A Prospective study of Changes in Pulmonary Function Tests and Diffusion Coefficient of Carbon Monoxide (DLCO) in Hodgkin Lymphoma and Germ cell tumor patients receiving Bleomycin containing chemotherapy

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CERTIFICATE

This is to certify that this dissertation on **"A Prospective study of changes in Pulmonary Function Tests and Diffusion Coefficent of Carbon Monoxide (DLCO) in patients with Hodgkin Lymphoma and Germ cell tumors receiving Bleomycin containing chemotherapy" ,** is a bonafide work done by Dr Manjunath Irappa Nandennavar , in the Department of medical Oncology , College of Oncological Sciences, Adyar, Chennai, under my overall supervision and guidance.

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Title of Dissertation:

A Prospective study of changes in Pulmonary Function Tests and Diffusion Coefficent of Carbon Monoxide (DLCO) in patients with Hodgkin Lymphoma and Germ cell tumors receiving Bleomycin containing chemotherapy

Abstract

Introduction: Bleomycin pulmonary toxicity has been quoted to range from 0-46% with a mortality of about 1-2%. Significant decline in PFTs (Pulmonary Function Tests) and DLCO (diffusion coefficient of Carbon monoxide) has been quoted to be predictive of Bleomycin induced lung injury. There are no published studies of PFTs and DLCO in Indian patients receiving bleomycin containing chemotherapy.

Aims: This is a prospective study to document the changes in serial PFTs and DLCO and also features of bleomycin lung toxicity in patients with Hodgkin lymphoma and Germ Cell Tumors receiving bleomycin containing chemotherapy.

Materials and Methods: Between June 2010 and October 2011, all the patients with a diagnosis of Hodgkin Lymphoma, Germ cell tumors of Ovary and Testis who were more than 15 yrs of age receiving Bleomycin containing chemotherapy were included in the study. These patients were followed up until Feb 2012 so that at least one set of interim PFTs is obtained of the patients enrolled in the later part of study period. PFTs and DLCO were done at baseline, interim (approximately 80-100U of Bleomycin delivered), end of treatment and at 6 months follow up.

Results: 76 patients (52 male, 24 female) were studied. 53 had Hodgkin lymphoma, 16 had Testicular GCT and 7 had Ovary GCT. Median age: 27 yrs, Median Hemoglobin 11.6 gm%(5.6-

`16.5 gm%), Median Creatinine 0.7mg% (0.5-1.1 mg%), Median cumulative dose of Bleomycin 180U (60-290U). The decline in serial DLCO and TLC values was not statistically significant. 10 (13%) had pulmonary toxicity. Dyspnea was the commonest symptom. Of these 10 patients, 3 didn't have PFT DLCO decline, 2 had asymptomatic drop in DLCO. Pulmonary toxicity did not develop in 4 other patients who continued to receive bleomycin even though they had significant DLCO drop. Age>30 yrs was the only significant predictive factor. No patient died of bleomycin pulmonary toxicity.

Conclusions: PFTs and DLCO alone are not predictive of Bleomycin Pulmonary toxicity. A combination of diligently looking out for pulmonary symptoms along with radiologic imaging and pulmonary function tests can pick up Bleomycin lung toxicity.

Key words: Bleomycin, Pulmonary function tests, Diffusion coefficient of Carbon monoxide, Bleomycin induced pulmonary toxicity.

INTRODUCTION

Bleomycin is an antibiotic agent with antitumor activity discovered by Umezewa et al in 1966.[1] Bleomycin was originally isolated from the fungus Streptomyces verticillus. It is commonly used as a part of treatment of several malignancies, such as germ cell tumors, Hodgkin lymphoma (HL), Kaposi's sarcoma and squamous cell carcinomas of head and neck.[2]

Bleomycin exerts its antitumor effects by inducing tumor cell kill. It acts by the induction of free radicals. These free radicals induce DNA breaks which ultimately cause tumor cell kill.[3] It is mainly eliminated by kidneys. Bleomycin is deactivated by the enzyme bleomycin hydrolase found in liver and skin.[4]Common toxic reactions include fever, rashes, mucositis, skin pigmentation, nausea and vomiting. The most feared and dose limiting toxicity is Bleomycin Pulmonary Toxicity (BPT). Bleomycin hydrolase may be absent in the lung and skin in some patients, leading to bleomycin toxicity predominantly occurring in these organs. Bleomycin (BLM) can cause various types of pulmonary toxicities viz BOOP (bronchiolitis obliterans with organizing pneumonia), acute chest pain syndrome, pulmonary venoocclusive

disease, acute eosinophilic hypersensitivity pneumonitis,bleomycin interstitial Pneumonitis (BIP) which may ultimately progress to fibrosis. [4,5]

Presently, Hodgkin Lymphoma can be cured in at least 80% of patients. Hence the major challenge is to cure the disease with minimal toxicity. Bleomycin is a part of various protocols used to treat Hodgkin Lymphoma. The standard regimen ABVD used to treat HL provides the best balance of effectiveness and toxicity. Bleomycin is also an important component in the systemic chemotherapy of Germ cell tumors of Testis and Ovary. These malignancies are highly curable with the present combined modalities of surgery, radiation therapy and multiagent chemotherapy. The pulmonary toxicity of Bleomycin usually consists of Pneumonitis or fibrosis, which may be fatal. Various studies quote different statistics for incidence of Bleomycin Pulmonary Toxicity (BPT). Bleomycin is known to cause fatal pulmonary toxicity in 1-2% of patients and non fatal pulmonary fibrosis in another 2-3% of patients.[6]Bleomycin induced Pneumonitis is stated to occur in 0-46% of patients who received BLM containing chemotherapy protocols.[4,5] The incidence of BPT depends upon the patient population studied, chemotherapy used and dose and mode of administration of Bleomycin and also the definition of bleomycin pulmonary toxicity.[7] Various studies have been carried out to try and detect early features of pulmonary toxicity and hence to prevent severe toxicity by stopping bleomycin administration. In general, most

of the reported pulmonary toxicity occurs at doses >450 mg.[2,4,7] There are a number of risk factors for the development of bleomycin pulmonary toxicity. However, there is no test which can reliably predict for significant lung toxicity. There are contradicting studies in the literature regarding role of pulmonary function tests (PFTs) and CO diffusion coefficient (DLCO) in predicting bleomycin induced lung toxicity. Many of these studies are retrospective studies, case series or prospective studies with small number of patients. There is no published Indian study of changes in PFTs in patients receiving bleomycin containing chemotherapy.

This study is designed to document the changes over time in Pulmonary Function tests and DLCO at various bleomycin doses. Clinical features of bleomycin pulmonary toxicity were documented. Furthermore, these tests were repeated 6 months post completion of therapy to document the status of these changes.

AIMS AND OBJECTIVES:

PRIMARY OUTCOMES

1. To prospectively study the changes in Pulmonary Function Tests (PFTs) and Carbon Monoxide diffusion coefficient (DLCO) in patients with Hodgkin Lymphoma and Germ cell tumors of Ovary and Testis receiving Bleomycin containing chemotherapy.

SECONDARY OUTCOMES

1. To document the risk factors likely to be associated with Bleomycin pulmonary toxicity – tobacco smoking status, renal function status, age, Granulocyte colony stimulating factor usage (G CSF).
2. To evaluate the effect of time on pulmonary function tests and pulmonary symptoms
3. To document the clinical features of bleomycin pulmonary toxicity.

REVIEW OF LITERATURE

Bleomycin

Bleomycin is the generic name for a group of antibiotics isolated from the fungus Streptomyces verticillus. Umezawa and colleagues, while searching for newer antineoplastic drugs, isolated a mixture of small glycopeptides from culture broths of fungus Streptomyces verticillus.[1] This compound consisting of sulfated glycopeptides was separated by paper chromatography into two fractions designated "A" and "B". [4,8] The drug has a unique mechanism of action and has virtually no hematologic toxicity. A major disadvantage is the bleomycin induced pneumonitis.

Structure of Bleomycin:

Bleomycin is a mixture of peptides with a molecular weight of approximately 1,500. All of them have the unique structural component – bleomycinic acid, while differing only in the terminal alkylamine group. [1,4] The predominant active component of this mixture of glycopeptides is Bleomycin A2.[1,9] The remaining bleomycins differ only in the terminal amine. Bleomycin causes single and double strand DNA damage.Bleomycin consists

of three regions that include a carbohydrate moiety, a metal binding domain, and a DNA binding domain (Figure 1).[4,8,9]

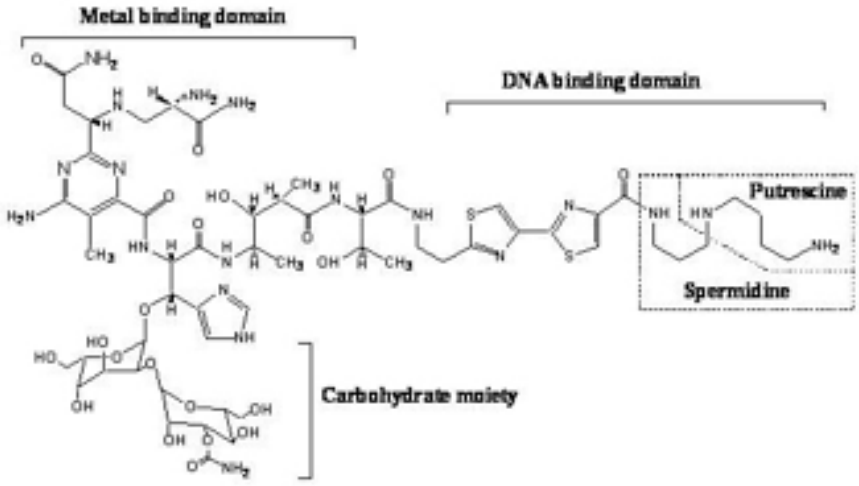


Fig1 : Structure of bleomycin-A5 depicting several domains. The metal binding domain binds to reduce iron and in the presence of oxygen forms a free radical that attacks the DNA. While the polyamine-like region is involved in DNA binding, the function of the carbohydrate moiety is unknown.

Mechanism of Action:

Bleomycins are isolated from Streptomyces verticillus as copper (II) complexes and also show high affinities for divalent ions of iron, nickel, cobalt and zinc. Bleomycin is selectively toxic to cells in the M and G2 phases of the cell cycle, and generally more effective against actively dividing rather than resting cells.

Mechanism of action of bleomycin is unique because unlike other DNA damaging drugs it neither attacks nucleic bases nor the phosphate linkages. Initially, activated Fe (II) – bleomycin- oxygen complex is formed. The drug binds to guanosine-cytosine-rich portions of DNA via association of the "S" tripeptide and by partial intercalation of the bithiazole rings. A group of five nitrogen atoms arranged in a square-pyramidal conformation binds divalent metals including iron, the active ligand, and copper, an inactive ligand. Molecular oxygen, bound by the iron, can produce highly reactive free radicals and Fe (III). The free radicals produce DNA single-strand breaks at 3'-4' bonds in deoxyribose. The intercalation of bleomycin with linear DNA results in either lengthening or relaxing of supercoiled circular DNA. Then the activated Fe (II) – Bleomycin complex acts as ferrous oxidase with the oxidation of Fe (II) to Fe (III). This short-lived oxygenated molecule causes at least four cleavage events for every single bleomycin molecule.[10] This ultimately leads to release of all four bases (adenine, guanosine, cytosine, thymine). Bleomycin produces both single and double strand breaks in a ratio of approximately 10:1.

Bleomycin produces these bi-stranded DNA lesions at certain specific sequences, such as CGCC, which are generated when the Fe.bleomycin complex creates an AP site on one strand, and a directly opposed single strand break on the complementary strand.[11]

Metabolism:

Bleomycin is rapidly absorbed following either subcutaneous, intravenous, intrapleural or intraperitoneal administration. It is widely distributed throughout the body with a mean volume of distribution of 17.5 L/m2.

The cellular uptake of bleomycin is slow. A bleomycin binding membrane protein has been identified which leads to internalization of bleomycin. Once internalized it can either damage DNA or be degraded by hydrolases.

Metabolic inactivation of bleomycin is a mechanism of resistance to the drug in some cells and may influence toxicity in normal tissues. Bleomycin hydrolase activity is low in lungs and skin, the two major sites of normal tissue toxicities, and levels of this enzyme have been elevated in some but not all tumor cell lines selected for resistance to bleomycin. The capacity to repair or withstand single and double strand DNA breaks may also be an important determinant of resistance to the drug. Single nucleotide polymorphism (SNP) studies of Bleomycin hydrolase have been done.[12] De Haas EC et al noted a decreased survival in patients with variations in bleomycin hydrolase gene. [13] They noted that survival curves were 20% inferior in patients with SNP changes resulting in GG allele homozygosity. They also noted more

pulmonary toxicity in this group of patients. This study had several flaws viz. patients with GG allele had received more cumulative dose of BLM (360 U) compared to other patients (270 U), the SNP analysis doesn't necessarily tell the exact level of activity of BLM hydrolase.

