[CANCER RESEARCH 39, 5076–5080, December 1979]
0008-5472/79/0039-0000$02.00

Role of Single-Breath Carbon Monoxide-diffusing Capacity in Monitoring the Pulmonary Effects of Bleomycin in Germ Cell Tumor Patients[1]

Robert L. Comis,[2] Mitchell S. Kuppinger, Sandra J. Ginsberg, Stanley T. Crooke, Robert Gilbert, J. Howland Auchincloss, and Archie W. Prestayko

Section of Oncology [R.L.C., S.J.G.] and Pulmonary Section [M.S.K., R.G., J.H.A.], Department of Medicine, State University of New York Upstate Medical Center, Syracuse, New York 13210, and Office of the Director of Research and Development, Bristol Laboratories, Syracuse, New York 13201 [S.T.C., A.W.P.]

ABSTRACT

Serial pulmonary function tests including single-breath carbon monoxide-diffusing capacity (DLco), forced vital capacity (FVC), and forced expiratory volume in 1 sec were performed in a relatively homogeneous group of male patients with germ cell tumors treated with vinblastine, bleomycin, and *cis*-diamminedichloroplatinum. Of the pulmonary function tests used, the DLco was shown to be the most sensitive indicator of subclinical bleomycin pulmonary effects. Decreases in DLco were both total dose and schedule dependent. Patients receiving their total dose of bleomycin at a rate of 25 ± 2 (S.D.) units/week developed a linear decrease in DLco with increasing total doses of bleomycin. Changes in FVC did not correlate with bleomycin total dose. Although both the mean DLco and FVC decreased after completion of bleomycin therapy, the mean FVC returned to base-line levels rapidly, whereas the decrease in mean DLco was persistent for several months. When routine volumetric tests (FVC and forced expiratory volume in 1 sec) and DLco are used in a systematic manner, DLco is the most sensitive indicator of the subclinical pulmonary effects of bleomycin in germ cell tumor patients treated with vinblastine, bleomycin, and *cis*-diamminedichloroplatinum.

INTRODUCTION

Bleomycin is a nonmyelosuppressive antitumor antibiotic with documented activity in testicular carcinoma (2). It has become an important component of recent combination chemotherapy programs for testis cancer. Current therapeutic regimens using bleomycin combined with either vinblastine or vinblastine plus DDP[3] are capable of inducing long-term complete responses in 40 to 60% of patients with metastatic disease (7, 14).

Pulmonary fibrosis is the primary dose-limiting toxicity of bleomycin. Fatal pulmonary fibrosis occurs in 1 to 2% of patients, and an additional 2 to 3% of patients develop well-documented interstitial fibrosis related to bleomycin (2, 3). Clinical manifestations of bleomycin pulmonary toxicity occur sporadically at total doses of 100 to 500 units. There is a significant increase in the incidence of bleomycin pulmonary toxicity when total doses in excess of 500 units are administered (3). The incidence of subclinical pulmonary toxicity manifested by unexpected findings of pulmonary fibrosis at autopsy parallels the incidence of clinically appreciated pulmonary toxicity, raising the possibility that the incidence of subclinical disease is at least as great as that of the clinically apparent disease (3).

Several studies have used pulmonary function tests in an attempt to monitor pulmonary toxicity. Most studies have been performed in heterogeneous groups of patients receiving bleomycin in various doses and by various schedules and routes of administration (10, 13, 17).

Male patients with metastatic germ cell tumors represent a relatively homogeneous group of previously healthy young patients. With current treatment regimens which include bleomycin, these patients can be expected to survive for prolonged periods of time. The present study was designed to evaluate the efficacy of standard, serial pulmonary function tests in a series of such patients receiving a standard treatment program including bleomycin administered by a single route of administration and with the same dosage and schedule in an attempt to assess whether any pulmonary function test would be a useful predictor of clinically apparent bleomycin pulmonary toxicity or indicator of subclinical bleomycin pulmonary toxicity.

MATERIALS AND METHODS

Patient Characteristics. Eleven male patients, 9 with testicular carcinoma and 2 with primary mediastinal germ cell tumors, were evaluated. The mean and median age was 25 years (range, 19 to 47 years). Three patients had pulmonary metastases. Three patients were cigarette smokers. All smokers were instructed to refrain from smoking prior to pulmonary function tests. Both patients with primary mediastinal germ cell tumors had received prior radiotherapy to the mediastinum. One patient received 6000 rads in 5 weeks to the mediastinum 6 months prior to initiating chemotherapy. The second patient presented with superior vena cava syndrome and received 1000 rads in 2 days to the mediastinum followed by an additional 3000 rads in 3 weeks. The 2 patients who received prior radiotherapy are analyzed separately, since it has been reported that prior radiotherapy increases the risk of developing bleomycin pulmonary toxicity (8, 15).

