Dissertation on

A PROSPECTIVE STUDY OF EARLIER PREDICTION OF LUNG TOXICITY IN PATIENTS RECEIVING BLEOMYCIN FOR HODGKIN'S LYMPHOMA & GERMCELL TUMOURS

Submitted in partial fulfillment of requirements of

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The Tamilnadu Dr.M.G.R. Medical University

CHENNAI, TAMILNADU

APRIL - 2019

BONAFIDE CERTIFICATE

This is to certify that this dissertation entitled **"APROSPECTIVE STUDY OF EARLIER PREDICTION OF LUNG TOXICITY IN PATIENTS RECEIVING BLEOMYCIN FOR HODGKIN'S LYMPHOMA & GERMCELL TUMOURS"** is the bonafide original work of Dr. R.Rajakumari , in partial fulfillment of the requirement for M.D. GENERAL MEDICINE Branch I examination of the Tamilnadu Dr.M.G.R. Medical university to be held in April 2019.

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This is to certify that this dissertation work titled "**APROSPECTIVE STUDY OF EARLIER PREDICTION OF LUNG TOXICITY IN PATIENTS RECEIVING BLEOMYCIN FOR HODGKIN'S LYMPHOMA & GERMCELL TUMOURS"** is the bonafide original work of the candidate **Dr.R.Rajakumari** with registration number 201611114, Post graduate Resident in Department of General medicine , Madurai Medical College , Madurai ,was done for the award of **Doctor Of Medicine (M.D) Branch- I General Medicine**.

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This is to certify that this dissertation work titled **"APROSPECTIVE STUDY OF EARLIER PREDICTION OF LUNG TOXICITY IN PATIENTS RECEIVING BLEOMYCIN FOR LYMPHOMA & GERMCELL TUMOURS** "of the candidate Dr.R.Rajakumari with registration number 201611114 for the done for award of **Doctor Of Medicine (M.D) Branch-I General Medicine**. I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file contained from introduction to conclusion pages and result shows 2% of plagiarism in the dissertation.

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DECLARATION

I, Dr. R.RAJAKUMARIhereby solemnly declare that, this dissertation titled **"APROSPECTIVE STUDY OF EARLIER PREDICTION OF LUNG TOXICITY IN PATIENTS RECEIVING BLEOMYCIN FOR LYMPHOMA & GERMCELL TUMOURS"**Is a bonafide record of work done by me at the Department of General Medicine, Government Rajaji Hospital, Madurai under the guidance of Professor DR R.BALAJINATHAN M.D., in Department of General Medicine, Madurai Medical college, Madurai from March 2018 to August 2018. I also declare that this bonafide work or a part of this work was not submitted by me or any others for any award, degree, diploma to any other University Board either in India or in abroad.

This dissertation is submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of the rules and regulations for the

award of Degree of Doctor of Medicine (M.D.) General Medicine Branch-I examination to be held in April 2019.

Place: Madurai **DR. R.RAJAKUMARI**

Date:

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CONTENT

INTRODUCTION

Bleomycin is one of the important chemotherapy commonly given for the treatment of Hodgkin lymphoma and germ-cell tumours, the most highly curable cancers. But bleomycin can cause severe life-threatening lung injury, which ranges from hypersensitivity pneumonitis and bronchiolitis obliterans organizing pneumonia (BOOP) to acute interstitial pneumonia and progressive pulmonary fibrosis .

Pulmonary toxicity is a known side effect of chemotherapy , but a 10% death rate of bleomycin is judged unacceptable for patients with curable cancers .Toxic effects of bleomycin are generally due to formation of free

radicals and organ specificity is driven by the bleomycin catalysing hydrolase, which is absent in lung and skin tissue, rendering these organs vulnerable to toxicity.

Hence early diagnosis , treatment, and prevention of limiting toxicities such as bleomycin-induced lung injury, is very important. This study aims at of earlier prediction of bleomycin induced lung toxicity in hodgkin's lymphoma & germcell tumours

AIMS AND OBJECTIVES:

To identify the patients developing lung toxicity for Bleomycin in hodgkin's lymphoma and germcell tumourby PFT and HRCT.

REVIEW OF LITERATURE

HODGKIN’S LYMPHOMA

Hodgkin's disease (HD) is a clonal malignancy of the lymphatic system .Its diagnosis is based on the identification of characteristic multinucleated giant cells within an inflammatory milieu. These cells—termed Reed-Sternberg (RS) or diagnostic cells—represent the body of the tumour. The tumoral population also includes a variable number of mononuclear elements—Hodgkin's cells (HCs)—showing similar

cytological features to RS cells and neoplastic cell variants, each corresponding to a specific subtype of HD. Molecular studies have recently shown that in most if not all cases RS cells, Hodgkin's cells, and cell variants belong to the same clonal population, which is derived from peripheral B and T cells in about 98% and 2% of cases, respectively. Accordingly, HD has been included among malignant lymphomas and the term "Hodgkin's lymphoma" (HL) has been proposed.

WHO CLASSIFICATION OF HODGKIN'S LYMPHOMA

Nodular lymphocyte predominant HL

Classical HL

- Nodular sclerosis
- Lymphocyte rich
- Mixed cellularity
- Lymphocyte depleted

BIOLOGY OF HODGKIN'S LYMPHOMA

CELL OF ORIGIN

Molecular studies of isolated tumor cells have demonstrated that lymphocyte-predominant (LP) cells of nodular Hodgkin's lymphoma (NLPHL) are derived from antigen-selected germinal center (GC) B cells, whereas ReedSternberg (RS) cells in classic HL (cHL) appear to be derived from preapoptotic crippled GC B cells . Molecular features of LP cells

include the presence of clonally rearranged and somatically mutated immunoglobulin (Ig)V gene cells, with signs of ongoing somatic hypermutation in a fraction of cases . These data linked the origin of LP cells in NLPHL to GC B cells. Another important feature supporting this linking was the immunohistochemical expression of BCL6 (a typical GC B-cell marker) in LP cells .Accordingly, LP cells can morphologically be observed in an environmental architecture resembling the structure of a secondary follicle, which contains a reactive GC.

In fact, in the early phases of NLPHL, LP cells can be found in follicular structures in association with follicular dendritic cells (FDC) and GC type T-helper cells, in this regard resembling GC. The derivation of NLPHL from GCs is supported by the following features:

(1) the expression of the BCL6 gene product and CD40 by LP cells
(2) the occurrence of numerous CD4+/ CD57+/PD1 T cells surrounding the LP cells, as seen in normal GCs and progressively transformed GCs (PTGC)6
(3) the presence of an FDC meshwork (CD21+/CD35+) within the tumor nodules
(4) the global gene expression profile

Conversely, molecular features of RS cells in cHL demonstrate that they are probably derived from GC B cells that have acquired disadvantageous immunoglobulin variable chain gene mutations and normally would have undergone apoptosis. In parallel to molecular investigations, biologic markers identifying distinct subsets of mature B cells have been used to study the cell of origin . According to the differential expression of these markers, LP and RS cells resemble mature B cells deriving from different stages of B-cell differentiation (i.e., GC and post-GC, respectively).