Bleomycin is rapidly eliminated primarily by renal excretion. This accounts for approximately half of a dose. In patients with renal compromise or extensive prior cisplatin therapy, the drug half-life can extend from 2 to 4 hours up to 21 hours. Thus, dose adjustments are needed when creatinine clearance is less than or equal to 50 mL/min.[14]Finally, resistance to bleomycin in normal tissues can be correlated with the presence of a bleomycin hydrolase enzyme, which is in the cysteine proteinase family. The enzyme replaces a terminal amine with a hydroxyl, thereby inhibiting iron binding and cytotoxic activity. The low concentration of enzyme in the skin and lung may explain the unique sensitivity of these tissues to bleomycin toxicity.

Bleomycin Toxicity:

Bleomycin does not cause myelosuppression except in patients with severely compromised bone marrow function due to extensive chemotherapy. Bleomycin commonly causes skin toxicity. It also causes acute allergic reactions, fever (usually occurring within 48 hours of administration) and the more dreaded pulmonary toxicity.

Bleomycin pulmonary toxicity:

Bleomycin may cause several different types of pulmonary toxicity viz. an acute chest pain syndrome, acute hypersensitivity pneumonitis, bronchiolitis obliterans with organizing pneumonia (BOOP), pulmonary venoocclusive disease, dose dependent interstitial pneumonitis progressing to chronic interstitial fibrosis.[4,5] There are various criteria used to define Bleomycin induced pulmonary toxicity. Bleomycin induced pneumonitis may occur in 0-46% of patients, depending upon the criteria used.

The mechanism of bleomycin toxicity in the lung is not fully understood. Meticulous animal experiments were carried out in the 1970s and 1980s, which could throw some light on the pathogenesis of pulmonary toxicity, however human data is scarce. Adamson et al[15] published their observations on pulmonary toxicity seen in mice treated with varying doses of Bleomycin. The pathogenesis was studied in detail with particular emphasis on sequential cellular responses. The first event noted was endothelial damage of the lung vasculature accompanied by edema. Electron microscopy revealed that endothelium of pulmonary veins and arteries developed subendothelial blebs which bulged into the vascular lumen.[16] Lesions in large pulmonary vessels were well developed much before changes were noted in the smaller vessels. Diffuse interstitial edema was noted. The mechanism of endothelial damage is not clearly known. Cytokines and free radicals have been

implicated in causing the initial insult. Induced cytokines activate the lymphocytes and upregulate the adhesion molecules on endothelial cells. Gasse Pamela et al[17]in their animal experiments have proposed that the lung pathology caused by Bleomycin administration is by the inflammasome and IL-R1/ MyD88 signaling. BLM induced lung cell injury results in sensing of the stress signals by the cells. Activation of the inflammasome leads to activated IL 1β. Activated IL 1β then activated the IL R1/ My D88 complex **(Fig 2).**This is followed by influx of inflammatory cells such as lymphocytes, neutrophils andmacrophagesinto the lung parenchyma. The adhered lymphocytes to the endothelium also induce apoptosis of endothelial cells. Fibroblasts are also seen in the later stages leading to pulmonary fibrosis. Lung fibroblast activation leads to collagenous deposition and eventually lung fibrosis. In humans, TNF α is also induced by bleomycin.[18,19] The free radicals that contribute to lung damage are produced by bleomycin directly after oxidation of bleomycin Fe (II) complex. Administration of dexrazaxone and amifostinefrequently prevents the development of Bleomycin Induced Pneumonitis.

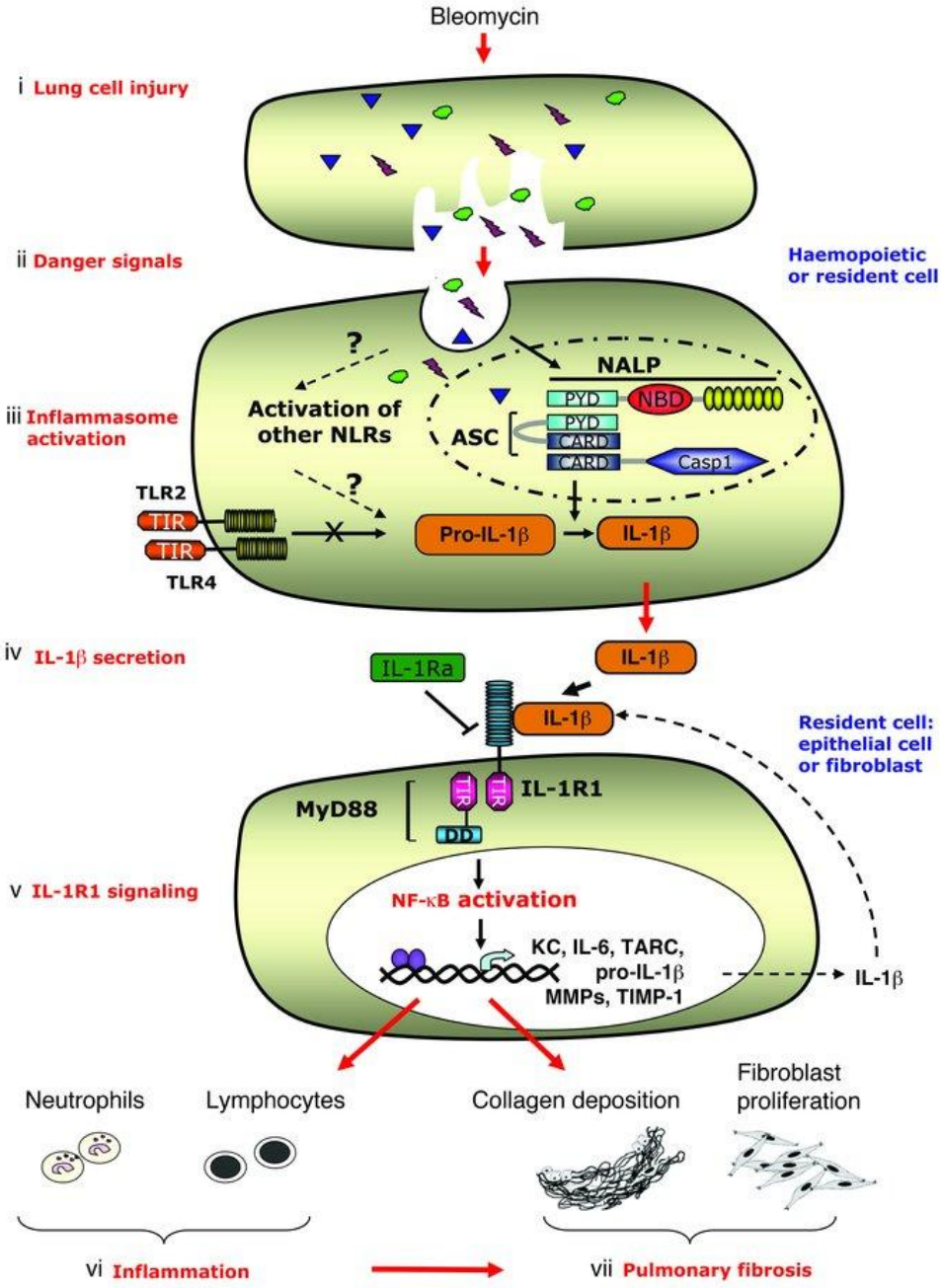


Fig 2. Schematic diagram showing the signalling pathways and the specific cascade after bleomycing lung injury.[17]

Clinical features of pulmonary toxicity:

Bleomycin Induced Pneumonitis (BIP) begins gradually during treatment. There are no pathognomonic clinical features of BIP and hence, the diagnosis of BIP is a diagnosis of exclusion. Other common clinical scenarios like pneumonia, lung metastases, pulmonary embolism or lymphangitic carcinoma have to be considered and then ruled out while contemplating BIP.[20]

Patients with pulmonary toxicity may present with nonspecific complaints like nonproductive cough, dyspnea, tachypnea, cyanosis and fever. Clinical examination may reveal fine bibasilar crepitations, tachypnea and sometimes pleural rub. Chest radiography reveals bibasilar infiltrates, sometimes diffuse interstitial and alveolar infiltrates.[21] Occasionally patients might have unilateral signs and imaging findings. Chest CT scan may reveal nodular opacities.[21,22] Transbronchial lavages are usually inconclusive. Sputum culture and sensitivity are usually done to rule out pneumonia. Patients are then treated on antibiotics before this entity is suspected.

Various studies have been carried out to document the risk factors for development of pulmonary toxicity and to find out a suitable test which can predict the development of pulmonary toxicity. Several studies have been conducted to study the changes in PFTs and DLCO. Among all the various

tools to detect BPT, PFTs and DLCO seem to be the most appropriate tool to try and detect preclinical lung toxicity. Although there have been conflicting reports, most experts agree that if DLCO drops by more than 40%-60% of baseline value then the drug should be stopped.

Pulmonary Function Tests:

These are tests that evaluate one or more major aspects of the respiratory system- Lung volumes, airway function and gas exchange. Pulmonary function tests consist of various components:

1. Spirometry
2. Diffusion studies – DLCO (CO diffusion coefficient)
3. Residual volume and Total Lung capacity

PFTs are used to evaluate and monitor diseases that affect heart and lung function, to monitor the effects of environmental, occupational and drug exposures and to assess risks of surgery.[23]

Spirometry:

Spirometry is the measurement of air into and out of lungs during various breathing maneuvers as a function of time. John Hutchison (1811-1861) invented the spirometer and the term Vital capacity. Original spirometer consisted of a calibrated bell turned upside down in water.

Spirometry can yield the following values:

1. Forced Vital Capacity (FVC) and its derivatives (FEV1, FEV 25-75)
2. Forced inspiratory vital capacity
3. Peak Expiratory Flow rate (PEFR)
4. IC, IRV and ERV
5. Pre and Post bronchodilator studies

There are 4 volumes- Inspiratory reserve volume (IRV), Tidal volume (TV), Expiratory reserve volume (ERV) and Reserve volume. 2 or more volumes comprise a capacity. There are 4 capacities – Vital capacity (VC), Inspiratory capacity (IC), Functional residual capacity (FRC) and Total Lung capacity (TLC).

DLCO	Diffusing capacity of the lung; the capacity of the lungs to transfer carbon monoxide (mL/min/mm Hg)
DLCOc	The DLCO adjusted for hemoglobin (mL/min/mm Hg)
DLVA	The DLCO adjusted for volume (mL/min/mm Hg/L)
DLVC	The DLCO adjusted for both volume and hemoglobin (mL/min/mm Hg/L)
ERV	Expiratory reserve volume; the maximum volume of air that can be exhaled from the end-expiratory tidal position (L)
FET	Forced expiratory time; the amount of time the patient exhales during the FVC maneuver (seconds)
FEV_1	Forced expiratory volume in 1 second; volume of air forcibly expired from a maximum inspiratory effort in the first second (L)
FEV_1/FVC ratio	Ratio of FEV_1 to FVC
FRC	Functional residual capacity; the volume of air in the lungs following a tidal volume exhalation = ERV + RV (L)
FVC	Forced vital capacity; the total volume that can be forcefully expired from a maximum inspiratory effort (L)
IC	Inspiratory capacity; the maximum volume of air that can be inhaled from tidal volume end-expiratory level; the sum of IRV and VT (L)
IRV	Inspiratory reserve volume; the maximum volume of air that can be inhaled from the end-inspiratory tidal position (L)
LLN	Lower limit of normal; the lowest value expected for a person of the same age, gender, and height with normal lung function
PEF	Peak expiratory flow; the highest forced expiratory flow (L/second)
RV	Residual volume; the volume of air that remains in the lungs after maximal exhalation (L)
TLC	Total lung capacity; the total volume of air in the lungs at full inhalation; the sum of all volume compartments (IC + FRC or IRV + V_T + ERV + RV) (L)
TV or VT	Tidal volume; the volume of air that is inhaled or exhaled with each breath when a person is breathing at rest (L)
VC	Vital capacity; the maximum volume of air that can be exhaled starting from maximum inspiration, TLC (L) can be measured either as slow vital capacity (SVC) or forced vital capacity (FVC)

Fig 3. Definitions of Lung volumes and Capacities used in PFTs.[24]