Chemotherapy. All patients were treated i.v. with a 3-drug regimen including vinblastine (0.2 mg/kg/day for 2 days), DDP (20 mg/sq m/day for 5 days), and bleomycin (30 units weekly for 12 doses). No "synchronization" of vinblastine and bleomycin doses was used. Chemotherapy was withheld in any patient having a leukocyte count of less than 1000 cells/cu mm or if a documented infection was present. Bleomycin therapy was reinstituted upon resolution of leukopenia and/or the infectious process.

[1] This work was supported in part by a grant from Bristol Laboratories, Syracuse, N. Y., and Training Grant HL05954 from the National Heart, Lung, and Blood Institute.

[2] To whom requests for reprints should be addressed.

[3] The abbreviations used are: DDP, *cis*-diamminedichloroplatinum; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 sec; DLco, single-breath carbon monoxide-diffusing capacity.

Received January 8, 1979; accepted September 17, 1979.

Changes in pulmonary function tests were, in general, not used in the decision to terminate bleomycin therapy. Bleomycin therapy was discontinued if: (*a*) radiological findings consistent with bleomycin pulmonary toxicity developed; (*b*) the patient developed dyspnea on exertion during routine activity, in the absence of a significant anemia; or (*c*) fine crackling rales were apparent on routine weekly physical examination. If no pulmonary toxicity as defined above was observed, bleomycin was discontinued after 360 units had been administered. After discontinuing bleomycin, therapy consisted of vinblastine (0.3 mg/kg every 4 weeks) in patients in complete remission. Patients who experienced a partial response continued vinblastine and DDP until a complete remission occurred.

Pulmonary Function Tests. FVC and FEV_1 were measured by standard spirometric techniques using a Med-Science wedge spirometer recording on a Tektronics storage oscilloscope. DLco was determined by the method of Ogilvie *et al.* (12) as modified by Mitchell and Renzetti (11). DLco measurements were performed in duplicate. Determinations were required to agree within 2 ml/min/mm Hg. If the first 2 determinations did not meet these criteria, further measurements were made until 2 such values were obtained; these were then averaged. All DLco values were corrected for hemoglobin concentration by the method of Dinakara *et al.* (6).

Baseline pulmonary function tests were performed prior to initiating therapy and serially thereafter at 1- to 3-week intervals. Changes in pulmonary function tests were analyzed both as a percentage of the initial pretreatment value and as a change in the percentage of the predicted values. Predicted values for DLco were obtained from the formulae given by Bates *et al.* (1), while predicted values for FVC and FEV_1 were based upon age and height (9). The initial DLco was 98 ± 9% (S.D.) of the predicted value in the 9 patients who had no prior radiotherapy, and the initial FVC was 100 ± 16% of the predicted value. The data, except when otherwise noted in the text, are expressed as the percentage of the initial pretreatment value.

Statistical Analysis. Statistical analyses were performed using the Student *t* test for paired samples, the analysis of variance for groups with unequal replication (16), and linear regression analysis, where appropriate.

RESULTS

Chart 1 shows the effects of increasing total doses of bleomycin on the mean DLco and FVC in the 9 patients who had not received prior radiotherapy. A significant decrease in the mean DLco was noted after a total dose of 60 units of bleomycin had been reached. The mean DLco decreased progressively with increasing bleomycin doses. No statistically significant changes occurred in the mean FVC values with increasing total doses of bleomycin, and no patient developed changes in FEV_1 independent of changes in FVC.

In most patients, a linear decrease in DLco was observed with increasing doses of bleomycin, and this preceded any significant decrease in FVC. An illustrative case is presented in Chart 2.