Morphologic, Phenotypic and Virologic Features of Reed-Sternberg Cells of Classic Hodgkin's Lymphoma, and Lymphocyte-Predominant Cells of Nodular Lymphocyte Predominant Hodgkin's Lymphoma[25]

Features/ Expression	cHL/RS Cells	NLPHL LP Cells
Tumor cells	Diagnostic RS cells	LP or "popcorn" cells
Pattern	Diffuse, interfollicular, nodular	Nodular
Background	Lymphocytes, (T cells > B cells) histiocytes, eosinophils, plasma cells	Lymphocytes, (B cells > T cells) histiocytes
Fibrosis	Common	Rare
CD15	+	–
CD19	+ (20%–30%)	+
CD20	+ (20%–30%)	+
CD22	+ (20%–30%)	–
CD30	+	–
CD40	+	+
CD45	–	+
EMA	–	+
IRF4/MUM1	+	+
BCL6	+ (30%)	+
EBV infection	+ (30%–40%)	–

Reed-Sternberg Cells Lack Common B-Cell Markers

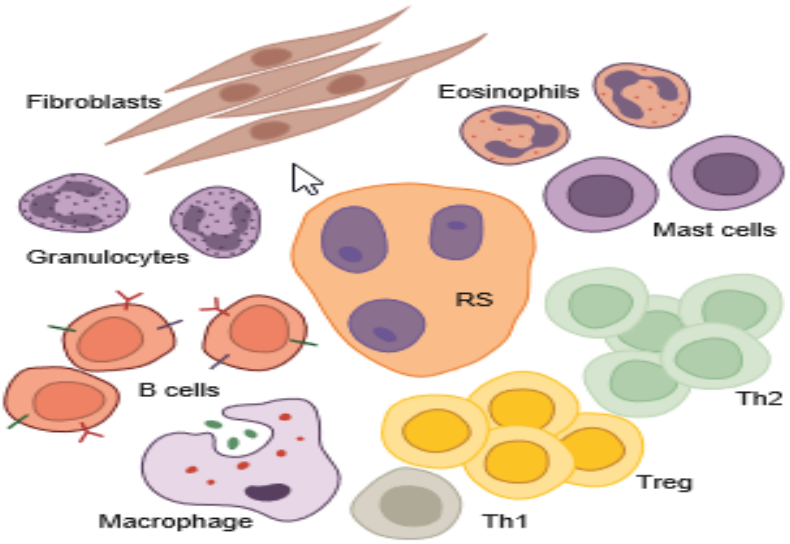


Figure 102.4 Reed-Sternberg (RS) cell and its microenvironment. An RS cell is shown within a rich, polymorphic cellular microenvironment that expresses members of the TNFR family protein and is embedded in a network of cytokines and chemokines. Treg, regulatory T cell; Th1, T-helper cell type 1); Th2, T-helper cell type 2.

The loss of the B-cell phenotype in RS cells is unique among human lymphomas in the extent to which the lymphoma cells have undergone reprogramming of gene expression. As shown in gene expression profiling (GEP) studies, RS cells have lost the expression of most B-cell–typical genes and acquired expression of multiple genes that are typical for other types of cells in the immune system.

Moreover, RS cell gene expression is most similar to that of Epstein-Barr virus (EBV)-transformed B cells, and cell lines derived from diffuse large-cell lymphomas showing features of in vitro activated B cells. The deregulated expression of inhibitors of B-cell molecules (inhibitor of differentiation and DNA binding 2 [ID2], activated B-cell factor 1 [ABF1],

and notch 1), the downregulation of B-cell transcription factors (OCT2, BOB1, and PU.1), and the epigenetic silencing of B-cell genes (CD19 and immunoglobulin H [IgH]) all seem to be involved in the loss of the B-cell phenotype in RS cells.

Multiple Signaling Pathways and Transcription Factors Have Deregulated Activity in Reed-Sternberg Cells

Very recently, biologic studies on HL cell lines using new technologies have shown that multiple signaling pathways and transcription factors have deregulated activity in RS cells. Involved pathways and transcription factors included nuclear factor kappa B (NF-+B), Janus kinase/signal transducers and activators of transcription (Jak-Stat), phosphoinositide 3-kinase (PI3K)–Akt, extracellular signal-regulated kinase (ERK), activating protein-1 (AP-1), notch 1, and receptor tyrosine kinases.

Functional studies have shown that in normal B GC cells, the activation of the CD40 receptor leads to NF-κB–mediated induction of the interferon regulatory factor 4/multiple myeloma oncogene 1 (IRF4/MUM1) transcription factor. CD40 engagement in HL cell lines by both soluble (s) CD40L and membrane-bound (mb) CD40L upregulates IRF4/MUM1 expression by HL cells.

CD40 engagement in HL cells by both sCD40L and mbCD40L enhances both clonogenic capacity and colony cell survival of HL cell lines, stimulates proliferation and rescue from apoptosis, mediates in vitro rosetting of activated CD4+ T cells to HL cells, and increases ERK phosphorylation and cell survival.

CLASSIC HODGKIN'S LYMPHOMA

Morphology The so-called RS cell is the diagnostic key for this lymphoma because of its typical morphology: a giant cell with bi- or multinucleation and huge nucleoli. The typical morphology of binucleated and multinucleated RS cells and their mononuclear variant, the so-called Hodgkin's cell, are not specific to cHL, because they can also be observed in B-NHL (especially in diffuse large B-cell lymphoma [DLBCL] of the anaplastic variant), but they are pathognomonic for cHL in conjunction with an abundant cellular background composed of a varying spectrum of nonneoplastic inflammatory cells.

Based on the characteristics of the reactive infiltrate, four histologic subtypes have been distinguished:

- ✓ Lymphocyte-rich chl (LRCHL),
- ✓ Nodular sclerosis (NS) chl,
- ✓ Mixed cellularity (MC) chl,
- ✓ Lymphocyte depletion (LD) cHL.

LRCHL accounts for only a small fraction (3% to 5%) of all HLs. Most LRCHLshave a better prognosis than do other cHLs and are characterized histologically by a small number of RS cells expressing a cHL immunophenotype. Based on these histologic and clinical features, there is no clear consensus on whether LRCHL represents a distinct disorder or just an early presentation of cHL. On the other hand, LRCHL cases display features intermediate between those of cHL and NLPHL.

PHENOTYPE

Phenotypically, RS cells of cHL are consistently positive for CD30, CD15, CD40, and IRF4/MUM1

MICROENVIRONMENT

cHL is a lymphoid neoplasm, derived from B cells, composed of mononuclear Hodgkin's cells and multinucleated RS cells residing in an abundant cellular microenvironment. In cHL, microenvironmental cell types include T- and B-reactive lymphocytes, eosinophils, mast cells, histiocytes/macrophages, plasma cells, and granulocytes .In addition, a great number of fibroblast-like cells and interdigitating reticulum cells are detectable, often in association with RS cells, within the collagen bands of NS cHL.

