is calculated by ANOVA. NS: not significant. %PV: percentage of predicted value.

Windows 17th version (IBM Corp., New York, USA). The continuous variables are expressed with mean ± standard error (SE) and were compared between the three groups with an ANOVA test (age, height, weight, FVC, FEV1, FEV1/FVC, FRC, TLCO, TLNO, VA, KCO, VI, TLCO/VI%, Dc, Vc, and the TLNO/TLCO ratio). The nominal variables were compared using Chi-square tests (gender, smokers, nonsmokers, and exsmokers). The results were considered statistically different when *P* value was under 0.05. Correlations between KCO or TLCO/VI% in one hand and DM or VC in the other hand used simple regressions. For regression calculation, the three groups were pooled in order to have normal and pathological values.

3. Results

3.1. Characteristics of the Three Studied Groups. The main characteristics of the three studied groups are given in Table 1. As expected, the sex ratio was different between the three groups. The rate of smokers was not significantly different in the three groups. The BLM group showed a younger mean age than the MTX group. The mean height was greater in

the BLM group than in the MTX one. The weight was not significantly different between the three considered groups.

3.2. Physiological Functional Tests' Results. The results of the physiological functional tests are shown in Table 2. FVC was lower in the MTX and BLM compared to the normal group. FEV1 was lower in MTX and in BLM groups compared to the N group. FEV1/CV was lower in BLM group compared to the N one (*P* = 0.03). No significant difference was observed between the MTX and the N groups regarding FRC.

3.3. Results of CO and NO Diffusing Capacities. The results of CO and NO diffusing capacities are reported in Table 3. TLNO was lower in the MTX (66.3% ± 1.7%) and BLM (66.1% ± 4.2%) groups compared to the N one (90.9% ± 1.8%). VA was not significantly different in the three groups. KCO was lower in the MTX and BLM groups compared to the N one and between the BLM and the MTX groups. TLCO/VI% was significantly reduced in the MTX compared to the N group only. Dm and Vc were significantly reduced in both of the MTX and the BLM groups compared to