A significant alteration in the rate at which the total bleomycin dose was administered (dose rate) accounted for some of the variability in the mean values of DLco presented in Chart 1. Of the 9 patients who had not received prior radiotherapy, 6

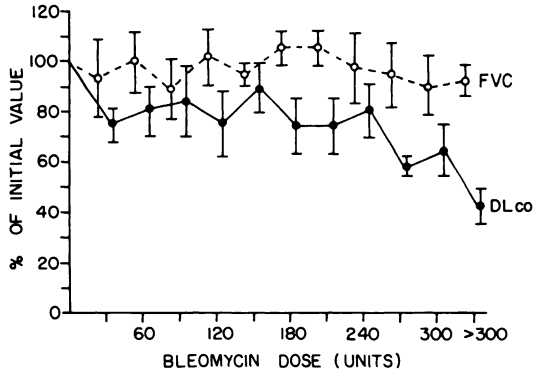

Chart 1. Mean values of DLco and FVC at increasing total doses of bleomycin while on therapy. Mean DLco determinations at cumulative doses of ≥60 units were significantly lower than the initial value ($p < 0.05$). There were no statistically significant differences in mean FVC values while on therapy. *Bars*, S.D.

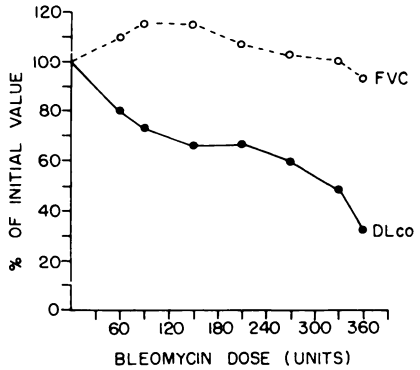

Chart 2. Changes in DLco and FVC in a patient while on bleomycin therapy. The DLco progressively decreased with increasing total doses of bleomycin, whereas no significant change in FVC occurred.

received their total bleomycin dose on a schedule approximating the projected 30 units/week dose rate (25 ± 2 (S.D.) units/week). Three other patients received their total bleomycin dose at a significantly reduced dose rate (16 ± 1.6 units/week) because of severe and persistent leukopenia during therapy.

A linear regression analysis of the DLco determinations of the 6 patients who received an average weekly dose of bleomycin of 25 ± 2 units is presented in Chart 3. The relationship between decreasing DLco and increasing total doses of bleomycin was linear ($p < 0.001$). No correlation existed between FVC and bleomycin dose (data not shown). Those patients who received the total bleomycin dose at a rate of 16 ± 1.6 units/week experienced either a delayed or decreased rate of decline and/or recovery to control values during delays in therapy, followed by a fall in DLco upon reinstitution of bleomycin at a rate of 30 units/week. An example of the effects of an alteration in the scheduling of bleomycin is presented in Chart 4. Because of severe myelosuppression, this patient received only 120 units of bleomycin in the first 9.4 weeks of therapy, an average of 13 units/week. During this period, the DLco initially declined and returned to baseline levels. Upon reinstitution of bleomycin at a dose of 30 units/week, the DLco decreased rapidly.

The relationship between the total dose of bleomycin administered and changes in DLco and FVC is presented in Chart 5. There was a statistically significant relationship between the total bleomycin dose administered and the change in DLco (*p*

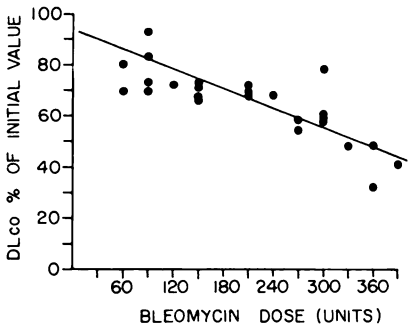

Chart 3. Linear regression analysis of all DLco determinations on the 6 patients receiving the total bleomycin dose at a dose rate of 25 ± 2 (S.D.) units/week; $r = 0.895$; $p < 0.001$ ($y = 94.36 - 0.1316x$; S.E. = ± 8.27).

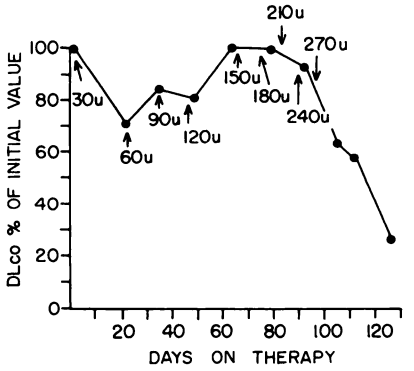

Chart 4. The effect of schedule on DLco. During the first 60 days of therapy, the patient received 120 units (*u*) of bleomycin (13 units/week), during which time the DLco decreased and then recovered. With reinstitution of therapy at 30 units/week, the progressive fall in DLco developed.