Fibrosis—considered a common morphologic feature of HL lesions—is found more frequently in cHL subtypes than in NLPHL. An abnormal network of cytokines and chemokines and/ or their receptors in RS cells is involved in the attraction of many of the microenvironmental cells into the lymphoma background .

Nonmalignant inflammatory/immune cellular components of the HL microenvironment express molecules involved in cancer cell growth and survival, such as CD30L or CD40L, or in immune escape, such as programmed death 1 (PD-1).

The nonmalignant cells that compose most of the cellular background of cHL are recruited and/or induced to proliferate by tumor cells. They in turn produce soluble or membrane-bound molecules involved in tumor cell growth and survival. Numerous molecules are involved directly or indirectly in the recruitment and/or proliferation of cells constituting the cHL microenvironment.

Normal cells may be recruited by cytokines/chemokines produced by RS cells or by T cells fibroblasts activated by RS cells. RS cells produce molecules capable of inducing proliferation and/or differentiation of eosinophils, Treg cells, and fibroblasts.

EPSTEIN-BARR VIRUS INFECTION

Generally, in the different histologic subtypes of cHL, the immunophenotypic and genetic features of RS cells are identical, whereas their association with EBV shows differences. EBV is found in RS cells in about 40% of cHL cases in the Western world, mostly in cases of MC and LD HL, and less frequently in NS and LRCHL. Conversely, EBV is found in RS cells in nearly all cases of HL occurring in patients infected with HIV.

Independent studies have recently demonstrated that EBV can transform antigen receptor–deficient GC B cells, which enables their escape from apoptosis. The continued survival of the rescued preapoptotic B cells allows their proliferation. The EBVencoded latent membrane protein (LMP) 2A is likely to function as the surrogate receptor through which B-cell signaling is triggered.

This mechanism of EBV/LMP2A-induced the escape of antigen receptor-deficient GC B cells from apoptosis offers an intriguing model of lymphomagenesis. EBV infection might also affect the microenvironment composition by increasing the production of molecules involved in immune escape and T-cell recruitment, such as interleukin 10 (IL-10), CCL5, CCL20, and CXCL10.36 LMP1 could have an interacting role with the microenvironment.

Recent evidence indicates that EBV can manipulate the tumor microenvironment through the secretion of specific viral and cellular components into exosomes, small endocytically derived vesicles that are released from cells. Exosomes produced by tumor cells from EBV-infected nasopharyngeal carcinoma contain LMP1, which can activate critical signaling pathways in uninfected neighboring cells, suggesting messenger functions of virus-modified exosomes.

Moreover, in B-cell lines, EBV-modified exosomes would activate cellular signaling mediated through integrins, actin, interferon, and NFκB.38 Further insights in these mechanisms are emerging from and the understanding of the capability of EBV to modulate the (tumor-like) microenvironment.

EARLY-STAGE HODGKIN'S LYMPHOMA

The management of early-stage Hodgkin's lymphoma exemplifies several important principles of oncology. These include the progressive improvement of cure rates through careful clinical research; the identification of prognostic features and new markers of optimal response; the refinement of treatment by the exploration of multimodality approaches; the vital importance of long-term follow-up; and a holistic analysis of the outcomes of treatment.

Overall, this is one of the success stories of modern oncology, with modern treatment achieving high initial cure rates (up to 90% with the first-line of therapy) and good overall survival at around 95% after 5 years or more. Because it most often affects younger people in the 2nd to 4th decade of life, this has important implications for the goals of treatment, which must include not only the maximization of initial tumor control but also the avoidance of preventable long-term side effects.

PROGNOSTIC FEATURES

The relatively orderly progression of cHL has long been recognized. It generally develops through involvement of adjacent nodes in the same anatomical site, then in adjacent nodal areas, and it is extremely rare to find isolated deposits in two distant nodes. The same is not true for nodular lymphocyte-predominant disease, which, in this respect, more closely resembles a low-grade non-Hodgkin's lymphoma: It often presents with a single isolated node in the neck, but if it does progress, the dissemination is often to distant sites without intervening nodal involvement.

The predictable spread of cHL has allowed for the construction of a staging system based on anatomical extent, so that early-stage disease is defined by involvement of nodal groups on one side of the diaphragm only, more usually the thorax. Stage I disease is confined to a single anatomical

nodal group (cervical, supraclavicular, axillary, anterior mediastinal, etc.), whereas a disease affecting more than one such group is stage II.

Beyond this division on the basis of nodal involvement, many studies have identified further prognostic features through retrospective analyses of large series of patients in clinical trials, mostly treated with extended field radiotherapy.

This has allowed for the subdivision of early-stage disease into favorable and unfavorable categories. These do not represent biologically distinct processes, but act as a useful indicator of the severity of the illness and its optimum management, even though the current approaches to treatment are different to those in use when the factors were identified.

Although a variety of stratification systems have been devised, common features include the presence of bulky disease (usually in the mediastinum), more advanced age (with a cutoff of 40 or 50 years of age), elevated erythrocyte sedimentation rate (ESR), systemic symptoms, and multiple or extranodal sites of involvement.

Criteria Used to Stratify Early-Stage Hodgkin's Lymphoma

	EORTC	GHSG	NCIC/ECOG	NCCN 2010
Risk factors	a) Large mediastinal mass (>1/3) b) Age ≥50 years c) ESR ≥50 without B symptoms or ≥30 with B symptoms d) ≥4 nodal areas	a) Large mediastinal mass b) Extranodal disease c) ESR ≥50 without B symptoms or ≥30 with B symptoms d) ≥3 nodal areas	a) Histology other than LP/NS b) Age ≥40 years c) ESR ≥50 d) ≥4 nodal areas	a) Large mediastinal mass (>1/3) or >10 cm b) ESR ≥50 or any B symptoms c) ≥3 nodal areas d) >1 extranodal lesion
Favorable	CS I–II (supradiaphragmatic without risk factors	CS I–II without risk factors	CS I–II without risk factors	CS I–II without risk factors
Unfavorable	CS I–II (supradiaphragmatic with ≥1 risk factors	CS I or CS IIA with ≥1 risk factors CS IIB with c) or d) but without a) and b)	CS I–II with ≥1 risk factors	CS I–II with ≥1 risk factors (differentiating between bulky disease and other risk factors for treatment guidelines)

EORTC, European Organisation for Research and Treatment of Cancer; GHSG, German Hodgkin's Lymphoma Study Group; NCIC, National Cancer Institute of Canada; CS, Clinical stage.