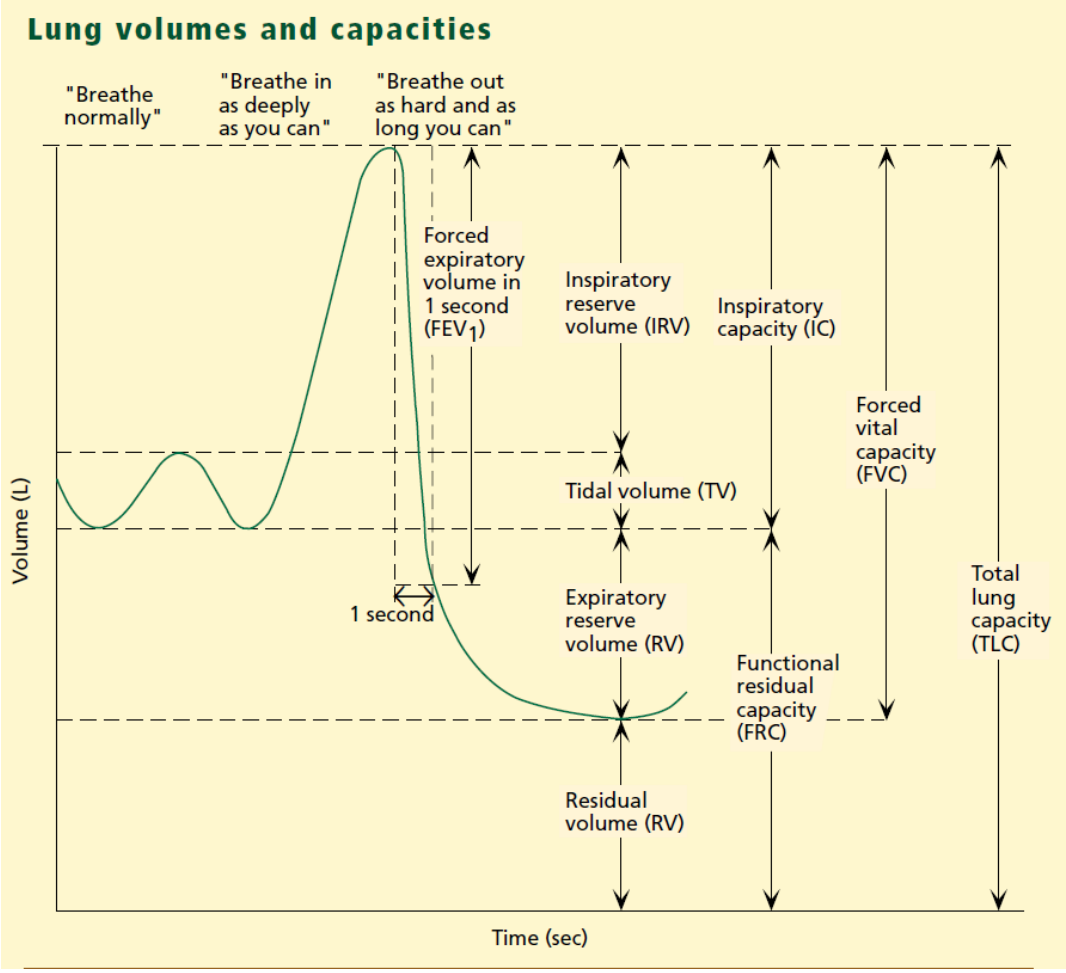


Fig 4. Lung Volumes and capacities depicted on a volume time spirogram.[24]

DLCO:

DLCO, also known as the transfer factor for CO (TLCO) is a measure of the ease of transfer of CO molecules from alveolar gas to the hemoglobin of the red blood cells in the pulmonary circulation. The single breath diffusion capacity of the lung for carbon monoxide was first described by Marie Krogh in 1915.

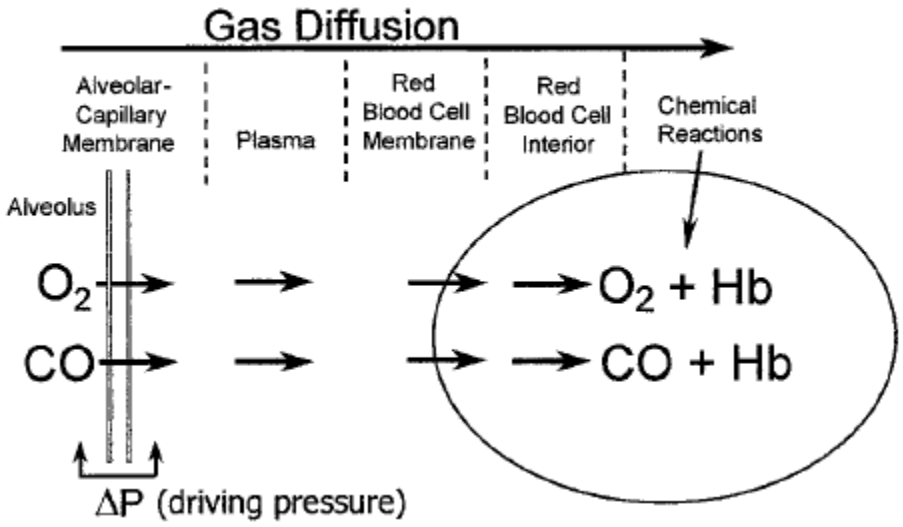


Fig 5. The gas diffusion pathway is similar for Carbon Monoxide (CO) and oxygen (O2).[25] Hb = Hemoglobin.

The efficiency of lung in transporting Oxygen from the alveoli to the Hemoglobin across the alveolocapillary membrane can be assessed by DLCO test. CO follows the same diffusion pathway as that of O_2. CO molecules bind to the same site in the hemoglobin molecule as that of the Oxygen molecule. Calculation of the DLCO has become simple now with the advent of computerized and automated testing machines. This is calculated during a single breath maneuver.

A tracer gas (helium, neon or methane) is used to measure the initial dilution of the inhaled CO. DLCO is calculated from this formula: [25]

$$D_{LCO} = \frac{\dot{V}_{CO}}{P_{ACO}}$$

VCO : rate of disappearance of CO

PACO : Alveolar concentration of CO, which is determined from the exhaled gas, after the anatomical dead space has been cleared.

In North America, the unit for DLCO is mL CO/ min/mmHg.

The SI unit is mmol/min/kPa, and in Europe it is called the CO transfer factor TLCO.[25]

DLCO = 2.986x TLCO

The patient inhales a gas comprising of 0.3% CO and a tracer (usually 0.3% CH4, 0.5% Ne or 1-5% He) and a measurement is made of exhaled concentration of CO and the tracer after a breath hold of 10 seconds. Several things are important to obtaining a proper DLCO. While measuring DLCO, several things are to be noted:

- There are interactions between subject and testing device
- There are interactions between technician and the subject
- There are interactions between technician and the measuring device

The patient:

- Should not have had meal within 2 hours of performing the test
- Should not have had recent strenuous exercise
- Should not have any ongoing or recent lung infections
- Should be fully cooperative
- Should understand the full instructions
- Should be able to be seated for the procedure

The patient should always be seated during the procedure. The use of nose clips is mandatory. The mouth piece and valves should be placed at a comfortable position for the patient. Almost all devices display the instructions for the pattern of breath holding to be followed by the patient.

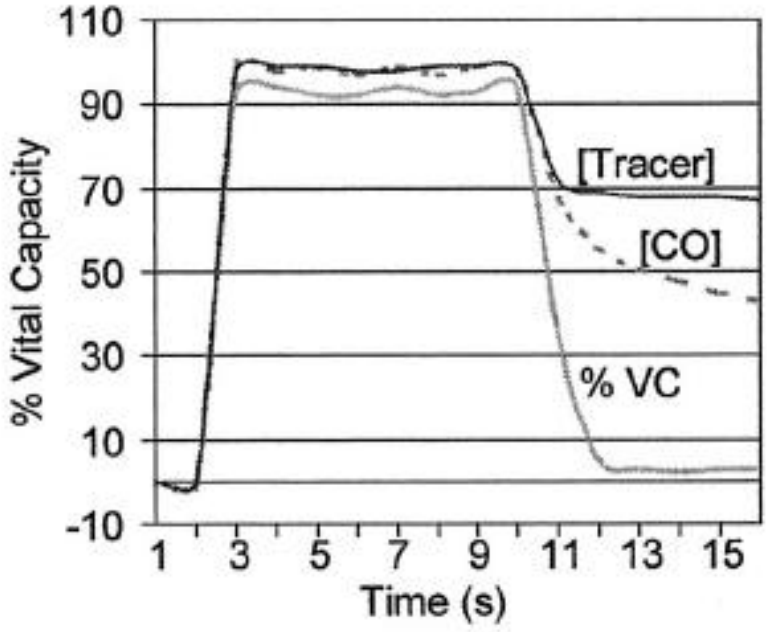


Fig 6.Ideal tracing of DLCO test. [25]

The DLCO can be affected by various factors: [25,26,27]

- Inspired volume
- Calibration
- Sufficient washout volume
- Representativeness of sample gas

Inspired volume is critical to the correct measurement of DLCO. CO uptake will be inappropriately reduced due to reduced membrane expansion.[26,27,28] Thus the inhalation must be at least 90% of the maximum measured vital capacity. In the present generation of newer machines, *calibration* is routinely done prior to each DLCO test by the machine itself. The washout volume is the volume of gas that must leave the lungs so that all remaining gas exiting the lungs is gas from the alveolar gas exchanging regions and not from the anatomical dead space. The ATS standards recommend minimum of 650 mL of washout volume. When problems are identified, it may be necessary to perform at least 2 but not more than 5 DLCO tests.[29,30,31] The reported DLCO range should be average of the first 2 tests that meet the reproducibility criteria. There should be at least 5 minutes gap between successive DLCO tests. Proper DLCO results may be obtained by paying attention to proper calibration of equipment, training of personnel, patient education and maintenance of quality control of the process. [29,30,31,32]

Radiologic Features:

Chest radiographs may vary from no abnormalities to reveal linear streaking, to bilateral basal reticulonodular infiltrates, diffuse interstitial and alveolar infiltrates.[21,33] Diffuse alveolar damage manifests radiographically as bilateral hetero- or homogeneous opacities usually in the mid and lower lungs. Computed tomographic (CT) scans reveal scattered or diffuse areas of ground glass opacity. Very rare isolated case reports of pneumothorax and pneumomediastinum have also been attributed to bleomycin containing chemotherapy.[33] BOOP appears on chest radiography as heterogenous and homogeneous peripheral opacities in both upper and lower lobes. CT scan may reveal BOOP as a nodular consolidation and centrilobular nodules.[21,33]

Risk factors for development of Lung Toxicity:

There are several clinical characteristics that may increase the likelihood of developing Bleomycin pulmonary toxicity. These include cigarette smoking, increasing age, renal dysfunction, concurrent chest irradiation, oxygen administration, usage of Granulocyte colony stimulating factor (G-CSF), cumulative dosage of bleomycin, mode of administration.[4,5,35] Carlson et al mention that continuous infusion of Bleomycin has lesser pulmonary toxicity.[36] Bleomycin administered by intramuscular route also causes lesser toxicity than bolus route. There is only 2-3% chance of lung

toxicity if the cumulative dosage <300mg, however the risk increased to 20% if cumulative dosage>500 mg. [5,37]

Linear dose toxicity relationship has been seen in animals. However, in humans, fatal Bleomycin toxicity has occurred at doses below 100 U, while in others >500 U of Bleomycin has not caused any toxicity.

Table 1. Incidence of Bleomycin pulmonary toxicity with increasing total dose of BLM.[38]

Total dose (mg)	% Toxicity	Mortality (%)
0-49	**3**	**-**
50-149	**4**	**-**
150-249	**4**	**<1**
250-349	**5**	**-**
350-449	**5**	**-**
450-549	**13**	**5**
>550	**17**	**10**

Bauer K et al observed that the incidence of lung complications were more in patients who received Bleomycin as part of combination chemotherapy regimens than when they received Bleomycin as single agent.[39] They observed that only a median dose of 36 units was causing a high

incidence (18% of patients) of reversible acute pulmonary reactions in patients receiving M BACOD regimen for Hodgkin Lymphoma. This may imply that interactions with other antineoplastic agents may potentiate its toxicity. Bauer et al also observed that younger patients receiving PVB regimen for germ cell tumors had a 6.3% incidence (5 of 79 patients) of having lung toxicity. An additional 7% of the patients had asymptomatic radiographic abnormalities.