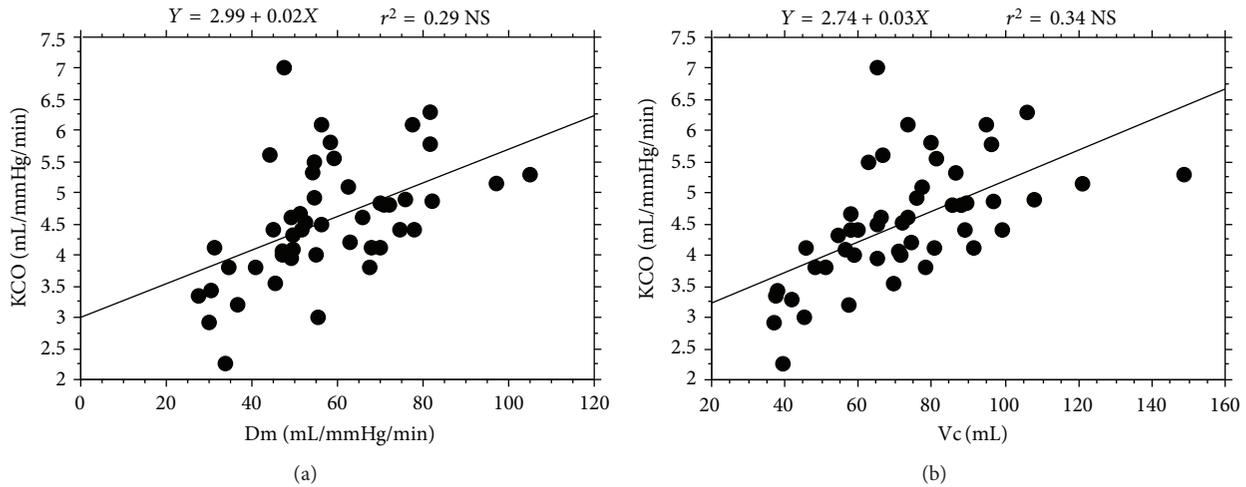


FIGURE 1

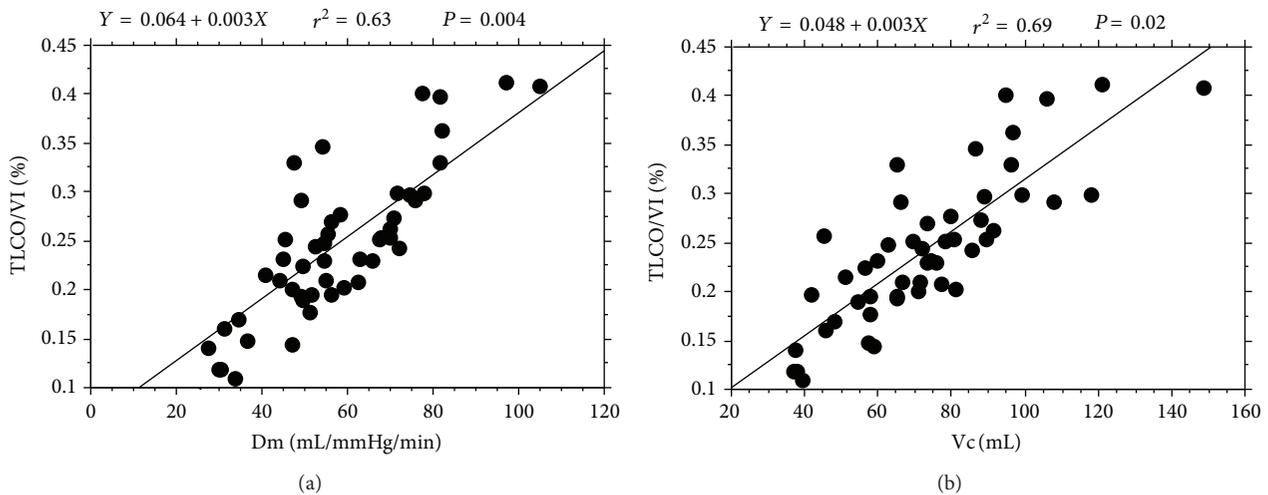


FIGURE 2

the N one. The TLNO/TLCO ratio was higher in the BLM group compared to the N group and compared to the MTX group. No significant difference was observed between the MTX and the N groups.

The results of the correlation between KCO and Dm and KCO and Vc are shown in Figure 1. KCO is correlated neither with DM ($r^2 = 0.26$, NS) nor with VC ($r^2 = 0.34$, NS).

By contrast, TLCO/VI% is significantly correlated with both DM and VC (see Figure 2) ($r^2 = 0.63$, $P = 0.004$ and $r^2 = 0.63$, $P = 0.02$, resp.).

4. Discussion

The difference observed in sex ratios may be explained by the type of pathology treated in the patients included in both groups such as testis cancer in the BLM group ($n = 6$) or autoimmune disorders (affecting mainly women). The younger mean age in the BLM group may be explained similarly (testis cancer and Hodgkin lymphoma affect mainly young adults).

Considering the first aim of the study, both MTX and BLM worsen FVC and FEV1, while FEV1/FVC ratio is worsened only in the BLM group, suggesting that BLM could cause more pulmonary obstruction than MTX. Both NO and CO diffusing capacities are decreased, much more in the BLM group. However, in animals, BLM-induced lung injury and fibrosis are known to be more serious in males than in females [28]. So, the greater proportion of females in the N and MTX groups could induce a bias. In further studies of this kind, it should be relevant to stratify experimental groups on sex and age.

In order to avoid MTX-induced pulmonary toxicity, the lowest dose of MTX should be delivered. Actually, a low dose of MTX (7.5 mg/week) proved to be efficient and to induce fewer adverse effects in patients suffering from rheumatoid arthritis [29].