RADIATION THERAPY

The effective treatment of HL by radiotherapy began with the work of Gilbert in the 1920s. He introduced the rationale for treating both the evident sites of nodal involvement and adjacent but clinically uninvolved lymph nodes, on the basis that these were likely to contain microscopic disease. Peters took the same approach at the Princess Margaret Hospital in the 1940s, publishing a landmark paper in the American Journal of Roentgenology in 1950, which described the cure of limited HL by high-dose, fractionated radiation.

She reported 5- and 10-year survival rates of 88% and 79%, respectively, for patients with stage I disease, which transformed the outlook for an illness previously thought to have no long-term survivors. In the early

days, radiation therapy utilized fields that included the entire lymphatic system, total lymphoid irradiation (TLI), to the development of the nodular sclerosis subtype. In this regard,relatively higher biologic radiation dosages compared to contemporary treatment.

Extended field radiation therapy (EFRT) included all nodal sites using three radiation fields classically known as mantle, para-aortic–spleen, and inverted Y. A variation of EFRT was also used known as subtotal nodal irradiation (STNI). This was effective, and in many cases curative, but was accompanied by important long-term toxicities, especially the induction of second malignancies and accelerated cardiovascular disease.

It remained the principal approach to treatment of early disease until clinical trials demonstrated that a combination strategy with chemotherapy could produce superior cure rates with much less irradiation, leading to a reduction of the irradiated field size to only the involved field (IF); the latter was based on a series of studies aimed at minimizing the toxicity of radiation therapy treatment.

The German Hodgkin's Lymphoma Study Group (GHSG) HD8 showed in a randomized trial that reducing the treatment volume from EFRT to involved field radiation therapy (IFRT), when combined with chemotherapy, is equally effective. The European Organisation for Research

and Treatment of Cancer (EORTC) H7 showed a similar outcome comparing IFRT to STNI.

The developments in functional imaging, treatment planning, and image-guided radiation therapy have made it possible to better define and further decrease the radiation fields. Thus, IFRT, which is based on anatomic landmarks and encompassing adjacent uninvolved nodal stations, is no longer appropriate.

Based on the fact that most recurrences occur in the original nodal sites, involved node irradiation therapy (INRT) was suggested; the field, in this case, is confined to the macroscopically involved nodes on imaging studies at diagnosis. Although this requires a significant margin around the node to allow and ensure adequate coverage, it can still result in significantly lower exposure to adjacent critical structures.

No formal comparison has been made to the results with IFRT, but multiple studies have shown no loss of efficacy with INRT .Using INRT requires acquiring images at diagnosis in treatment positions and prior to the start of chemotherapy to minimize anatomic position variations between diagnostic and radiation treatment planning imaging.

Because that is not practical in most cases, new guidelines defining involved site radiation therapy (ISRT) has been introduced by the

International Lymphoma Radiation Oncology Group (ILROG). The new standard of care represents a significant reduction in the volume included in the previously used IFRT by using modern imaging and radiation planning techniques to limit the amount of normal tissue being irradiated.

COMBINED MODALITY THERAPY

The recognition that HL is highly sensitive to cytotoxic chemotherapy led to the testing of systemic treatment in early stage disease. By administering limited doses of chemotherapy, it has been shown possible to reduce both the extent and dose of radiotherapy, while still maintaining high cure rates. The success of this approach has depended on the different treatment of favorable and unfavorable disease, with results in favorable groups excellent even after low impact chemotherapy, such as two cycles of doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD) or the attenuated EBVP regimen.

The EORTC H7-F study compared STNI to six cycles of EBVP followed by IFRT (36 to 40 Gy), with better results from the combined modality treatment: 10-year eventfree survival was 88% versus 78%, and overall survival was 92% in both arms. The GHSG HD10 study in favorable early disease compared results in a 2 × 2 randomization between two or four cycles of ABVD and 20 or 30 Gy of IFRT. All four groups had very high cure rates, with progression-free survival of 92% and overall survival of 97%

at 5 years, suggesting that two cycles of ABVD and 20 Gy of IFRT is sufficient treatment for carefully selected favorable disease.

A slightly different picture has emerged from studies of unfavorable early disease, where many patients present with bulky mediastinal nodes. Here, there is a threshold of treatment intensity below which the results become less favorable, with an apparent interaction between the efficacy of chemotherapy and the dose of irradiation used.

Attenuated use of either modality can be compensated by the other, but if both elements are reduced too far, the freedom from treatment failure is lowered as the result of the excess of early recurrences. The EORTC H8-U trial showed the equivalence of either six or four cycles of MOPP-ABV when given before IFRT (36 to 40 Gy), or four cycles of MOPP-ABV before STNI, with 5-year event-free survivals of 84%, 88%, and 87%, respectively, and 10-year overall survival estimates of 88%, 85%, and 84%, respectively, indicating that treatment more intensive than four cycles of MOPP-ABV and IFRT was unnecessary, and that less toxic treatment might be possible.

More recently, the GHSG HD11 study has tested a 2 × 2 randomization between four cycles of ABVD and four cycles of the baseline bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone (BEACOPP) regimen before either 20 Gy or 30 Gy IFRT. The

least intensive arm, four cycles of ABVD and 20 Gy, showed inferior 5-year progression-free survival at 82%, compared to 87%, although overall survival was unaffected, at 94.5%.

This suggests that for the unfavorable early-stage group, it may hazardous to reduce treatment below a threshold of four cycles of ABVD and 30 Gy IFRT, unless some means can be found to select those patients for whom further deintensification can be attempted, such as the use of functional imaging.

CHEMOTHERAPY ALONE

Recognition of the long-term toxicity of extended field irradiation has led many investigators to test approaches by which radiotherapy may be omitted altogether from the treatment of early HL. Two large randomized trials have been performed, in pediatric and adult patients, respectively, and both demonstrated that the omission of radiotherapy slightly reduced control of the disease, reflected in lower progression-free survival, but had no adverse impact on overall survival.

The North American Children's Oncology Group study CCG 5942, tested the omission of low-dose IFRT (21 Gy) for those in complete remission after four cycles of COPP-ABV chemotherapy. The study was

closed prematurely when an interim analysis showed a difference in the progression rates in the two arms.

With a median 7.7 years follow-up, the event-free survival favored the radiotherapy group (93% versus 83%; p = 0.004), with most recurrences in the chemotherapy-alone group seen at the sites of original disease. There was, however, no difference in overall survival, estimated at 97% at 10 years74.

In adults with early-stage nonbulky disease, the intergroup Eastern Oncology Cooperative Group (ECOG)/National Cancer Institute of Canada (NCIC) study tested treatment with ABVD alone to either 35 Gy STNI in favorable disease, or two cycles of ABVD followed by STNI in unfavorable cases. The first report of this study, with a median follow-up of 4.2 years, showed inferior freedom from progression in the chemotherapy-alone arms (87% versus 93%), with the unfavorable group particularly disadvantaged by the omission of radiotherapy.

The initial analysis showed no difference in overall survival, but with longer follow-up, a different picture emerged, with inferior 10-year survival among the patients who had received radiotherapy (87% versus 94%, respectively; p = 0.04).