Enhanced pulmonary toxicity has been described in patients with renal insufficiency. Bleomycin is cleared rapidly with a terminal half life of 1 to 1.5 hours. However, if creatinine clearance is 10ml/min then, the half life of Bleomycin is 21 hours. McLeod et al[40] reported a case of 54 yr old gentleman who developed fatal pulmonary toxicity after a cumulative dosage of only 60 U of Bleomycin during chemotherapy of Non Hodgkin Lymphoma. Other investigators also noted renal dysfunction as a significant predictive factor for BPT.[41,42]

Cigarette smoking is a risk factor for development of Bleomycin lung. Senan Suresh et al assessed 71 patients who had completed chemotherapy for testicular germ cell tumor.[34] Vital capacity (VC), Total Lung capacity (TLC) and single breath diffusion study of CO (KCO) were studied. Smokers had a significantly less Total Lung Capacity (TLC) and Vital Capacity (VC). A slower rate of recovery also was seen post 2 yr in smokers. Impaired lung

function measurements recovered completely in non smokers, however, smokers had persisting lung function abnormalities on follow up. A possible explanation for this lung function abnormality is the production of hydrogen peroxide by alveolar macrophages, which is not the case with alveolar macrophages in non smokers.

Some investigators have reported that administration of growth factors (GCSF) may increase the chance of Bleomycin toxicity. Mathews et al described 5 patients with Hodgkin lymphoma who received ABVD chemotherapy and GCSF. Four of these five patients developed features of pulmonary toxicity after cumulative dosage of 70U only.[43] Saxmanet al[44] retrospectively analyzed 2 groups of patients who received BEP chemotherapy with or without GCSF support. They did not find any significant difference in the incidence or severity of lung toxicity in these 2 groups of patients.

Elderly patients have an increased likelihood of bleomycin lung toxicity. Simpson et al mention that the incidence of BPT increases with every decade after 30 years of age.[45]

Hirsch et al[46] prospectively evaluated the effect of ABVD chemotherapy alone and of ABVD with chest irradiation (mantle or mediastinal RT) in 60 patients with early Hodgkin Lymphoma. PFTs were

done upfront, during, at completion of planned therapy and also at various intervals in the follow up period. During chemotherapy, cough and dyspnea were noted in 32 of 60 (53%) patients and lung function measurements occurred in 22 of 60 (37%) patients. While 72% of patients developed either clinical symptoms or PFT decline, only 11 patients (18%) had both clinical symptoms and PFT decline, however this association was not significant. Patients had decline in FVC following chemotherapy. Following RT a further significant decline in FVC occurred, however the DLCO remained stable. This did not significantly alter the functional status of the patients.

Prevention and Treatment of Bleomycin Pulmonary Toxicity:

One of the most effective ways of preventing Bleomycin pulmonary toxicity is to decrease the cumulative dose of Bleomycin administered. The same has been tested by various cooperative groupsin multiple trials. It has been shown by Einhorn et al that 270 U of BLM is sufficient in good prognosis germ cell tumors.[47,48] In patients with high risk of development of BPT, alternative chemotherapy regimens like VeIP (for GCTs of testis and ovary patients) and non Bleomycin protocols like COPP,AVD or GVD may be considered in HL. Several agents have been tested in animals to treat BLM toxicity vz. soluble Fas antigen, Il 1 antagonists, Keratinocyte growth factor, cyclosporine and antibodies against TNF. None of these have however been successful in human studies. When clinical BPT occurs, steroids have been

used in high doses (Prednisolone 60-100 mg/day). There are conflicting opinions regarding role and efficacy of steroids in Bleomycin pulmonary toxicity. White Dorothy et al[49]described their observations about 10 patients who developed severe BIP and the outcome of steroid administration. 7 of the 10 patients were started on high dose steroids. There was a clear improvement in signs and symptoms when steroids were started. Steroids needed to be given for several months before being tapered. 3 of these 7 patients died later. If a patient receiving BLM chemotherapy suddenly develops features of BPT then steroids can be safely started.[4,5,50] However, in patients who develop insidious onset dyspnea, cough, then role of steroids is uncertain.

Pulmonary function assessments:

Given the serious nature of pulmonary toxicity of Bleomycin, various attempts have been made to indentify the patients at risk. Screening strategies for early Bleomycin lung toxicity included biomarkers, bronchoalveolar lavage cell counts, chest radiographs, CT scans of the chest and PFTs. There are conflicting reports regarding the usefulness of PFTs to predict the toxicity of BLM. Most of these studies either had small patient size or had heterogenous group of patients receiving different chemotherapeutic protocols. Wolkowicz et al studied the usefulness of serial PFTs in Non seminomatous Germ cell tumor patients receiving BVP chemotherapy (BLM, vinblastine, CDDP). They found that Total Lung Capacity (TLC) is far more specific in

identifying patients who are developing symptomatic or radiologic BIP. DLCO could not differentiate patients with BIP from those without. Lewis et al[52] noted that 50% of patients studied (11 out of 21 patients) had false elevations of DLCO and FVC. These values may be affected by generalized weakness or anemia which may lead to false negatives. Hence they concluded that routine pulmonary function testing and DLCO during Bleomycin therapy may be ineffective and may be potentially misleading. MacKeage et al[53] also made similar observations. They retrospectively analyzed serial PFTs done in 81 patients receiving Bleomycin containing chemotherapy. Six of 81 patients developed clinical features of BPT. DLCO predicted the development in only 1 patient (sensitivity 16%). Respiratory symptoms and chest x-ray abnormalities were the earliest manifestations in the other five patients. Seventy-five of 81 patients did not develop clinically significant bleomycin lung, and 12 of these had major falls (greater than or equal to 35% pretreatment level) in DLCO (specificity, 63 of 75 patients; 84.0%). Bleomycin was continued after a major fall in DLCO in eight patients, and none developed clinically significant lung toxicity. In this study, the DLCO failed to predict the development of serious bleomycin lung toxicity in all but one case. Furthermore, in some patients, bleomycin may be stopped inappropriately after low DLCO measurements. Hence they concluded that clinicians should watch for respiratory symptoms and chest x-ray abnormalities during bleomycin treatment as these will be the earliest signs of

lung toxicity in most cases. Bell et al[54] also observed that DLCO could not predict the fatal pulmonary toxicity seen in 2 patients. They also observed that the other patients who had a fall in DLCO never showed any features of toxicity (either clinical or radiological). Although all these data are conflicting, experts advise stopping Bleomycin when DLCO decreases by more than 40-60% of the pretreatment value.

In conclusion, BPT is a very serious and sometimes fatal side effect of Bleomcin. Identification of risk factors also reduces the morbidity and mortality of BPT. Combined with clinical signs and symptoms, PFTs and DLCO are probably suitable for picking up BPT at early stage.

STUDY	Nature of study	No of pts	Diagnosis	Predictive Risk factors	Clinical Features	DLCO	VC	Outcome
Ng et al	**Prospective**	**52**	**HL**	**High baseline DLCO, Smoking**	**Cough dyspnea**	**Decreased>15%**	**NS**	**6 BIP**
Hirsch et al	**Prospective**	**60**	**HL**	**--**	**Cough dyspnea**	**Decreased**	**NS**	**1 death 14 pts BIP**
Sleijfer et al	**Prospective**	**27**	**Testis GCT**	**VC**	**Dyspnea cough**	**Decrease d**	**Significant**	**3BIP**
MacKeage et al	**Retrospective**	**81**	**Testis GCT, HL**	**Symptoms, Radiographic features**	**Dyspnea**	**PFTs not predictive**	**PFTs not predictive**	**6 (7.5%)BIP,**
Duggan et al	**Prospective EORTC**	**856**	**HL**	**Symptoms and radiographic features**	**dyspnea**	**PFTs not predictive**		**24% had BPT**

PATIENTS AND METHODS

STUDY DESIGN

A total of 76 patients were included in this single centre prospective study to document the symptomatology and changes in pulmonary function tests and single breath Carbon monoxide diffusion coefficient (DLCO) in patients with Hodgkin lymphoma and Germ cell tumors of ovary and testis who received Bleomycin containing chemotherapy regimens from June 2010 to Feb 2012.

Inclusion Criteria:

Between June 2010 and October 2011, all the patients with a diagnosis of Hodgkin Lymphoma, Germ cell tumors of Ovary and Testis who were more than 15 yrs of age and who received Bleomycin containing chemotherapy protocols (ABVD, Hybrid i.e COPP/ABV, BEACOPP, BEP) were included in the study. These patients were followed up until Feb 2012 so that at least one set of interim PFTs is obtained of the patients enrolled in the later part of study period.

METHODS:

Patients with Hodgkin Lymphoma received ABVD, Hybrid or BEACOPP chemotherapy as per the institute guidelines. The total number of chemotherapy cycles ranged from 2-8 cycles. Involved Field Radiation Therapy (IFRT) was given to patients with bulky sites as per protocol and after discussion in Medical Oncology board. Patients with germ cell tumors received 2-3 BEP chemotherapy cycles. The treatment given to each patient was according to institution guidelines which were also discussed in medical oncology board prior to administration to the patient.

Pulmonary function tests comprising of spirometry (VC, FVC, FEV1, FEV1/FVC) and Single breath carbon monoxide diffusion coefficient (DLCO) were done at the following intervals:

- **Baseline Evaluation**
- **Interim evaluation** (which was done after the patient has received approximately 80-100 U of Bleomycin)
  - In Hodgkin Lymphoma: prior to the 4^{th} cycle of ABVD, after 6^{th} cycle of Hybrid chemotherapy.
  - In germ cell tumors of ovary and testis: after the 1^{st} BEP chemotherapy cycle.
- **At the end of planned chemotherapy**

- **At 6 months of Follow Up**

A history and physical examination was done prior to each planned lung function testing to document any features of bleomycin induced pulmonary toxicity. Various parameters like symptomatology pertaining to BPT (cough, dyspnea, chest pain), risk factors like history of smoking, other lab parameters (Hemoglobin, creatinine) were noted. All patients received standard doses of protocol chemotherapy as mentioned below:

ABVD	
Adriamycin	25 mg/m² IV bolus D1 & D15
Vinblastine	6 mg/m² IV bolus D1 & 15 (Cap at 10 mg)
Bleomycin	10mg/m² IV bolus D1 & 15
Dacarbazine	375mg/m²IV infusion D1 &15
Hybrid COPP & ABV	
Cyclophosphamide	800 mg/m² IV infusion D1
Vincristine	1.4 mg/m² IV bolus D1
Procarbazine	100 mg/m² Oral D1-7 PO
Prednisolone	40 mg/m² Oral D1-14 PO
Adriamycin	35 mg/m² IV bolus D8
Vinblastine	6 mg/m² IV bolus D8
Bleomycin	10mg/m² IV bolus D8

BEACOPP - 21 day cycles	
Bleomycin	10 mg/m² IV D8
Etoposide	100 mg/m² IV D1-3
Adriamycin	25 mg/m² IV D1
Cyclophosphamide	650 mg/m² IV D1
Vincristine	1.4 mg/m² IV D8
Procarbazine	100 mg/m² D1-7 PO
Prednisolone	40 mg/m² D1-14 PO
BEACOPP - Escalated 21 day cycles	
Bleomycin	10 mg/m² IV d8
Etoposide	200 mg/m² IV d1-3
Adriamycin	35 mg/m²IV d1
Cyclophosphamide	1250 mg/m² IV d1
Vincristine	1.4 mg/m² IV d8
Procarbazine	100 mg/m² d1-7 PO
Prednisolone	40 mg/m² d1-14 PO
G - CSF	s/c d8+

Bleomycin Pulmonary toxicity Definition:

A patient was diagnosed to have BPT if he had the following features:

Clinical Symptoms:

- Dry Cough
- Dyspnea
- Chest pain
- Bibasilar crepitations

Radiographic Features: (infection has to be ruled out)

- Fine bibasilar reticular infiltrates
- Bibasilar interstitial infiltrate (producing ground glass appearance)
- Progressive lower lobe involvement
- Subpleural nodular opacities

PFT changes:

- Drop in absolute level of corrected DLCO (Kco) >20%
- Drop in level of corrected DLCO >40% of pretreatment value

Other causes of fever, cough, dyspnea, chest pain like pneumonia, pulmonary thromboembolism, pulmonary hemorrhage, progressive disease etc were ruled out prior to entertaining diagnosis of Bleomycin Pulmonary Toxicity (BPT).