KCO is more strongly decreased in the BLM group. Both Dm and Vc are reduced by MTX and BLM. These results match the literature data [1–3, 7, 8, 13]. However, in sarcoidosis, the limitation of gas diffusing capacities is mainly

located in the membrane barrier, although recruitment of microvascular reserves is modestly impaired. These results suggest that in sarcoidosis, the thickening of the alveolocapillary membrane is the main factor inducing a decline of the gas diffusing capacities in lungs [30].

The TLNO/TLCO ratio is another critical point. Actually, it is only increased in the BLM group. This result suggests that BLM affects pulmonary capillary more than MTX does. As a matter of fact, this ratio has been suggested by some other authors to be reflective of a decreasing thickness of the capillary blood layer [27]. BLM could mimic this pattern. The TLNO/TLCO ratio is also known to increase after obstruction of the pulmonary artery in animals [31]. The authors of this work suggest that this rise may be the consequence of a better sensitivity of TLCO than TLNO in response to a local reduction in capillary blood flow.

Regarding the second aim of the study, KCO correlates neither with Dm nor with Vc in the pooled population. This observation may lead to a debate about the usefulness of this parameter to assess the alveolar membrane and capillary volume, as previously described [21, 25, 26]. This result could be explained by the way VA is calculated [21]. VA is calculated during the TLCO measurement and so expressed in standard temperature and pressure dry (STPD) conditions; while KCO calculation ($KCO = TLCO/VA$) is expressed in body temperature and pressure saturated (BTPS) conditions.

By contrast, the VI is directly measured by the device used for TLNO and TLCO measurements.

5. Conclusion

This study shows that TLCO/VI% correlates significantly with both Dm and Vc. This result suggests that this parameter could be taken into account to investigate pulmonary alveolar membrane impairment. Further studies are needed to confirm this observation. Several studies have demonstrated the great interest of a combined TLCO and TLNO measurement in many different diseases such as liver cirrhosis [27], idiopathic pulmonary hypertension [32], or chronic renal failure [33]. As far as we know, it has been poorly studied in patients receiving agents inducing lung toxicity such as MTX or BLM. This study shows that this technique is useful and confirms that combined TLNO and TLCO could be a relevant test and could be routinely performed as suggested by other authors [34].

Conflict of Interest

The authors declare no conflict of interests.

Authors' Contribution

C. Viart-Ferber and S. Couraud are contributed equally to this work.

Acknowledgments

The authors thank Emrah Arslan for his help in the redaction of the paper. They also would like to thank Hervé Truchet,

Henriette Brias, Sylvie Curtis, Laurence Gonnet, Chrystèle Guyot, Geneviève Lardelier, and Eliane Tranchard for their technical assistance.