The risk of death from lymphoma was not different between the arms, but the risk of death from other causes was more than threefold higher among

those treated with radiotherapy, and much of the excess was due to second cancers.

It is important to note, however, that this protocol involved much more extensive irradiation than is currently in use, making extrapolation of the results difficult. In the absence of direct comparative trials between modern combined modality therapy and chemotherapy alone, a metaanalysis was performed using the intergroup study ABVD-alone group and the comparable patients from the GHSG HD10 and HD 11 studies who received ABVD and IFRT.

This showed that the short-term disease control was inferior with ABVD alone, reflected in worse 8-year time to progression (93% versus 87%; hazard ratio [HR], 0.44; 95% confidence interval [CI], 0.24 to 0.78), but that overall survival was not adversely affected in these groups, with 95% alive in the long-term follow-up.

The impact of combined modality treatment was particularly apparent among patients who showed less than complete remission after chemotherapy, suggesting that some means of selecting those with chemosensitive disease for deescalation of therapy would be attractive, and might allow radiotherapy to be omitted without a loss of disease control.

Response-Adapted Treatment

Much interest has been generated in the possible use of functional imaging to give an early indication of chemosensitivity in HL. The technique most widely tested is 2-(18F)fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET), the application of which as an interim readout of efficacy has been enhanced by the development of a highly reproducible five-point scale for reporting the results .

This approach appears to improve the sensitivity for the detection of residual active lymphoma when compared to conventional computed tomography, but the data from prospective randomized studies using it as a guide to therapy are not yet mature enough for firm conclusions, and it is clear that there is a small but definite false-negative rate for FDG-PET, probably of the order of 5% to 10%.

Two studies have reported early results, with broadly similar outcomes . The United Kingdom National Cancer Research Institute RAPID study randomized patients with nonbulky early-stage disease who had an interim PET score of 1 or 2 after three cycles of ABVD to either 30 Gy IFRT or no further therapy, and found that the 3-year progression-free survival and overall survival were not significantly different.

There was, however, a trend toward inferior disease control, which became significant when patients who did not receive the radiotherapy as

allocated were excluded (97% versus 90.7%; HR, 2.39; p = 0.003). Similarly, the EORTC H10 study compared two strategies of therapy: standard treatment with ABVD and IFRT, stratified according to baseline prognostic factors, versus a nonradiotherapy approach, but using further chemotherapy, for those with negative FDG-PET scans after two cycles of ABVD.

The results with a short follow-up suggested inferior disease control in the experimental PET-directed arms, although the number of progressions was small and a much longer follow-up will be required to determine whether there is any detrimental effect on survival. Taken overall, the evidence suggests that for early HL, the use of combined modality treatment produces optimum results in terms of disease control, with a very high expectation of cure from the initial therapy.

There is, however, a large proportion of patients (around 90%) who will be curable with chemotherapy alone, and the number needed to treat with radiation in order to achieve 1 extra cured patient is between 15 and 30 according to these trials. Given these figures and the perceived risks of late toxicity from radiotherapy, many patients may prefer the slightly higher risk of recurrent lymphoma to the potential for longer term morbidity.

This will, of course, be subject to other variables such as their age, the sites of involvement (and thus the radiotherapy fields), and their baseline risk

category. In general, the results of treatment from either approach are very good, and it is reassuring that in almost all the trials carried out, a small reduction in disease control does not have any detrimental effect on overall survival, thanks to the excellent results of second-line therapy, when it is required.

POSITRON-EMISSION TOMOGRAPHY–DIRECTED APPROACHES

A strategy to potentially optimize therapy for HL, by possibly increasing efficacy and decreasing toxicity, is to utilize PET scans during treatment. Because changes in glucose metabolism precede changes in tumor size, responses can be assessed earlier during treatment with PET scans than with CT.

Early interim PET scan imaging after chemotherapy for HL has been shown to be a sensitive prognostic indicator of outcome in patients with advanced disease.123 In prospective studies, interim PET scans after two cycles of ABVD chemotherapy was a significant predictor of progression-free or event-free survival in patients with advanced-stage disease.

Similar findings were reported for patients treated with Stanford V or escalated BEACOPP. Current clinical trials are now testing whether patient outcomes can be improved by modifying treatment based on the interim PET scan results. In patients who have an inadequate response based on the interim PET scan, treatment is either intensified or salvage therapy is

contemplated. Initial studies testing whether deescalation to less intense or abbreviated therapy maintains efficacy in patients who have a complete response by the interim PET scan suggest that this approach is feasible.

Avigdor et al. treated advanced-stage HL patients with two cycles of escalated BEACOPP and deescalated to ABVD chemotherapy for four cycles if the PET scan after the initial two cycles was negative. Patients who did not achieve a negative scan were removed from the study and considered for salvage therapy followed by high-dose chemotherapy and autologous stem-cell transplantation.

Seventy-two percent of patients had a negative scan, and deescalation to ABVD resulted in a 4-year progression-free survival of 87%.In a similar fashion, the GHSG HD18 trial is testing whether the number of cycles of escalated BEACOPP can be reduced from six to four in patients with a negative interim PET scan.

An alternative approach is to intensify therapy in patients who do not have a negative interim PET scan. Initial studies have explored whether patients can start treatment with two cycles of ABVD and escalate to BEACOPP if the interim scan is positive. Initial reports suggest that this strategy, with BEACOPP intensification only in interim PET-positive

patients, showed better results than ABVD-treated historic controls, and spared BEACOPP toxicity in the majority of patients.

A similar strategy is being prospectively explored in the UK National Cancer Research Institute Response Adapted Therapy using FDG-PET imaging in the advanced HL (RATHL) trial. In this study, all patients receive two courses of ABVD chemotherapy, and PET-negative patients are randomized between ABVD and AVD, to test whether the omission of bleomycin reduces lung toxicity while achieving an equivalent outcome. Patients who remain PET positive undergo treatment escalation with BEACOPP, thereby attempting to improve remission rates. These studies are still in progress.

Five-Point Scale for the Interpretation of Interim FDG-PET Scanning

Score	PET/CT Result
1	No uptake above background
2	Uptake ≤ mediastinum
3	Uptake > mediastinum but ≤ liver
4	Uptake moderately increased compared to the liver at any site
5	Uptake markedly increased compared to the liver at any site
X	New areas of uptake unlikely to be related to lymphoma

CONSOLIDATION RADIOTHERAPY

An alternative strategy to modifying the initial treatment for advanced-stage HL is to attempt to consolidate the response following initial

chemotherapy. Radiotherapy is commonly used as consolidation following primary chemotherapy, with the goal of improving responses or preventing progression in patients with residual masses.

The precise subgroup of patients with advanced-stage HL who benefit from consolidative radiotherapy has changed over time with the use of different chemotherapy regimens and the routine use of PET scans in clinical practice. For patients treated with standard anthracycline-based chemotherapy, those with only a partial response to treatment as determined by conventional restaging may convert to complete remissions after consolidation radiotherapy.