Measurements of Spirometry, lung volumes and DLCO were done prospectively for each patient. Each patient was explained in detail about the procedure of the lung function tests. Every patient performed spirometry upfront (to get baseline lung function studies like vital capacity, forced vital capacity, Forced expiratory volume, FEV1/FVC, DLCO, Functional residual capacity and total lung capacity).The patient inhaled a gas comprising of 0.3% CO and a tracer (usually 0.3% CH4, 0.5% Ne or 1-5% He) and a measurement was made of exhaled concentration of CO and the tracer after a breath hold of 10 seconds. A tracer gas (helium, neon or methane) is used to measure the initial dilution of the inhaled CO. DLCO is calculated from this formula: [25]

$$D_{LCO} = \frac{\dot{V}_{CO}}{P_{ACO}}$$

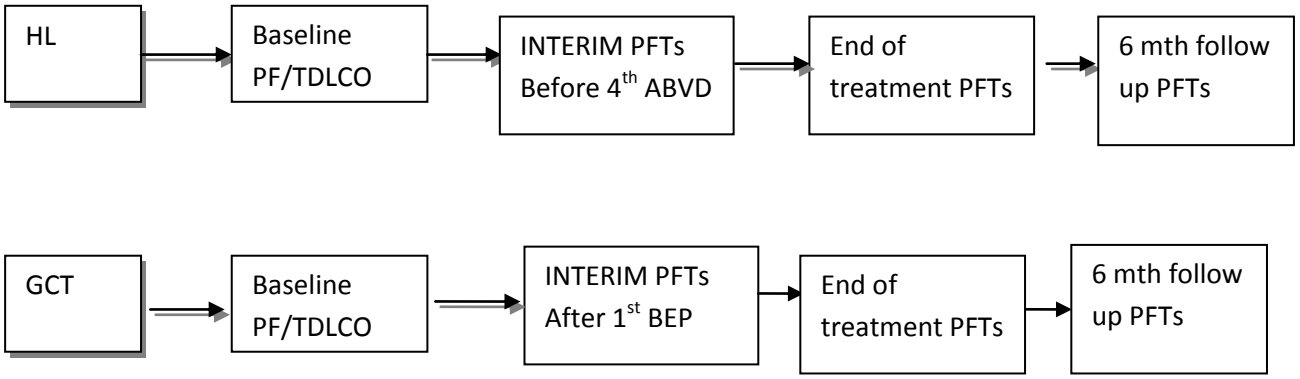
VCO **:** rate of disappearance of CO

PACO **:** Alveolar concentration of CO, which was determined from the exhaled gas, after the anatomical dead space has been cleared. The measured value was corrected for Hemoglobin. The correction is done by using the **Cotes' formula:** [25]

DLCO adj (KCO) = DLCO measured X (10.22+HB)/ 1.7 X HB

[Kco= Corrected DLCO, HB= Hemoglobin in gm/dL]

These studies were then repeated prior to the 4th ABVD and 7th Hybrid chemotherapy in patients with Hodgkin Lymphoma. In patients with Germ Cell Tumors, these tests were done before and after the 1st BEP chemotherapy. These lung function tests were repeated after the completion of planned chemotherapy. If the patient was suspected to have BPT, then the PFTs and DLCO were repeated and values documented. The basic clinical details, laboratory parameters, radiographic features of BPT and the PFTs were documented. The same tests were done at 6 months of follow up of the patient. PFTs were performed on an automated unit. A significant decline in PFTs was defined as a reduction from a baseline FVC of greater than 15%, FEV1 greater than 15%, or DLCO greater than 20%. Pulmonary symptoms of cough and dyspnea were noted regularly during each visit and during follow up visits. Radiographic evidence of bleomycin pulmonary toxicity was defined as pulmonary interstitial infiltrate or fibrosis on plain chest X ray or CT chest (in the absence of infection).



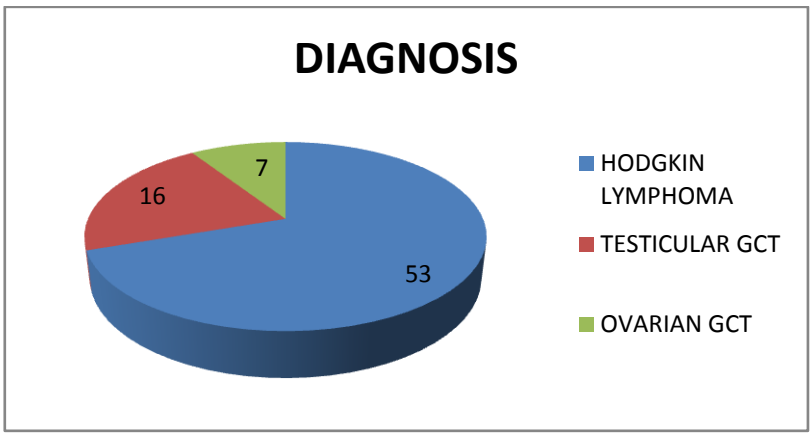
STATISTICAL ANALYSIS:

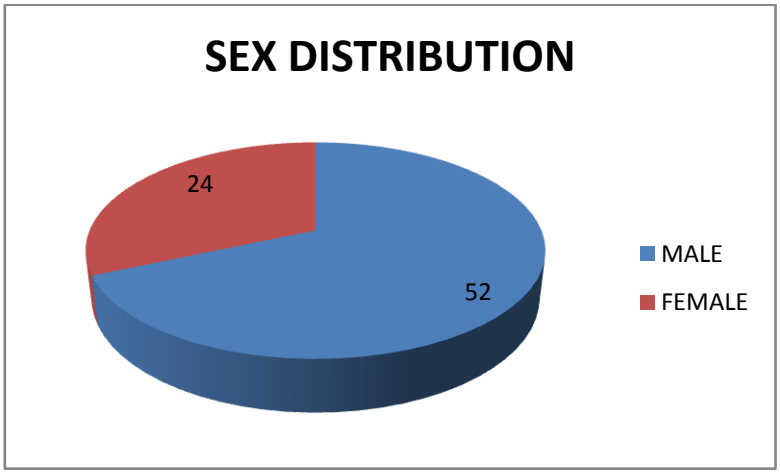
Descriptive statistics were used to describe patient characteristics as frequencies. Statistical analysis was carried out using the SPSS version 13 software. The actual values of the lung assessments are mentioned in mean values (+/- SD) or median values (range) and categorical data are presented as proportions. In all statistical analyses, $p<0.05$ is considered as significant. The association of incidence of abnormal PFT declining values with patient factors, respiratory symptoms and radiologic changes was examined. The serial PFT and DLCO values were compared using the paired sample "T" test.

RESULTS

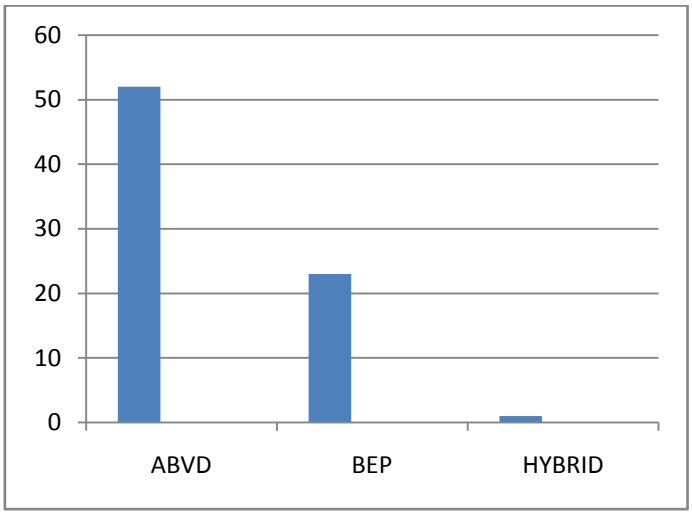
76 patients who received Bleomycin containing chemotherapy for Hodgkin Lymphoma, Germ cell tumors of Testis and Ovary were included in the study.

Distribution of Diagnosis:





Distribution of chemotherapy:



Age Distribution:

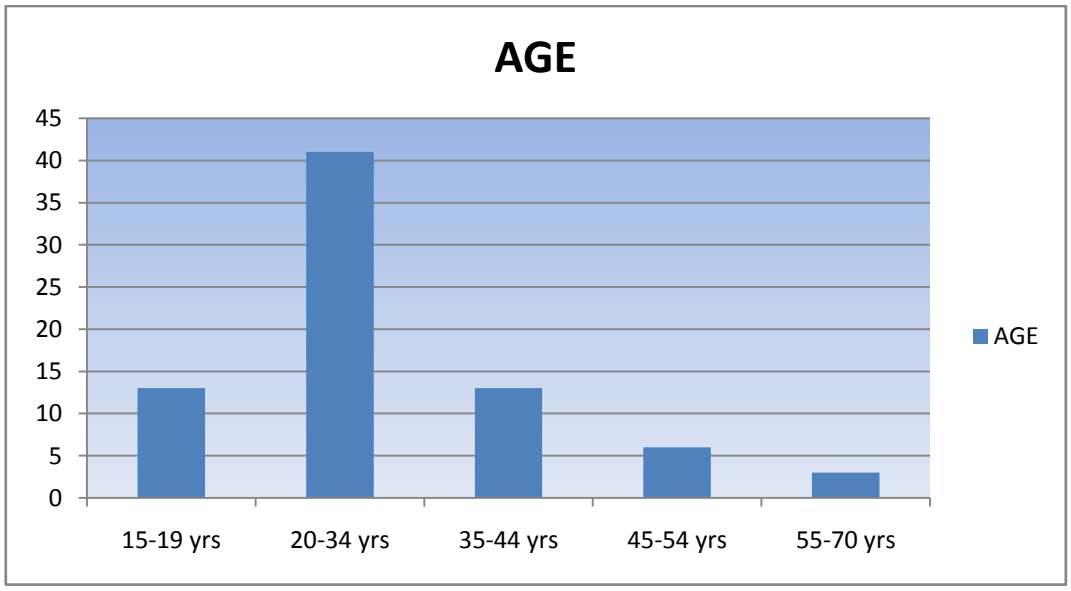


Table 2.Details of the patients in the study.

Parameters	N (%)	Median value (range)
Age	76 patients	27 (15-70)
Male	52 (68%)	
Female	24 (32%)	
Diagnosis HL	53	
GCT testis	16	
GCT Ovary	07	
Smokers	66 (14%)	
Chemotherapy ABVD	52 (68%)	
Hybrid	01 (1.3%)	
BEP	23 (30%)	
Hemoglobin (gm/dL)		11.6 (5.6-16.5)
Creatinine (mg/dL)		0.7 (0.5-1.1)
Chest irradiation	4 (5.2%)	

Chemotherapy Cycles:

Patients received a minimum of 1 bleomycin containing cycle to a maximum of 8 cycles (ABVD/ Hybrid/BEP). Median number of chemotherapy cycles delivered was 6.

NO OF CHEMOTHERAPY CYCLES	NO OF PATIENTS
1	1
2	9
3	18
4	15
5	4
6	23
8	6

The minimum cumulative bleomycin dose received by a patient was 60 U. A total of 6 patients received cumulative dose of 120 U of BLM. 10 patients received total of 120 U of Bleomycin. 18 patients received a total dose of 180 U of bleomycin. 16 patients received a dose of 270 U of bleomycin. Median dose received by the patients was 180 U.

Pulmonary Function Tests:

Pretreatment (Baseline) pulmonary function tests:

Various spirometry parameters were documented when the PFTs were done by the patients. The following table gives the information about the values of the lung tests.

Table 3.baseline evaluation of pulmonary function (n=76)

	VC 1	FVC 1	FEV11	DLCO1	KCO1	TLC1
Mean	64.91	65.61	74.01	84.49	94.42	82.47
Median	66	66	74	85.5	95.5	85
Std. Deviation	15.02	13.777	15.867	16.907	17.711	16.667
Minimum	25	24	21	41	52	33
Maximum	93	93	118	124	134	131
Expected N=76						

VC1: Pretreatment Vital Capacity, FVC1: Pretreatment Forced Vital Capacity,

FEV11: Pretreatment Forced Expiratory Volume in 1 second, DLCO1: Pretreatment Diffusion Coefficient

of CO, TLC1: Pretreatment Total Lung Capacity

Patients had median baseline values of VC (66 %), FVC (66%), DLCO (85%), KCO (95%) and a TLC (85)

Interim Pulmonary Function Assessment:

Following table depicts the interim changes in the pulmonary function parameters, which was approximately done when the patient has received about 80-100 U of Bleomycin.

Tab 4 Median Values of Interim Lung Function Parameters (N= 71)

	VC2	FVC2	FEV12	DLCO2	KCO2	FRC2	TLC2
Mean	65.96	67.27	74.58	83.2	90.39	91.93	84.92
Median	68	67	73	84	90.5	88	87
Std. Deviation	14.383	12.284	13.814	13.978	13.467	26.615	14.261
Range	73	63	85	63	57	157	87
Minimum	32	33	36	51	60	10	46
Maximum	105	96	121	114	117	167	133
Expected N=76							

VC2: Interim Vital Capacity, FVC2: Interim Forced Vital Capacity, FEV12: Interim Forced Expiratory Volume in 1 second, DLCO2: Interim Diffusion Coefficient of CO, TLC2: Interim Total Lung Capacity

End of Treatment Pulmonary Function Tests:

The following table depicts the PFT parameters done at the completion of planned bleomycin containing chemotherapy.