< 0.05), but no relationship was found between FVC changes and the total dose of drug administered.

Chart 6 shows the mean values of monthly determinations of DLco and FVC after bleomycin was discontinued. Six patients were in apparent complete remission; 2 were in partial remission and were being treated with vinblastine and DDP. One patient had died from bleomycin pulmonary toxicity. The decrease in mean DLco was persistent. DLco determinations at 7 months were significantly lower than pretreatment values ($p < 0.05$). On the other hand, after 1 month there was no statistically significant difference between mean FVC determinations and initial pretreatment values. In 6 of 8 patients, recovery of the FVC to ≥90% of the initial value was observed. Only 2 of 8 patients developed recovery of the DLco to ≥90% of the initial value, at 1 and 7 months postbleomycin, respectively.

The 2 patients who received radiotherapy prior to bleomycin treatment developed precipitous decreases in DLco after receiving 90 and 30 units of bleomycin, respectively. The patient who had received 6000 rads to the mediastinum 6 months prior to chemotherapy developed a fall in DLco to 65% of the pretreatment level after having received only 90 units of bleomycin in 3 weeks. No further bleomycin was administered because of the precipitous fall in DLco and the development of dyspnea on exertion. In spite of the development of a complete clinical remission of metastatic pulmonary disease for 11 months and improvement in the FVC, a significant decrease in DLco (60% of predicted) persisted throughout the period of observation (12 months). The second patient presented with severe superior vena cava syndrome which required emergency radiotherapy. No preradiotherapy pulmonary function values were available. Thirty units of bleomycin were administered 1 month after completion of radiotherapy. Within 10 days, the DLco fell to 51% of the predicted value, and diffuse bilateral pulmonary infiltrates developed within and outside the radiation therapy portal. Seven months later, the DLco and FVC were both 70% of the predicted value, and the patient was in a complete clinical remission.

Pulmonary toxicity, comfirmed by light and electron microscopy studies, developed in 2 patients. Serial DLco and FVC determinations for these 2 patients are shown in Chart 7. Both patients developed precipitous decreases both in DLco and FVC after the last dose of bleomycin therapy. The patient shown in Chart 7*A* had a persistent large abdominal mass after 3 full courses of therapy including 360 units of bleomycin. An additional 30 units of bleomycin was administered when the DLco and FVC were 41 and 84% of the initial values, respectively. At that time, the patient had no pulmonary symptoms and no physical or radiographic findings consistent with pulmonary toxicity. Within 1 month, the patient developed respiratory failure and died. The second patient (Chart 7*B*) had received the first 120 units of bleomycin at a decreased dose rate as shown in Chart 4. After reinstitution of bleomycin at a dose of 30 units/week, a rapid decrease (10% of the initial value per week) in both DLco and FVC developed. One month

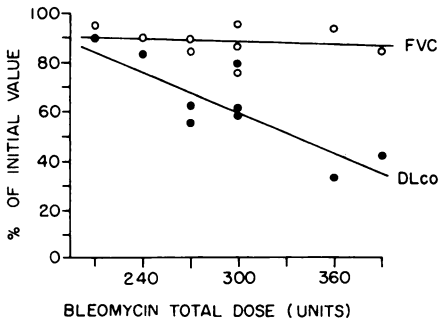

Chart 5. Linear regression analysis of DLco and FVC as a percentage of the initial value related to the total dose of bleomycin administered. For the 9 patients who did not receive radiotherapy, the relationship between total dose and DLco was linear; $r = 0.77$; $p < 0.05$ ($y = 147.29 - 0.289x$; S.E. = ± 10.59).

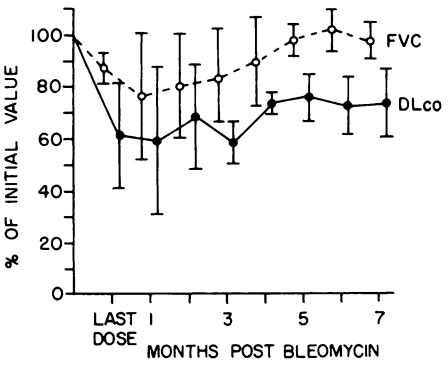

Chart 6. Serial mean values for DLco and FVC after discontinuing bleomycin. Both the mean DLco and FVC decreased significantly after the total bleomycin dose had been administered. The decrease in DLco was still present in patients followed for 7 months. No significant difference in mean FVC determinations was present 1 month after discontinuing bleomycin. Bars, ± S.D.