Patients with a complete response to initial treatment, however, do not appear to benefit from consolidation radiotherapy.As more intensive regimens have been used, resulting in more complete responses, the need for consolidation radiotherapy has decreased. This may be particularly true when intensive approaches are coupled with a PET-based evaluation of residual masses to confirm a complete response.

In three successive GHSG trials for advanced HL, the use of radiotherapy was reduced in each study as treatment was intensified and a PET scan analysis was included. In the HD9 trial, two-thirds of patients treated with COPP/ABVD or BEACOPP received radiotherapy. In contrast,

in the HD15 trial where PET scans guided the decision, only 11% of patients were treated with radiotherapy after escalated BEACOPP without compromising patient outcome.

These studies suggest that the use of radiotherapy can possibly be restricted to patients with PET-positive residual masses after escalated BEACOPP treatment; however, the exact role of radiotherapy in ABVD-treated patients in the era of PET scans is not well defined

COMPLICATIONS OF TREATMENT

The initial treatment of patients with HL with chemotherapy, often in combination with radiotherapy, results in a significant proportion of patients who are cured of their disease. The toxicity of treatment, however, is a significant limitation to its use. Although early toxicities of therapy are commonly manageable and of short duration, late toxicities are often irreversible and may result in lifethreatening complications.

The late effects of treatment determine the long-term morbidity, mortality, and quality of life of patients with HL. In the first 10 years after treatment, most deaths are due to disease progression or relapse, but beyond this time point, deaths due to late effects predominate. Acute hematologic toxicity, with possible infectious complications and treatment-related

mortality, is associated with the intensity of the treatment combination, the age of the patient, and their comorbid conditions.

These toxicities are commonly managed by dose modifications and growth factor support. For patients receiving bleomycin, pulmonary toxicity is a concern. Bleomycin lung toxicity is a potentially life-threatening complication and may be more prevalent in patients receiving ABVD chemotherapy.

A significant complication after treatment is the development of second malignancies. These can involve solid organs (most commonly lung, skin, breast, or gastrointestinal) or be hematologic (leukemia, myelodysplasia, or secondary lymphomas). The risk of second malignancies is highest after treatment for childhood HL.

In those patients treated for HL before adulthood, the risk of developing a second malignant disease has been estimated to be almost 20 times greater than the general population, with a 30-year cumulative risk of 18% for male patients and 26% for female patients.

The most common second malignancy in female patients is breast cancer. Important risk factors for therapyassociated breast cancer are age of younger than 20 years at the time of treatment and treatment with extended field radiotherapy that includes the mediastinum.

The risk of breast cancer is estimated to be approximately 30% in patients who received 40 Gy to the mediastinum before 25 years of age.Chemotherapy drugs, especially alkylating agents, contribute to the risk of hematologic malignancies, particularly acute myeloid leukemia (AML) and myelodysplasia. The cumulative risk of developing AML is approximately 1.5% for patients treated for advanced stage Hodgkin lymphoma with chemotherapy regimens such as ABVD.

There may be an increase in the incidence of myelodysplasia and AML when more intensive regimens such as escalated BEACOPP are used. The overall rate of other second malignancies, however, appears similar when more intensive and less intensive chemotherapy regimens are compared.

Other late effects include infertility, cardiac effects, endocrine dysfunction, peripheral neuropathy, and local effects from radiotherapy. Alkylating agents may induce male and female sterility, but this is far less frequent in patients treated with ABVD-like regimens than alkylating-containing regimens such as BEACOPP.

An increase in myocardial infarction, congestive cardiac failure, asymptomatic coronary disease, valvular dysfunction, and stroke have been

recorded after treatment for HL, and the risk of cardiac mortality may persist for many years after completing therapy.

GERM CELL TUMOURS

INTRODUCTION

Germ cell tumours are derived from the primordial germ cells .during embryonic development germ cells migrate from the caudal part of the yolk sac , along the dorsal part of the hindgut before being incorporated into thesex cord of the developing gonads. Although germ cell tumour can arise from extra gonadal sites like mediastinum and retroperitoneum , most arise from

undifferentiated germ cells in the gonads. In men germ cell tumour represents more than 95% of testicular cancers.

RISK FACTORS

Men whose family history includes a first-degree relative with testicular germ cell tumor or who have a personal history of cryptorchidism are at increased risk of developing germ cell tumor.Testicular cancer survivors are also at increased risk of a second primary cancer in the contralateral testicle.

These risk factors suggest a genetic or developmental etiology for testicular germ cell tumors. No postnatal environmental risk factors have yet been identified. In the United States, testicular cancer is rare among African Americans and most common among Caucasian men. This racial disparity in testicular cancer diagnosis and its geographic distribution suggest a genetic linkage with the Caucasian phenotype, but no high penetrance allele has yet been identified.

Testicular germ cell tumors are commonly accompanied by intratubular germ cell neoplasia (ITGCN). It is thought that all adult germ cell tumors, with the exception of spermatocytic seminoma, arise from ITGCN. The widely accepted theory is that ITGCN begins in utero. The

multifocality of ITGCN suggests a field effect within the testicle and provides a mechanistic explanation for cases of bilateral testicular cancer.

Men without a history of testicular cancer are occasionally found to have ITGCN on testicular biopsy or orchiectomy that was performed for other reasons. The incidental finding of testicular microlithiasis on ultrasound may also provide evidence of ITGCN in an otherwise healthy man.The risk of testicular cancer for such individuals has not been determined, but they should be counseled regarding a potential increased risk and to report any new testicular symptoms.

INITIAL PRESENTATION AND MANAGEMENT

Symptoms and Signs

- Testicular swelling.
- Pressure-like sensation
- Heaviness
- Mild-to-moderate testicular pain.

Testicular ultrasound should be obtained as soon as a neoplasm is suspected, and it will appear as one or more hypoechoic lesions within the testicle. Ultrasound distinguishes a solid from cystic mass, and intratesticular from intrascrotal/extratesticular location. A solid intratesticular mass on ultrasound is presumed to be a neoplasm and is an indication for radical inguinal orchiectomy.

Approximately half of patients present with a testicular mass and no clinical evidence of metastasis (clinical stage I). Others present with metastatic disease that may also be symptomatic. Primary tumors can be small and asymptomatic, even in the presence of metastatic disease, and occasionally they “burn out” leaving only a fibrotic scar (burned-out primary).

Metastases can be the source of clinical symptoms on presentation in such cases, and include back pain, shortness of breath, cough, gynecomastia, hemoptysis, and weight loss. Ultrasound of the testicles is helpful in establishing the diagnosis even if there is no palpable testicular mass. The detection of elevated serum tumor markers can also be helpful when an occult testicular primary or extragonadal germ cell tumor is suspected.