Tab 5. End Of treatment median PFT values (N= 59)

	VC3	FVC3	FEV13	DLCO3	KCO3	FRC3	TLC3
Mean	65.39	68.31	75.08	81.46	87.23	93.05	81.17
Median	67	71	74	80	87	96	85
Mode	73	71	56(a)	69(a)	95	110	80(a)
Std. Deviation	14.385	15.094	15.949	16.46	16.327	26.824	15.487
Range	58	65	69	76	71	149	76
Minimum	33	37	43	45	50	31	41
Maximum	91	102	112	121	121	180	117
Expected N=76							

VC3: End of treatment Vital Capacity, FVC3: End of treatment Forced Vital Capacity, FEV13: End of Treatment Forced Expiratory Volume in 1 second, DLCO3: End of treatment Diffusion Coefficient of CO, TLC3: End of treatment Total Lung Capacity.

6 month Follow Up Pulmonary Function Assessments:

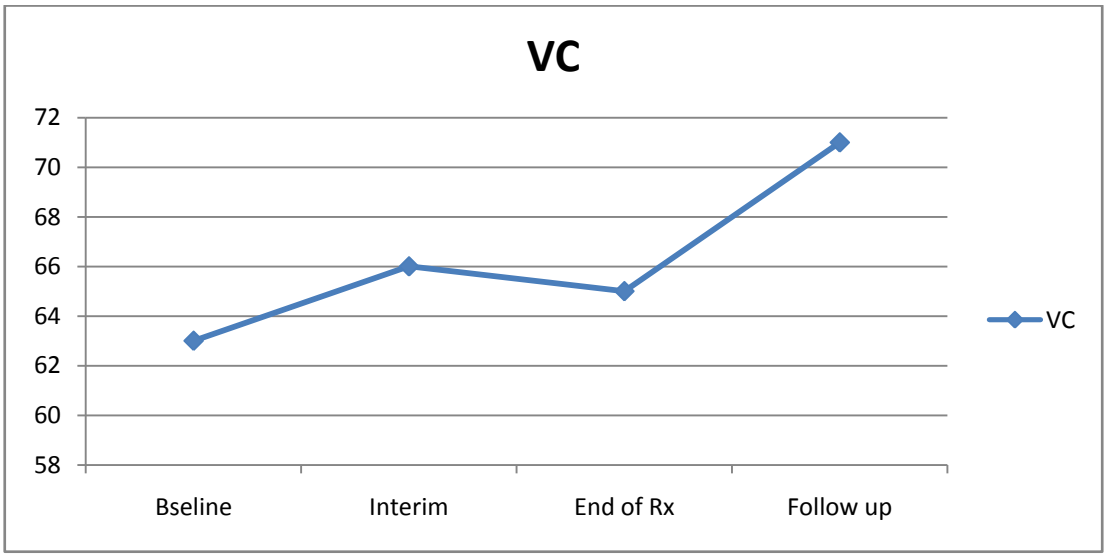
Tab 6. 6 month Follow up median PFT values (N=28)

	VC4	FVC4	FEV14	FEV1/ FVC4	DLCO 4	KCO	FRC4	TLC4
Mean	71.89	70.29	77.86	115.36	86.64	84.25	406.37	83.81
Median	68	70	78	117	86	86.5	95	80
Std. Deviation	13.566	11.585	15.335	8.202	13.423	14.119	1592.87	14.15
Range	57	50	67	41	73	47	8317	64
Minimum	42	42	36	89	54	59	58	63
Maximum	99	92	103	130	127	106	8375	127

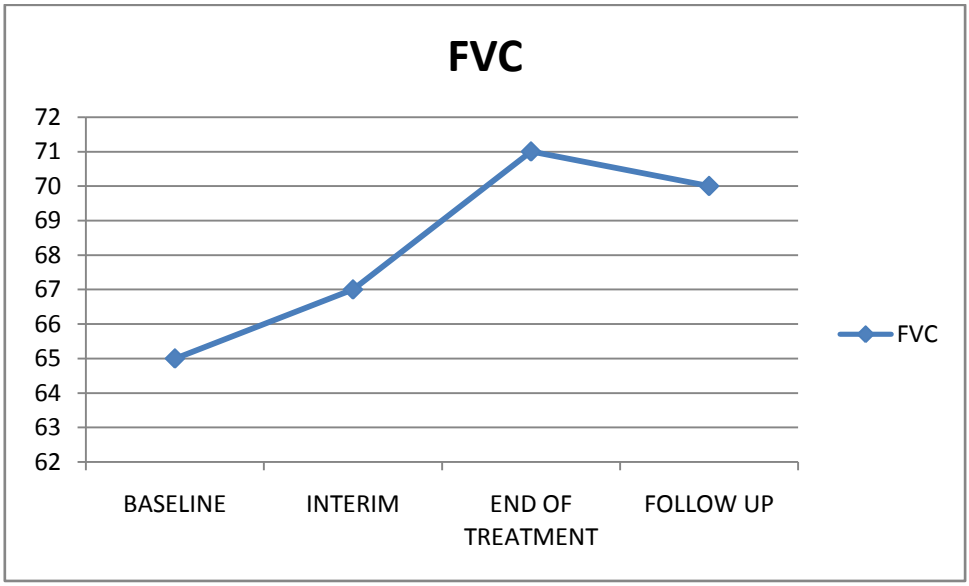
Trends in the Changes of Parameters over time:

The serial median values of the lung function parameters (VC, FVC, FEV1, corrected DLCO, TLC) have been plotted to understand the trends of their changes over time. Following are the line charts that depict these changes. The X axis has numerics 1-4 which denotes the four time points when these PFTs were repeatedand the Y axis plots the median values of each of the variables.

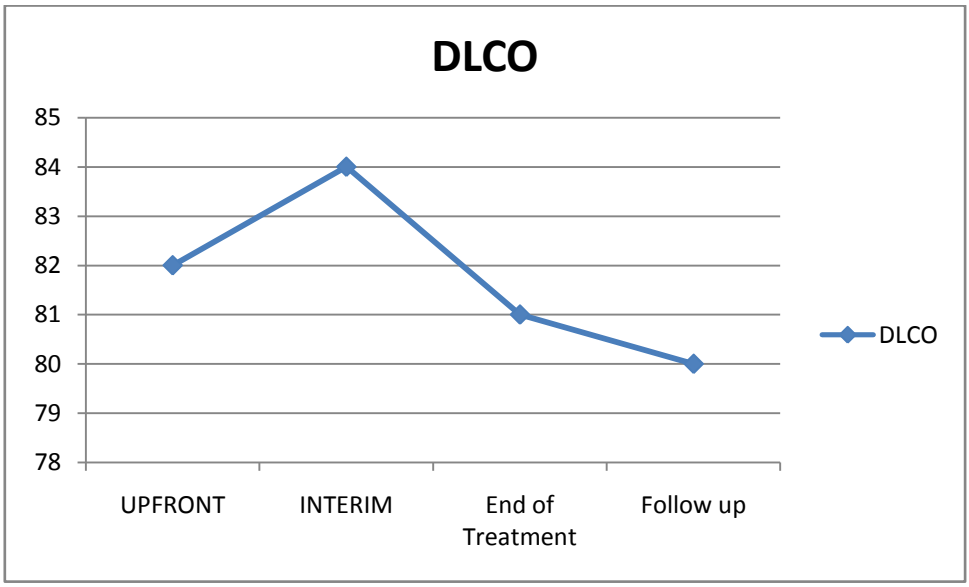
Vital Capacity



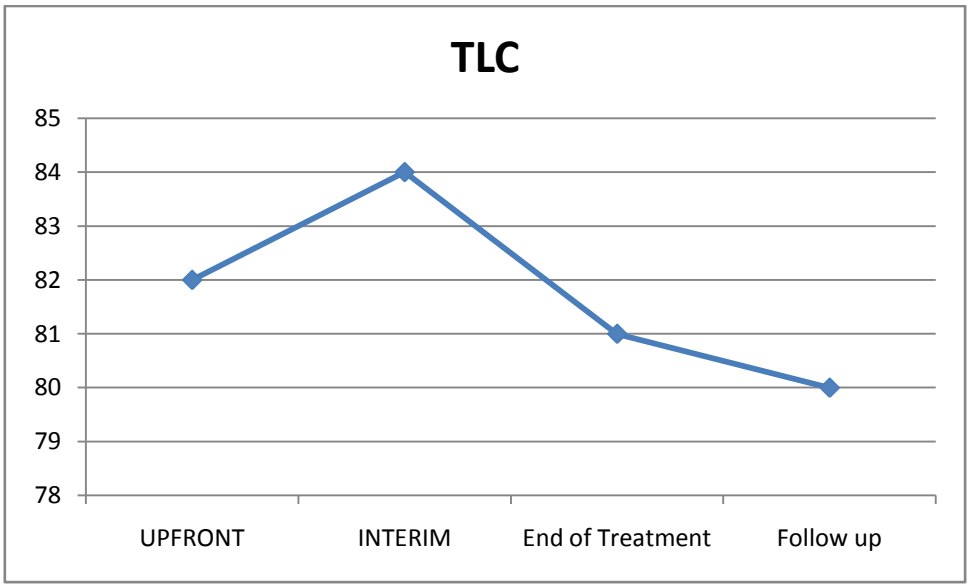
Forced Vital Capacity:



Corrected DLCO:



Total Lung Capacity (TLC):



Significance of changes of PFTs over time:

Tab 7 Significance of Upfront and Interim PFT values

	Mean	SEM	Sig (2 tailed) p Value
VC 1 - VC2	-1.563	1.727	0.368
FVC 1 - FVC2	-2.257	1.558	0.152
TLC1 - TLC2	-1.972	2.004	0.329
DLCO1 - DLCO2	1.606	1.54	0.301
FEV11 - FEV12	-1.056	1.773	0.553

SEM: Standard Error of Mean

There was no statistical significance when the median values of the parameters of the upfront and interim PFTs were compared with the 2-tailed test.

Tab 8 Significance of Upfront and End Of treatment PFTs

	Mean	SEM	Sig (2- tailed) p value
VC 1 - VC3	-2.288	2.135	0.288
FVC 1 - FVC3	-4.466	2.071	0.035
TLC1 - TLC3	-0.475	2.118	0.824
DLCO1 - DLCO3	1.898	2.311	0.415
FEV11 - FEV13	-3.458	2.261	0.132

When the values of upfront and end of treatment PFTs were compared with the 2 – tailed test then only the change in FVC was found to be significant.

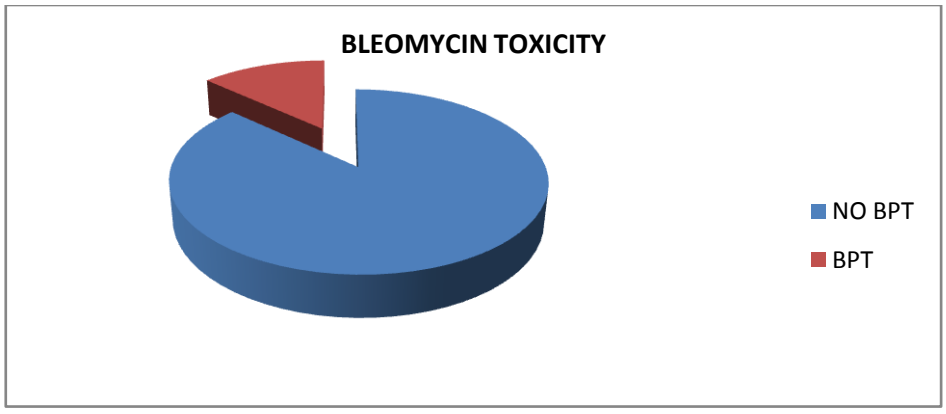
Tab 9 Significance of values of Upfront and Follow up PFTs

	Mean	**SEM**	**Sig (2- tailed) p values**
VC 1 - VC4	-8.107	2.993	0.012
FVC 1 - FVC4	-4.741	2.566	0.076
TLC1 - TLC4	-1.926	4.249	0.654
DLCO1 - DLCO4	-0.5	3.045	0.871
FEV11 - FEV14	-4.643	3.019	0.136