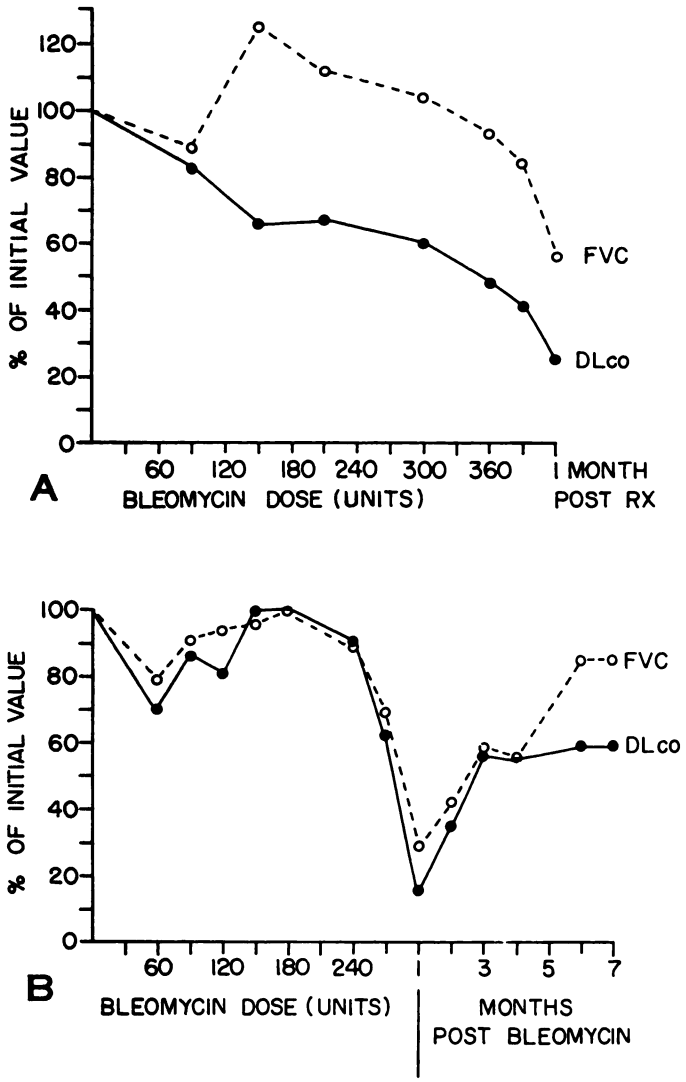

Chart 7. *A*, DLco and FVC changes in a patient who developed lethal bleomycin pulmonary toxicity. *B*, DLco and FVC changes in a patient who developed severe, nonlethal, bleomycin pulmonary toxicity. See text for details.

after receiving the last dose of bleomycin (total dose, 270 units), diffuse bilateral pulmonary infiltrates developed with accompanying severe dyspnea on exertion and hypoxemia. The pulmonary infiltrates gradually resolved concomitant with the initiation of steroid therapy. Throughout treatment of this patient with bleomycin, the changes in DLco were paralleled by changes in FVC.

DISCUSSION

Previous studies examining the relationship between changes in pulmonary function tests and bleomycin administration have been performed in groups of patients heterogeneous with respect to age, sex, type of cancer, and the presence or absence of pulmonary metastases or other nonmalignant pulmonary disease (10, 13, 17). Within these studies, there has also been substantial variability in bleomycin dose, rate of drug delivery, route of administration, and the total dose of drug administered. In addition, the timing of the pulmonary function testing relative to the total dose of bleomycin or time from discontinuation of therapy has, in general, not been well defined.

With these constraints, most previous studies have demonstrated that detectable abnormalities in pulmonary function tests occur during bleomycin therapy when no detectable radiographic findings are apparent. Yagoda *et al.* (17) and Krakoff *et al.* (10) have reported that decreases in total lung capacity and DLco occur in most patients treated with bleomycin, although no correlation was found between changes in these parameters and the total dose administered. The majority of patients studied by Pasqual *et al.* (13) developed a decrease in FVC and/or DLco "before and during" therapy with bleomycin, but the relationship between these changes and bleomycin dose was obscured by the presence or absence of pulmonary metastases. Samuels *et al.* (15) have stated that the FVC is the most reliable indicator of bleomycin pulmonary toxicity, but the relationships among the dose of bleomycin, timing of FVC measurements, and the type of other pulmonary function tests performed were not presented.