DIAGNOSIS

Radical inguinal orchiectomy is the standard diagnostic and therapeutic procedure for a solid intratesticular mass. Transscrotal orchiectomy or biopsy is specifically contraindicated because of the risk of tumor cell seeding of the inguinal and pelvic lymphatic drainage. Biopsy is also of limited value because testicular germ cell tumors are heterogeneous.

Removal of the entire organ is necessary to properly identify the histologic type(s) present and to select the appropriate therapy. It is reasonable to perform needle biopsy of a metastatic site in cases of occult testicular primary, burned-out primary, or extragonadal germ cell tumor; although, the results of needle biopsy must always be interpreted with caution due to sampling error. The pattern of serum tumor marker elevation is also informative about the likely cell types present (seminoma or nonseminoma), as discussed in the next section.

HISTOLOGY

Germ cell tumors are broadly classified as seminoma and NSGCT Patients with pure seminoma are managed differently than patients with NSGCT or mixed histology tumors, although mixed tumors may have a component of seminoma. In that sense, when we refer to seminoma as a clinical diagnosis it is meant as pure seminoma, whereas seminoma as a histologic pattern may also be present in mixed NSGCT.

SEMINOMA

The microscopic appearance of seminoma is characterized by sheets of neoplastic cells with abundant cytoplasm, round, hyperchromatic nuclei and prominent nucleoli . A prominent lymphocytic infiltrate is common, such that it is sometimes confused with lymphoma until the surface immunophenotype has been determined. Most seminomas do not produce

serum tumor markers, but the presence of syncytiotrophoblastic giant cells in a minority of cases accounts for modest elevations of serum human chorionic gonadotropin (hCG).

Seminomas never produce alpha-fetoprotein (AFP), and patients whose tumors have the histologic appearance of seminoma and whose serum AFP is elevated should be considered to have a mixed NSGCT, even if a nonseminomatous histologic pattern cannot be identified.

Exceptions are cases in which there is another explanation for the elevated AFP, such as liver disease or a chronic nonspecific elevation. Immunohistochemistry is usually positive for placental alkaline phosphatase (PLAP), negative for CD30, AFP, and epithelial membrane antigen, and either negative or weak/focally positive for cytokeratin.

Histologic variations of seminoma such as “anaplastic” or “atypical” seminoma are of no known clinical relevance. Spermatocytic seminoma, however, is the one variant of seminoma that has a different natural history and is even of uncertain relation to other germ cell tumors. Spermatocytic seminoma usually occurs in older individuals and has low metastatic potential. Orchiectomy is the only treatment required. Unlike all other germ cell tumors, spermatocytic seminoma is not associated with ITGCN.

NONSEMINOMATOUS HISTOLOGIES

Germ cell tumors may be composed of a single histology or multiple histologic patterns. Through poorly understood processes of mutation and differentiation, a single clone beginning as ITGCN can develop into an undifferentiated neoplasm (seminoma), or to a primitive zygotic neoplasm (embryonal carcinoma).

Further differentiation from embryonal carcinoma results in somatically differentiated tumors (teratoma) or extraembryonal-differentiated tumors (yolk sac and choriocarcinoma). Nonseminomatous histologies are found in 55% of germ cell tumors. Male germ cell tumors that contain any histologic cell type other than seminoma or syncytiotrophoblasts are collectively referred to as NSGCT.

EMBRYONAL CARCINOMA

Embryonal carcinoma is the most undifferentiated type of germ cell tumor and is thought to be pluripotent. Cells are characterized by indistinct borders and scant cytoplasm, which can be arranged in solid sheets or in glandular or tubular structures . On immunohistochemical staining, embryonal carcinoma can be positive for cytokeratin, CD30, PLAP, AFP, and hCG. Modest elevations of serum AFP and/or hCG can be seen, and frequently it is marker-negative. Lactate dehydrogenase (LDH)

concentration in the serum is an important prognostic factor in metastatic embryonal carcinoma that is marker negative

CHORIOCARCINOMA

Choriocarcinoma is composed of both cytotrophoblasts and syncytiotrophoblasts.The cells strongly express hCG. The clinical presentation of a choriocarcinoma-predominant or pure choriocarcinoma tumor often includes very high serum hCG levels, widespread hematogenous metastases, and tumor hemorrhage . Syncytiotrophoblasts and syncytiotrophoblastic giant cells can be associated with other germ cell histologies, so the presence of cytotophoblasts is required for the diagnosis.

YOLK SAC TUMOR

Yolk sac tumor (endodermal sinus tumor) is commonly seen as a component of mixed NSGCT. Pure yolk sac tumors represent a significant proportion of mediastinal germ cell tumors, but are rarely seen in adult testicular cancer. Histologic patterns include papillary, solid, glandular, hepatoid, macrocystic, and microcystic types. Perivascular arrangements of epithelial cells can be seen in yolk sac tumor and are known as glomeruloid or Schiller-Duval bodies. Immunostains are diffusely positive for AFP and may also be positive for cytokeratin, SALL4, glipican-3, PLAP, and CD117. Yolk sac tumor is associated with high serum levels of AFP.

TERATOMA

Teratoma arises from a pluripotent malignant precursor (embryonal carcionoma or yolk sac tumor) and contains somatic cells from at least two germ cell layers (ectoderm, endoderm, or mesoderm). Teratoma is commonly seen as a component of adult mixed NSGCT .A small percentage (2% to 3%) of postpubertal male germ cell tumors appear to have teratoma as the only histologic type, but these are always assumed to harbor a minor component of pluripotent NSGCT and are treated the same as a mixed NSGCT.

Immature teratoma shows partial somatic differentiation, whereas mature teratoma has terminally differentiated tissues such as cartilage, skeletal muscle, or nerve tissue, and frequently forms cystic structures. Although these cells can resemble normal tissues, teratoma is a low-grade malignancy and if untreated will grow until it is unresectable.

Moreover, teratomas can give rise to secondary somatic malignancy, such as rhabdomyosarcoma, poorly differentiated carcinoma, or primitive neuroectodermal tumor.These typically display the biology of their de novo counterparts and are treated accordingly. Teratoma does not produce elevated serum AFP or hCG. Patients with elevated serum AFP and/or hCG should be assumed to have a nonteratoma germ cell tumor component, unless the elevation can be otherwise explained.

MECHANISM OF GERM CELL TRANSFORMATION

ITGCN is thought to derive from malignant transformation of primordial germ cells or gonocytes during fetal development. Primordial germ cells migrate from the proximal epiblast (yolk sac) through the hindgut and mesentery to the genital ridge, and become gonocytes. The precise molecular events underlying transformation to ITGCN are not well understood. The most consistent genetic finding in germ cell tumors is a gain of material from chromosome 12p. The majority of NSGCT and seminomas contain i(12p), an isochromosome comprised of two fused short arms of chromosome 12.

The remaining i(12p)-negative germ cell tumors also have a gain of 12p sequences in the form of tandem duplications that may be transposed elsewhere in the genome.