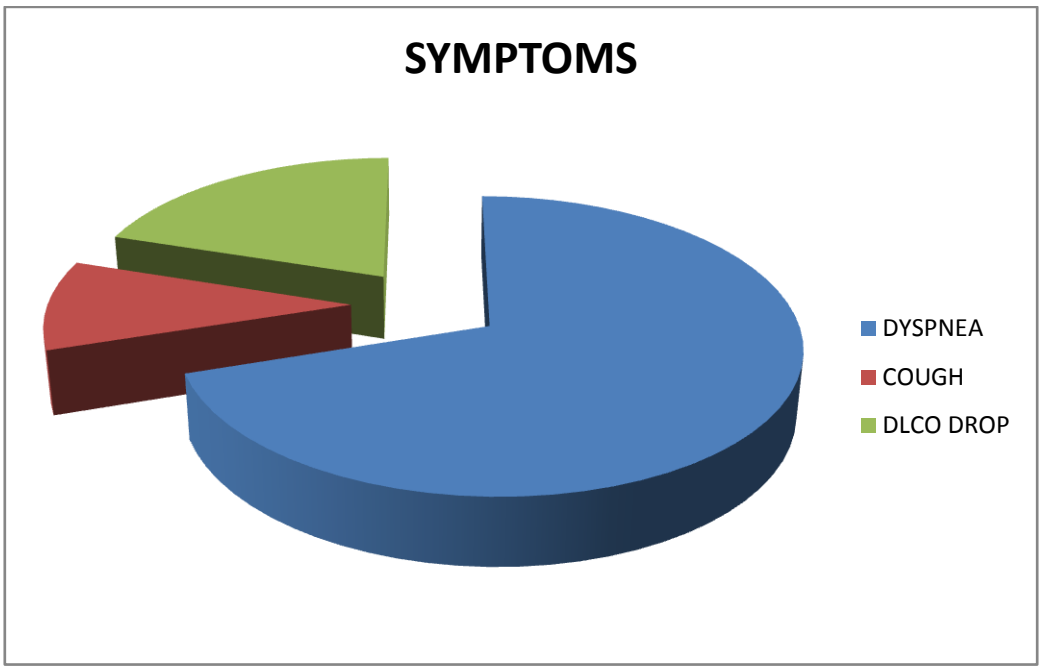
When the values of upfront and follow upPFTs were compared with the 2 – tailed test then only the change in VC was found to be significant.

Bleomycin Pulmonary Toxicity:

BPT defined either by clinical, radiologic or PFTs occurred in 10 of the 76 patients (13%).



SYMPTOMS	NO OF PATIENTS	%
DYSPNEA	7	70
COUGH	1	10
ONLY DLCO DROP	2	20



Age of patient and Bleomycin Pulmonary Toxicity:

Age as a risk factor for developing bleomycin toxicity is well known. We had only 2 patients of age <30 years who developed BPT. There were 2 patients aged 65 and 70 years who developed BPT. Age >30 yrs was a statistically significant risk factor for development of Bleomycin lung toxicity ($p < 0.02$).

Cumulative dose of bleomycin and pulmonary toxicity:

Cumulative dose of BLM in BPT pts	No of Patients
60	1
90	1
144	1
150	1
180	2
190	2
240	1
280	1

Details of patients with pulmonary toxicity:

A total of 10 (13%) patients developed pulmonary toxicity. 9 patients were receiving ABVD chemotherapy for HL. 8 patients presented with pulmonary symptoms (7 with dyspnea and 1 with cough) and 2 had only drop in DLCO. 2 of these 10 patients did not have any significant lung function parameter decline after development of pulmonary symptoms. Only 2 patients had radiographic features suggestive of bleomycin pulmonary toxicity. Renal dysfunction, smoking status and GCSF usage were not significant risk factors for predicting development of pulmonary toxicity. Age >30 years was the only significant risk factor for development of bleomycin lung toxicity.

Table 10.Risk factors in the 10 patients who developed BPT.

Risk Factors	No of Patients
Chemotherapy ABVD BEP	9 1
GCSF usage	5
Renal dysfunction	0
Smoking	1
Chest Irradiation	1
Significant DLCO drop prior to pulmonary symptoms/radiographic features	0

Trend of fall of Median Values of DLCO in patients with BPT :

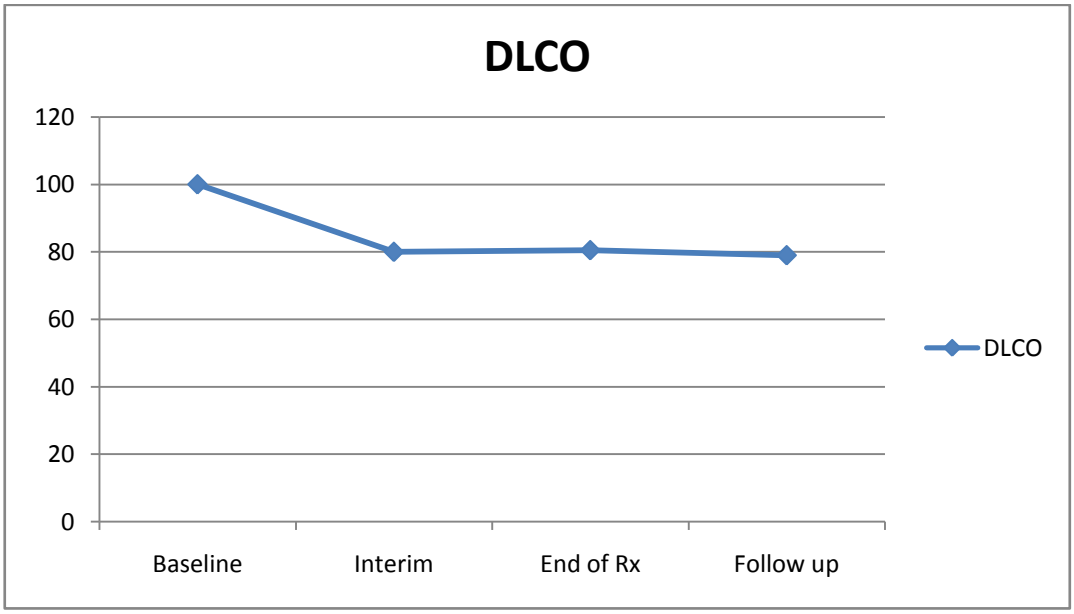


Table 11. Details of the patients with Bleomycin Pulmonary toxicity

Patient	**Diagnosis**	**Age**	**Sex (M/F)**	**Hb (gm%)**	**Dose of BLM at which BPT Occurred**	**DLCO Drop %**	**Bleomycin Discontinued**	**Outcome**
1	HL	70	M	13.2	90U	No Decline	YES	CR
2	HL	44	M	11.8	280U	24%	YES	CR
3	HL	37	M	9.6	150U	25%	YES	CR
4	HL	65	M	7.4	190U	27%	No further chemo	CR
5	HL	28	M	9.0	190U	34%	YES	CR
6	HL	35	M	10.0	180	27%	YES	CR
7	GCT Ovary	34	F	12.8	60	No decrease	Same chemo given	CR
8	HL	23	F	9.1	180	No decrease	YES	CR
9	HL	42	F	8.6	144	36%	YES	CR
10	HL	47	M	9.3	160	66%	YES	CR

Patient 1: 70 yr old gentleman, non smoker, was diagnosed to have dual malignancy. Pt was initially diagnosed to have poorly differentiated carcinoma lung, and treated with Radiation and chemotherapy. Patient had persisting mediastinal lymphadenopathy which on biopsy revealed Hodgkin lymphoma. Patient developed cough after 3rd cycle of ABVD. He had received G CSF during his chemotherapy. He did not have any significant decline in any of his lung function parameters. There were no radiographic features of Bleomycin lung. He received AVD chemotherapy and is in CR and on follow up.

Patient 2: 44 yr old gentleman, smoker (15 pack years), diagnosed to have Hodgkin Lymphoma, had an asymptomatic drop in DLCO (24%) after his 6thABVD. Patient did not have any radiographic changes suggestive of bleomycin lung. BLM was discontinued, patient received AVD chemotherapy. DLCO marginally improved, but not yet reached pretreatment level. Other lung parameters were not suggestive of lung toxicity.

Patient 3: 37 yr old gentleman, diagnosed to have Hodgkin lymphoma, developed dyspnea after 3 cycles of ABVD. He developed features of Bleomycin lung toxicity before the planned interim PFTs were due. He had received 150 U of BLM. PFT done after the symptoms revealed DLCO

drop(25%). He was then treated with AVD and is currently in CR and on follow up. Subsequent PFT shows normalization of DLCO values.

Patient 4:65 yr old gentleman, non smoker, diagnosed to have Hodgkin Lymphoma, developed dyspnea after 3 cycles of ABVD. He developed features of Bleomycin lung toxicity before the planned interim PFTs were due. He had received cumulative 190U of Bleomycin. PFT done after the symptoms revealed significant DLCO drop (27%). He was then treated with AVD and is currently in CR and on follow up. Subsequent PFT shows improvement of DLCO values but not reaching to the pretreatment value. Other lung function parameters were not indicative of BPT.

Patient 5: 28 yr old gentleman, non smoker, diagnosed as Hodgkin Lymphoma, developed dyspnea after 4^{th} cycle of ABVD chemotherapy. Interim PFT had not shown any significant decline. Bleomycin was discontinued. He did not have any radiographic abnormalities. PFTs done after the development of dyspnea showed significant DLCO drop (absolute value 34% below the pretreatment value). Chemotherapy was changed to AVD. Patient is in CR and is on follow up. DLCO done during 6 months follow up also show persistently low DLCO. However patient doesn't have any symptoms of BPT.

Patient 6: 35 year old gentleman, non smoker, diagnosed as Hodgkin Lymphoma. Pt developed asymptomatic drop in DLCO in the interim PFT done after the 4th cycle of ABVD. DLCO dropped by 27% from the upfront value. Pt had received a total of 180U of BLM. He had not received G CSF during chemotherapy. There were no radiographic abnormalities. Chemotherapy was changed to AVD. Pt is in CR and on follow up. End of treatment PFTs are due.

Patient 7:34 yr old lady diagnosed as having Germ Cell tumor of ovary, developed an acute hypersensitivity reaction manifesting as acute dyspnea and fever. Patient improved within few hours. Pt later went on to receive BEP chemotherapy again. She received total of 150U of BLM. Pt did not develop any further symptoms suggestive of Bleomycin lung toxicity. She did not have any significant declines in lung function parameters.

Patient 8: 23 year old lady, diagnosed as Hodgkin lymphoma, had bulky mediastinaladenopathy at presentation. Some of the lung function parameters like VC, FVC, FEV1, DLCO were low compared to predicted values. There was no significant drop of DLCO (just 3% below the pretreatment value). However TLC had a significant drop in the interim value. Pt developed cough and dyspnea after the 4th cycle of ABVD. PFTs did not reveal any significant decline of lung parameters. There were no radiographic

features suggestive of bleomycin lung toxicity. Patient was continued on Hybrid chemotherapy without BLM, he is in CR and on follow up. There are no symptoms of BPT on follow up.

Patient 9: 42 year old lady, diagnosed to have Hodgkin lymphoma, was started on ABVD. She has also received G CSF for febrile neutropenia. She developed dyspnea and cough after 3rd cycle of ABVD (cumulative BLM dose 144U), before she was due for the scheduled interim PFT evaluation. There were no radiographic features of BPT. DLCO continued to decline in the end of treatment PFTs and was persistently low in the 6 month Follow up PFTs.

Patient 10: 47 year old gentleman, developed mild dyspnea after receiving 5 cycles of ABVD chemotherapy (cumulative BLM dose 240U). Pt did not have any significant DLCO drop in interim PFTs. DLCO done after the pulmonary symptoms showed a 66% drop. (DLCO reports attached) CT chest showed basal interstitial infiltrates in both lung lower lobes and small subpleural nodularity. Patient improved with supportive care. Patient is on AVD chemotherapy now.

There were 4 patients, (1 patient with Testicular GCT, 3 with Hodgkin Lymphoma) in whom there was a significant DLCO drop. A 19 yr old patient with Germ cell tumor of ovary had only significant VC drop. BLM chemotherapy was continued in these patients. None of these 5 patients had any other features of BPT like pulmonary symptomatology or radiographic features. These parameters remained persistently low in follow up also.

Table 11 Details of patients with asymptomatic drop in DLCO who continued to receive BLM

Parameter	**Pt 1**	**Pt 2**	**Pt 3**	**Pt 4**	**Pt 5**
Age	26	19	48	38	22
Diagnosis	Testis GCT	Ovary GCT	HL	HL	HL
Chemo given	BEP	BEP	ABVD	ABVD	ABVD
Cumulative Dose of BLM	270	270	180	180	200
PFT decline	DLCO (24%)	VC(24%)	DLCO28%)	DLCO (23%)	DLCO (29%)
Radiographic features	NIL	NIL	NIL	NIL	NIL

DISCUSSION

Bleomycin, an antitumor antibiotic, is frequently used in several different combination chemotherapy schedules because of its lack of hematological toxicity. Bleomycin is active against Hodgkin and Non Hodgkin Lymphomas, testicular tumors and squamous cell carcinomas of the cervix, penis, esophagus and head and neck area. Though Bleomycin is very appealing because of its non myelosuppressive nature, it still has an unusual and potentially lethal pulmonary toxicity. This toxicity is manifested by interstitial fibrosis which increases in incidence with escalating dosage, age of patient, prior radiotherapy, renal dysfunction and smoking. Bleomycin pulmonary toxicity though well documented in literature still does not have an agreed definition.