The design of the present study differs from most previous investigations, primarily in that the patient population was a relatively homogeneous group of previously healthy young men with germ cell tumors. In addition, the dose and route of bleomycin administration was standardized; pulmonary function tests were performed serially, and the relationship between the timing of pulmonary function testing and bleomycin therapy is presented.

Under these conditions, DLco was found to be a more sensitive indicator of subclinical bleomycin pulmonary effects than were standard volumetric measurements. Decreases in DLco during bleomycin therapy were found to be both total dose and schedule dependent. The latter relationship may account for some of the variability reported previously in correlating changes in DLco with bleomycin therapy.

The persistence of the decrease in DLco after discontinuing bleomycin may explain our recently reported observation that patients with germ cell tumors who have received bleomycin 1 to 6 months prior to reinstituting a new therapeutic regimen including bleomcyin are more likely to develop bleomycin pulmonary toxicity than patients who have never received bleomycin (5).

Several reports have indicated that the risk of developing pulmonary toxicity is increased in patients who have received prior radiotherapy (8, 15). Both patients who had received radiotherapy in the present study developed precipitous and prolonged decreases in DLco after low total doses of bleomycin were administered.

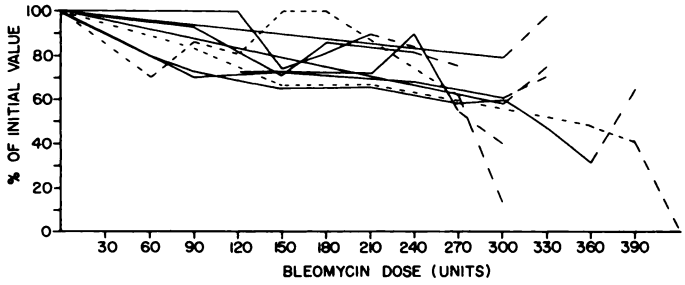

Chart 8. Changes in DLco while on therapy for 9 patients who did not receive radiotherapy. The 2 patients who developed severe bleomycin pulmonary toxicity (- - - -); those patients who did not develop severe toxicity (——); the measured DLco 1 month after discontinuing bleomycin (— —).

Chart 8 shows the changes in DLco of all patients, including the 2 patients who developed severe bleomycin pulmonary toxicity. It is apparent that more data are necessary to define whether the DLco will be of value in predicting which individual patient will develop severe bleomycin pulmonary toxicity. It would appear, however, that caution should be exerted in continuing bleomycin therapy in patients whose DLco has fallen to ≤40% of the initial or predicted value, regardless of FVC changes, or in patients who develop rapid, parallel decreases in both DLco and FVC while on therapy (Chart 7).

As used in this study, bleomycin was part of a 3-drug program including vinblastine and DDP. Therefore, the possibility exists that significant drug interactions may account for some of the results noted. Neither vinblastine nor DDP has been reported to be a clinically significant pulmonary toxin. It is unlikely, therefore, that a direct effect of either drug on the lungs was involved in the observed pulmonary function abnormalities. On the other hand, the major excretory pathway for bleomycin is renal, and we have shown that the elimination half-life of bleomycin varies inversely with changes in creatinine clearance (4). It is conceivable that alterations in bleomycin elimination secondary to DDP-induced nephrotoxicity might have contributed to the results observed in this study. We examined bleomycin pharmacokinetics in 5 patients. There was no correlation between the elimination half-lives in these patients and abnormalities in pulmonary function tests. Since only one or two pharmacokinetic studies were performed in an individual patient throughout the treatment program, the lack of a significant correlation may have resulted from inadequate sampling.

In contrast to previous conclusions by others, it appears that the DLco is a sensitive indicator of subclinical bleomycin pulmonary effects when the test is performed systematically in a relatively homogeneous group of young male germ cell tumor patients treated with vinblastine, bleomycin, and DDP.

ACKNOWLEDGMENTS

The excellent technical assistance of Sharon Bleb, who performed the pulmonary function tests, is appreciated. We also thank Dr. Yerach Daskal of Baylor University, who performed electron microscopy on the lung tissue of the patients with severe pulmonary toxicity.