References

- [1] N. Spinel, C. Ochoa, C. Saavedra et al., "Methotrexate-induced pulmonary toxicity in psoriatic arthritis (PsA): case presentation and literature review," *Clinical Rheumatology*, vol. 30, pp. 1379–1384, 2011.
- [2] H. Lioté, "Respiratory complications of new treatments for rheumatoid arthritis," *Revue des Maladies Respiratoires*, vol. 21, pp. 1107–1115, 2004.
- [3] K. Shidara, D. Hoshi, E. Inoue et al., "Incidence of and risk factors for interstitial pneumonia in patients with rheumatoid arthritis in a large Japanese observational cohort, IORRA," *Modern Rheumatology*, vol. 20, no. 3, pp. 280–286, 2010.
- [4] S. Imokawa, T. V. Colby, K. O. Leslie, and R. A. Helmers, "Methotrexate pneumonitis: review of the literature and histopathological findings in nine patients," *European Respiratory Journal*, vol. 15, no. 2, pp. 373–381, 2000.
- [5] L. Green, A. Schattner, and H. Berkenstadt, "Severe reversible interstitial pneumonitis induced by low dose methotrexate: report of a case and review of the literature," *Journal of Rheumatology*, vol. 15, no. 1, pp. 110–112, 1988.
- [6] D. Sáenz Abad, F. J. Ruiz-Ruiz, S. Monón Ballarín, J. Mozota Duarte, and A. Marquina Barcos, "Pneumonitis associated to methotrexate," *Anales de Medicina Interna*, vol. 25, no. 1, pp. 27–30, 2008.
- [7] H. Kameda, A. Okuyama, J. I. Tamaru, S. Itoyama, A. Iizuka, and T. Takeuchi, "Lymphomatoid granulomatosis and diffuse alveolar damage associated with methotrexate therapy in a patient with rheumatoid arthritis," *Clinical Rheumatology*, vol. 26, no. 9, pp. 1585–1589, 2007.
- [8] R. L. Mulder, N. M. Thinnissen, H. J. van der Pal et al., "Pulmonary function impairment measured by pulmonary function tests in long-term survivors of childhood cancer," *Thorax*, vol. 66, pp. 1065–1071, 2011.
- [9] E. F. Redente, K. M. Jacobsen, J. J. Solomon et al., "Age and sex dimorphisms contribute to the severity of bleomycin-induced lung injury and fibrosis," *American Journal of Physiology*, vol. 301, pp. L510–L518, 2011.
- [10] T. T. - Huang, M. M. Hudson, D. C. Stokes, M. J. Krasin, and S. L. Spunt, "Ness KK. Pulmonary outcomes in survivors of childhood cancer: a systematic review," *Chest*, vol. 140, pp. 881–901, 2011.
- [11] D. Jun, C. Garat, J. West et al., "The pathology of bleomycin-induced fibrosis is associated with loss of resident lung mesenchymal stem cells that regulate effector T-cell proliferation," *Stem Cells*, vol. 29, no. 4, pp. 725–735, 2011.
- [12] M. Usman, Z. S. Faruqui, N. ud Din, and K. F. Zahid, "Bleomycin induced pulmonary toxicity in patients with germ cell tumours," *Journal of Ayub Medical College Abbottabad*, vol. 22, pp. 35–37, 2010.
- [13] M. Tashiro, K. Izumikawa, D. Yoshioka et al., "Lung fibrosis 10 years after cessation of bleomycin therapy," *Tohoku Journal of Experimental Medicine*, vol. 216, no. 1, pp. 77–80, 2008.
- [14] A. K. Ng, S. Li, D. Neuberg et al., "A prospective study of pulmonary function in Hodgkin's lymphoma patients," *Annals of Oncology*, vol. 19, no. 10, pp. 1754–1758, 2008.