There are no studies which have prospectively studied the PFTs and DLCO in Indian patients receiving Bleomycin containing chemotherapy. Our study was designed to document prospectively the changes in PFTs and DLCO over time in Hodgkin lymphoma and germ cell tumor patients receiving Bleomycin containing chemotherapy. Our study had 76 patients: 53 Hodgkin lymphoma, 16 Germ cell tumor of testis and 7 patients with GCT of

ovary. Bleomycin pulmonary toxicity was documented in 10 patients (13%). Though not significant, 9 out of these 10 patients had received ABVD chemotherapy. There was a statistically significant increase in the median values of VC and FVC. However, various studies have documented fall in median values of VC and DLCO. A probable explanation for this increase in the values even after receiving Bleomycin chemotherapy is the possible improvement in the performance status of the patient after the delivery of chemotherapy enabling them to perform the PFTs better. Serial values of DLCO and TLC decreased, which were not however significant.

In our study, the cumulative dose of BLM at which BPT occurred ranged from 60U – 280U (median dose: 180U). In our study, serial PFTs could not predict for early BIP. In our study, there was an increase in median values of VC when the serial values were compared. Sleijfer[55] et al published their observations regarding changes in pulmonary function tests in patients with testicular germ cell tumors randomized to 4 cycles of BEP vs EP. Of the 27 patients treated with BEP, 3 patients developed BIP (Bleomycin Induced Pneumonitis). These 3 patients had received a total of 330 U of bleomycin compared to 360 U of bleomycin among the other patients. They noted a mean decrease of 20% in DLCO values. Vital capacity decreased by 15% among patients treated with BEP. PFTs and DLCO tests did not differ significantly in the 3 patients who developed BIP. The authors also noted similar decrease in

DLCO in patients receiving EP chemotherapy also. They concluded that changes in DLCO were not specific for detecting bleomycin induced lung toxicity, as it might reflect toxicity due to etoposide and cisplatin which are also part of the multiagent chemotherapy. They felt that Vital Capacity may be a better lung function test for detecting BPT. In our study, there was no significant decline in any of the parameters when comparing pretreatment and interim PFTs. We did not include patients receiving EP chemotherapy in our study. In our study, 10 (13%) patients had BPT. Bleomycin was discontinued in 9 of these 10 patients (1 patient was deemed to have acute hypersensitivity reaction and BLM was rechallenged without any toxicity). 2 of these 10 patients in our study had only DLCO drop. There was no fatal pulmonary toxicity noted. In our study we noted there was significant increase in the VC & FVC values, and insignificant decrease in the values of DLCO and TLC.

Hirsch et al[46] in their prospective study of a total of 60 patients noted that 32 of 60 patients (53%) developed features of BPT. Bleomycin needed to be discontinued in 14 of 60 (23%) patients. They noted that 43% had either symptoms of BPT or pulmonary function decline, only 11% had both pulmonary symptomatology and PFT decline. Bleomycin was discontinued for 14 patients, either because of PFT decline alone (4 patients), clinical symptoms of BPT alone (5 patients) or both pulmonary symptoms and PFT

decline (5 patients). They noted 1fatal pulmonary toxicity. Hirsch et al noted a significant decline in median FVC & DLCO.

In our study, 10 (13%) developed features of BPT. 7 had dyspnea, 1 had cough and 2 had only asymptomatic drop in DLCO. The average drop in DLCO in patients with BPT was 28%. There was no statistically significant drop in TLC values. The median dose at which BPT occurred in our study was 180 U. Wolkowiczet al[51]noted that 9 (15%) of patients developed BPT. 4 patients had dyspnea, one had chest pain, one had ARDS, chest tightness and cough (1 patient). These patients were treated with a tapering course of high dose steroids. The average drop of DLCO in the patients with BIP was 12.96 %, however this was similar to the 10.6% drop in DLCO in the patients without BIP. However, they noted a statistically significant difference in the reduction of TLC values. TLC dropped by12.9% in the patient with BIP compared with 1.73% drop in the group without any BIP. Wolkowicz et al concluded that DLCO is affected more frequently than TLC, however DLCO alone cannot reliably predict early BIP, as diagnosed by symptoms and radiographic abnormalities.

Comis et al[56] found that serial DLCO measurements were more sensitive than FVC in diagnosing bleomycin pulmonary toxicity. However this observation was done in a study with only 11 patients. BPT occurred in 2

patients. They noted a large decrease in mean DLCO after only 60 mg of BLM was given, which however was stable until 240 mg BLM was given. They also noted that both DLCO and FVC decreased by the end of therapy, but FVC rapidly returned back to normal. DLCO did not normalize even at 4 months of follow up. In our study there was no decline in mean of pretreatment and interim VC, FVC and DLCO parameters.

Our study had 16 patients with Testicular Germ cell tumor. We also noted no significant change in median values of VC and DLCO. None of our patients with GCT testis had BLM lung toxicity. Luursemaet al[57]studied the serial PFT parameter changes in18 patients of disseminated germ cell tumor testis patients. They noted that there were no significant changes in mean values of VC, DLCO from 0 mg to 150 mg. The first significant changes were noted after patients had been given 240 mg of Bleomycin. They also noted that the PFT changes did not precede or coincide with radiographic abnormalities.

In our prospective study of serial PFTs of 76 patients (53 Hodgkin Lymphoma, 16 Testicular and 7 Ovarian GCT), 10 (13%) patients developed BPT. BLM containing chemotherapy was continued in 4 other patients who had an asymptomatic fall in DLCO. No Bleomycin toxicity was noted in these patients in whom the chemotherapy was continued even though the DLCO

declined. Only 2 of our patients had radiographic features suggestive of BPT. MacKeageet al[53]did a retrospective analysis of serial PFTs of 81 patients (41testicular cancer and 26 malignant lymphoma) who received Bleomycin chemotherapy. 6 of 81 patients developed clinically significant bleomycin lung toxicity.12 of the remaining 75 patients developed DLCO abnormalities without manifesting any BPT (sensitivity 16.7% and specificity 84%). Bleomycin was continued in 8 of these 12 patients who had significant fall in DLCO (DLCO drop <65% of the pretreatment value). Clinically significant toxicity did not develop in these patients. They observed that respiratory symptoms and chest radiographic changes were more likely to detect Bleomycin lung toxicity than DLCO changes.

O'Sullivan et al[58]examined the serial DLCO of 835 germ cell tumor patients from 1982-1999. 57 patients (6.7%) had BPT. Of these 35 (61%) patients with BPT were diagnosed based upon chest radiographs and CT chest findings alone. 8 patients (1% of total cohort) died of bleomycin lung toxicity. Multivariate analysis revealed that the probability of developing BPT was strongly predicted by GFR<80 ml/min, age>40 years and cumulative BLM dose>300 U.Analysis of various parameters like smoking status, GCSF usage, baseline renal function, hemoglobin, cumulative dosage, age etc in our study revealed age>30 yrs to be the only statistically significant factor.

Our study had 10 patients with a history of smoking (5-15 pack year history). Bleomycin pulmonary toxicity occurred in 1 out of this 10 patient. The median values of VC, FVC, DLCO were better than the group of non smoking patients. The drop of DLCO in these smoking patients was not any greater than among the non smoker patients. SenanS et al[34] investigated the effect of smoking on VC, TLC and DLCO in 71 patients who had completed their BLM based chemotherapy. Significantly worse VC, TLC values were noted. They also observed much more DLCO decline among smokers when compared to non smokers.

Ng A et al[59] prospectively studied serial pulmonary function tests of 52 patients treated for Hodgkin lymphoma. They found the median %DLCO was 94%. On follow up tests, the DLCO decreased by average 12% by 1 yr.

The median Creatinine in our study was 0.7 mg% and it was not found to be a significant predictor of BLM toxicity. Dalgleish A et al[14] noted that renal dysfunction significantly increases the likelihood of bleomycin lung toxicity. 26 of 275 patients (10%) developed BPT. 20 of these 26 patients had renal dysfunction during or preceding the bleomycin administration. All their patients had pulmonary features like cough, dyspnea and bibasilar rales.

Comparison of studies documenting PFT & DLCO in Bleomycin containing chemotherapy

STUDY	Nature of study	No of pts	Diagnosis	Predictive Risk factors	Clinical Features	DLCO	VC	Outcome
OUR STUDY	Prospective	76	HL. Testis and ovary GCT	Age>30	Dyspnea, cough	Decrease by 28%	NS	No mortality
Ng et al	Prospective	52	HL	High baseline DLCO, Smoking	Cough dyspnea	Decreased>15%	NS	6 BIP
Hirsch et al	Prospective	60	HL	--	Cough dyspnea	Decreased	NS	1 death 14 pts BIP
Sleijfer et al	Prospective	27	Testis GCT	VC	Dyspnea cough	Decrease d	Significant	3BIP
MacKeage et al	Retrospective	81	Testis GCT, HL	Symptoms, Radiographic features	Dyspnea	PFTs not predictive	PFTs not predictive	6 (7.5%)BIP,

CONCLUSIONS

- In our study of prospective changes over time of PFTs of 76 patients receiving Bleomycin containing chemotherapy (Hodgkin lymphoma, GCTs of ovary and testis), we found that BPT occurred in 10 (13%) of the patients.
- Statistically significant increase in Vital capacity and Forced Vital Capacity were noted in the serial PFTs.
- Fall in DLCO and TLC median values in the serial PFTs were noted, which however was not statistically significant.
- There was **no mortality** among the patients with BPT.
- Patients receiving BLM containing chemotherapy need to be diligently assessed for pulmonary symptoms and radiologic features suggestive of BPT.
- PFTs and DLCO values alone are not predictive of Bleomycin pulmonary toxicity.
- Combination of diligently looking out for pulmonary symptoms along with radiologic imaging and pulmonary function tests can pick up Bleomycin lung toxicity.

- ❖ The strengths of this study is that it is a prospective study and one of the first study to document PFTs and DLCO in Indian patients receiving Bleomycin containing chemotherapy.
- ❖ Limitations of this study: small number of patients, and limited follow up of the patients.

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ABBREVIATIONS USED IN THIS DISSERTATION:

ADR	-	Adriamycin
BLM	-	Bleomycin
BPT	-	Bleomycin Pulmonary Toxicity
BIP	-	Bleomycin Induced Pneumonitis
CTX	-	Cyclophosphamide
DLCO	-	Diffusion Coefficient of Carbon Monoxide
EOT	-	End of Treatment
PFT	-	Pulmonary Function Test
PCZ	-	Procarbazine
PDN	-	Prednisolone
VBL	-	Vinblastine
VC	-	Vital Capacity
FEV1	-	Forced Expiratory volume in 1 Second
FVC	-	Forced Vital Capacity
TLC	-	Total Lung Capacity

DATA PROFORMA

NAME :

AGE: SEX UHID

INDEX NO:

DIAGNOSIS HL/GCT Testis/GCT Ovary STAGE

Contact PHONE NOS:

STATUS OF PATIENT: UNDER TREATMENT / FOLLOW UP/ DEFAULTED/ DEAD

CHEMOTHERAPY GIVEN ABVD/BEP/Hybrid/BEACOPP

NO OF CYCLES

SMOKING HISTORY

USAGE OF GCSF:

Upfront

interim

end of Rx

follow up

HB

Creatinine

DATA	DLCO	TLC	KCO	VC	FEV1	FEV1/FVC
UPFRONT						
INTERIM						
END OF TREATMENT						
FOLLOW UP						
REMARKS / BLM TOXICITY						

To enter the percentage of the predicted values of the PFT parameters in the columns above

Symptoms of Bleomycin toxicity: Yes / No

Symptoms: Dyspnea/Cough/chest pain/Asymptomatic drop DLCO

BLM Withheld: Yes/ No

BLM dose at which BPT occurred:

Significant DLCO drop: Yes/No

Radiographic features:

Chemotherapy changed to: AVD/Hybrid without BLM/COPP/No change

Follow up study due in

Any study missed

Status of BPT: Improved/ still symptomatic/persisting Low DLCO

Status at FU: CR/PD/Still on chemotherapy/Defaulted/Dead