REFERENCES

1. Bates, D. V., Macklem, P. T., and Christie, R. V. The normal lung: physiology and methods of study. *In:* D. V. Bates (ed.), Respiratory Function in Disease, pp. 93–94. Philadelphia: W. B. Saunders Co., 1971.
2. Blum, R. H., Carter, S. K., and Agre, K. A. A clinical review of bleomycin—a new antineoplastic agent. Cancer (Phila.), *31:* 1938–1942, 1973.
3. Comis, R. L. Bleomycin pulmonary toxicity. *In:* S. K. Carter, H. Umezawa, and S. T. Crooke (eds.), Bleomycin—Current Status and New Developments, pp. 279–291. New York: Academic Press, Inc., 1978.
4. Crooke, S. T., Comis, R. L., Einhorn, L. H., Strong, J. E., Broughton, A., and Prestayko, A. W. Effects of variations in renal function on the clinical pharmacology of bleomycin administered as an i.v. bolus. Cancer Treat. Rep., *61:* 1631–1635, 1978.
5. Crooke, S. T., Einhorn, L. H., Comis, R. L., D'Aoust, J. C., and Prestayko, A. W. The effects of prior exposure to bleomycin on the incidence of pulmonary toxicities. Med. Pediatr. Oncol., *5:* 93–98, 1978.
6. Dinakara, P., Blumenthal, W. S., Johnston, R. F., Kauffman, L. A., and Solnick, P. R. The effect of anemia on pulmonary diffusing capacity with derivation of a correction equation. Am. Rev. Respir. Dis., *102:* 965–969, 1970.
7. Einhorn, L. H., and Donohue, J. *cis*-Diamminedichloroplatinum, vinblastine, and bleomycin combination chemotherapy in disseminated testicular cancer. Ann. Intern. Med., *87:* 293–298, 1977.
8. Einhorn, L. H., Krause, M., Hornback, N., and Furnas, B. Enhanced pulmonary toxicity with bleomycin and radiotherapy in oat cell lung cancer. Cancer (Phila.), *37:* 2414–2416, 1976.
9. Kory, R. C., Callahan, R., Baren, H. C., and Snyder, J. C. Clinical spirometry in normal men. The Veterans Administration-Army Cooperative Study of Pulmonary Function, Am. J. Med. Vol. 30, pp. 243–258. 1961.
10. Krakoff, I. H., Cvitkovic, E., Currie, V., Yeh, S., and LaMonte, C. Clinical pharmacologic and therapeutic studies of bleomycin given by continuous infusion. Cancer (Phila.), *40:* 2027–2037, 1977.
11. Mitchell, M. M., and Renzetti, A. D. Application of the single breath method of total lung capacity of the calculation of the carbon monoxide diffusing capacity. Am. Rev. Respir. Dis., *97:* 581–584, 1968.
12. Ogilvie, C. M., Forster, R. E., Blakemore, W. S., and Marton, J. W. A standardized breath holding technique for the clinical measurement of the diffusing capacity of the lung for carbon monoxide. J. Clin. Invest., *36:* 1–17, 1957.
13. Pasqual, R. S., Mosher, M. B., Sikand, R. S., DeConti, R. C., and Bou Hoys, A. Effect of bleomycin on pulmonary function in man. Am. Rev. Respir. Dis., *108:* 211–217, 1973.
14. Samuels, M. L., Holoye, P. Y., and Johnson, D. E. Bleomycin combination chemotherapy in the management of testicular neoplasia. Cancer (Phila.), *36:* 318–326, 1975.
15. Samuels, M. L., Johnson, D. E. Holoye, P. Y., and Lanzotti, V. J. Larger dose bleomycin therapy and pulmonary toxicity. A possible role of prior radiotherapy. J. Am. Med. Assoc., *235:* 1117–1120, 1976.
16. Steel, R. G. D., and Torrie, J. H. Analysis of variance for groups with unequal replication and Dupnett's procedure for comparing all means with a control. *In:* R. G. D. Steel and J. H. Torrie (eds.), Principles and Procedures of Statistics, pp. 111–115. New York: McGraw-Hill Book Co., Inc., 1968.
17. Yagoda, A., Mukherji, B., Young, C., Etchumbana, E., LaMonte, C., Smith, J. R., Tan, C. T., and Krakoff, I. M. Bleomycin, an antitumor antibiotic. Clinical experience in 274 patients. Ann. Intern. Med., *77:* 861–870, 1972.