- [15] M. K. Ferguson, J. J. Dignam, J. Siddique, W. T. Vigneswaran, and A. D. Celauro, "Diffusing capacity predicts long-term survival after lung resection for cancer," *European Journal Cardio-Thoracic Surgery*, vol. 41, pp. 81–86, 2012.
- [16] B. Mahut, B. Chevalier-Bidaud, L. Plantier et al., "Diffusing capacity for carbon monoxide is linked to ventilatory demand in patients with chronic obstructive pulmonary disease," *Journal of Chronic Obstructive Pulmonary Disease*, vol. 9, pp. 16–21, 2012.
- [17] S. Trad, L. T. Huong du, C. Frances et al., "Impaired carbon monoxide diffusing capacity as a marker of limited systemic sclerosis," *European Journal of Internal Medicine*, vol. 22, pp. 80–86, 2011.
- [18] A. J. Lopes, D. Capone, R. Mogami, S. L. S. de Menezes, F. S. Guimarães, and R. A. Levy, "Systemic sclerosis-associated interstitial pneumonia: evaluation of pulmonary function over a five-year period," *Jornal Brasileiro de Pneumologia*, vol. 37, no. 2, pp. 144–151, 2011.
- [19] J. S. Park, H. K. Kim, K. Kim, J. Kim, Y. M. Shim, and Y. S. Choi, "Prediction of acute pulmonary complications after resection of lung cancer in patients with preexisting interstitial lung disease," *Thoracic and Cardiovascular Surgeon*, vol. 59, no. 3, pp. 148–152, 2011.
- [20] D. Launay, M. Humbert, A. Berezne et al., "Clinical characteristics and survival in systemic sclerosis-related pulmonary hypertension associated with interstitial lung disease pulmonary hypertension in scleroderma," *Chest*, vol. 140, no. 4, pp. 1016–1024, 2011.
- [21] J. Michael, B. Hugues, and N. B. Pride, "Examination of the carbon monoxide diffusing capacity (DLCO) in relation to its KCO and Va components," *American Journal of Respiratory and Critical Care Medicine*, vol. 186, pp. 132–139, 2012.
- [22] F. J. W. Roughton and R. E. Forster, "Relative importance of diffusion and chemical reaction rates in determining rate of exchange of gases in the human lung, with special reference to true diffusing capacity of pulmonary membrane and volume of blood in the lung capillaries," *Journal of Applied Physiology*, vol. 11, no. 2, pp. 290–302, 1957.
- [23] F. J. W. Roughton, R. E. Forster, and L. Cander, "Rate at which carbon monoxide replaces oxygen from combination with human hemoglobin in solution and in the red cell," *Journal of Applied Physiology*, vol. 11, no. 2, pp. 269–276, 1957.
- [24] H. Guenard, N. Varene, and P. Vaida, "Determination of lung capillary blood volume and membrane diffusing capacity in man by the measurements of NO and CO transfer," *Respiration Physiology*, vol. 70, no. 1, pp. 113–120, 1987.
- [25] B. Aguilaniu, J. Maitre, S. Glénet, A. Gegout-Petit, and H. Guénard, "European reference equations for CO and NO lung transfer," *European Respiratory Journal*, vol. 31, no. 5, pp. 1091–1097, 2008.
- [26] S. Guillot, J. Beillot, C. Meunier, and J. Dassonville, "Interpreting carbon monoxide transfer coefficient: significance and difficulties," *Revue des Maladies Respiratoires*, vol. 22, no. 5, pp. 759–766, 2005.
- [27] B. Degano, M. Mittaine, H. Guénard et al., "Nitric oxide and carbon monoxide lung transfer in patients with advanced liver cirrhosis," *Journal of Applied Physiology*, vol. 107, no. 1, pp. 139–143, 2009.
- [28] E. F. Redente, K. M. Jacobsen, J. J. Solomon et al., "Age and sex dimorphisms contribute to the severity of bleomycine induced lung injury and fibrosis," *American Journal of Physiology*, vol. 301, pp. L501–L508, 2011.
- [29] D. Capone, A. Spanò, A. Gentile et al., "Are there differences in methotrexate kinetics between responding and nonresponding patients with rheumatoid arthritis?" *BioDrugs*, vol. 13, no. 5, pp. 373–379, 2000.
- [30] A. R. Phansalkar, C. M. Hanson, A. R. Shakir, R. L. Johnson Jr., and C. C. W. Hsia, "Nitric oxide diffusing capacity and alveolar microvascular recruitment in sarcoidosis," *American Journal of Respiratory and Critical Care Medicine*, vol. 169, no. 9, pp. 1034–1040, 2004.
- [31] R. S. Harris, M. Hadian, D. R. Hess, Y. Chang, and J. G. Venegas, "Pulmonary artery occlusion increases the ratio of diffusing capacity for nitric oxide to carbon monoxide in prone sheep," *Chest*, vol. 126, no. 2, pp. 559–565, 2004.
- [32] C. Borland, Y. Cox, and T. Higenbottam, "Reduction of pulmonary capillary blood volume in patients with severe unexplained pulmonary hypertension," *Thorax*, vol. 51, no. 8, pp. 855–856, 1996.
- [33] J. Moinard and H. Guenard, "Membrane diffusion of the lungs in patients with chronic renal failure," *European Respiratory Journal*, vol. 6, no. 2, pp. 225–230, 1993.
- [34] C. Borland, "A place for $T_{L,NO}$ with $T_{L,CO}$?" *European Respiratory Journal*, vol. 31, no. 5, pp. 918–919, 2008.



Hindawi

Submit your manuscripts at
<http://www.hindawi.com>