Gain of 12p sequences has been found in ITGCN, indicating that it is an early event in testicular cancer pathogenesis. The acquisition of i(12p) is not thought to be the initiating event, however, because it is preceded by polyploidization. Overexpressed genes on 12p are likely to be important, and there are candidate genes on 12p including several that confer growth advantage (KRAS2, CCND2 [cyclin D2]), and others that establish or

maintain the stem cell phenotype (NANOG, DPPA3, GDF3). The exact genes that are critical to this step have not yet been identified.

Seminomas are usually hypertriploid, whereas NSGCT is more commonly hypotriploid. Other chromosome regions were found to have nonrandom gains or losses in germ cell tumors with less frequency than 12p. Single gene mutations are uncommon in germ cell tumors. The most commonly mutated genes are BRAF, KIT, KRAS, NRAS, and TP53.11 The KIT/kit ligand (KITLG) pathway has special relevance for gonadal development. The biologic function of this pathway is broad and includes development of hematopoietic cells, melanocytes, and germ cells.

KITLG is essential for primordial germ cell survival and motility, as are the chemokine SDF-1 (CXCL12) and its receptor CXCR4.11 Immunohistochemical markers found on primordial germ cells and gonocytes (PLAP, CD117 [KIT], OCT3/4 [POU5F1]) are also found on ITGCN, suggesting a transformation from these cells during fetal development.

The biallelic expression of imprinted genes in germ cell tumors has been reported, showing that they likely arose from primordial germ cells where the genomic imprinting is temporarily erased.31 Somatic mutations in

KIT as well as increased copy number have been described in 9% of testicular germ cell tumors and 20% of seminomas.

The somatic alterations in KIT found in germ cell tumors are predicted to upregulate pathway activity. KITLG plays a role in determining skin pigmentation and has undergone strong selection in European and Asian populations. Difference in the frequency of risk alleles for KITLG between European and African populations may provide an explanation for the difference in germ cell tumor incidence between Caucasian and African Americans. There is evidence that epigenetic regulation of gene expression plays a role in the pathogenesis of germ cell tumors.

The DNA methylation patterns are different among histologic types. Global hypomethylation is more common in seminomas than in NSGCT. In a study of 16 germ cell tumors, the methylation of CpG islands in NSGCT was similar to that observed in other tumor types, whereas it was virtually absent in seminomas.

Aberrant promoter methylation is generally associated with absent or downregulated expression of the methylated genes. This can result, for example, in the silencing of tumor suppressor genes.Methylation has also been correlated with germ cell tumor differentiation. The more differentiated tumors (yolk sac tumor, choriocarcinoma, teratoma) were consistently

hypermethylated, whereas seminoma and ITGCN were hypomethylated.Some of the observed methylation patterns may reflect normal development rather than germ cell tumor pathogenesis.

IMMUNOHISTOCHEMICAL MARKERS

SALL4 is expressed in almost all germ cell tumors and has been reported to be positive in ITGCN, classic seminoma, spermatocytic seminoma, embryonal carcinoma, yolk sac tumor, choriocarcinoma, and teratoma.OCT3/4 is variably expressed in ITGCN, classic seminoma, embryonal carcinoma, and yolk sac tumor. Spermatocytic seminoma, choriocarcinoma, and teratoma are usually negative for OCT3/4. CD117 (KIT) helps highlight ITGCN and classic seminoma.

CD30, SOX2, and keratin are helpful in the diagnosis of embryonal carcinoma, whereas SALL4 and Glypican-3 are often positive in yolk sac tumor.In tumors of unknown primary or those presenting as a retroperitoneal or mediastinal mass, SALL4, OCT3/4, CD117, SOX2, CD30, and low-molecular-weight keratins all may be useful in distinguishing germ cell tumors from non–germ cell tumors STAGING

The most widely used system for staging testicular cancer is the tumor, node, metastasis classification endorsed by the American Joint Commission on Cancer and the International Union Against Cancer . An important

distinction for germ cell tumors is the inclusion of “S” classification (S0 to S3), signifying serum tumor marker elevation.

There are three stage groupings of tumor, node, metastasis/serum tumor marker elevation classifications whereby, in general, stage I disease is confined to the testis, stage II is confined to the retroperitoneal lymph nodes with serum tumor markers in the good-prognosis range (S0 to S1), and stage III includes metastases that extend beyond the retroperitoneum or are extranodal in location. Stage III NSGCT also includes any patient with serum tumor markers in the intermediate- or poor-prognosis range (S2 to S3).

PATTERNS OF METASTASIS

Testicular cancers can undergo both lymphatic and hematogenous dissemination. The lymphatics arising from the testicle accompany the gonadal vessels in the spermatic cord. Some follow the gonadal vessels to their origin while others diverge and drain into the retroperitoneum. The landing zone for metastasis from the right testicle is in the interaortocaval lymph nodes just inferior to the renal vessels . The landing zone from the left testicle is in the para-aortic lymph nodes just inferior to the left renal vessels.

Large volume disease tends to progress in retrograde fashion to the aortic bifurcation and below, along the iliac vessels. Seminoma Seminoma can spread extensively through the lymphatic system to include

retroperitoneal, retrocrural, mediastinal, supraclavicular, and cervical lymph nodes, often in the absence of hematogenous metastasis. Metastasis to lungs (stage IIIA) is common; metastasis to nonpulmonary organs (stage IIIB) is less common. Serum tumor markers do not affect the stage (except in stage IS) or prognosis in seminoma.

Hematogenous metastasis to extrapulmonary organs (e.g., bone) in seminoma carries an intermediate prognosis. There is no stage IIIC or poor-prognosis designation in seminoma. Nonseminomatous Germ Cell Tumors Similar to seminoma, lymphatic spread is the most common and usually the earliest type of dissemination in NSGCT. Stage groupings depend on both the anatomic extent of disease and serum tumor markers.

Stage IIIB is distinguished from stage II or IIIA on the basis of tumor markers being in the intermediate-prognosis range (S2). Stage IIIC NSGCT carries a poor prognosis, and more often than seminoma it involves multiple organs such as liver and brain. Embryonal carcinoma, in some cases, exhibits hematogenous metastasis to lungs or nonpulmonary viscera without clinical involvement of retroperitoneal lymph nodes.

Computed tomography (CT) of the chest is necessary for complete staging workup of tumors that have a high percentage of embryonal carcinoma. Serum tumor markers are an important part of the staging system

for germ cell tumors. Three markers, namely AFP, hCG, and total LDH, are considered for establishing the correct prognostic classification (good, intermediate, or poor prognosis).

Markers should be assessed after orchiectomy and before the start of chemotherapy. Markers that are elevated prior to orchiectomy and then normalize appropriately have no prognostic significance. Markers that do not normalize in a patient without any other clinical evidence of metastatic disease are considered stage IS. In the absence of residual disease, the expected half-life of postoperative serum tumor marker decline is 2 to 3 days for hCG and 5 to 7 days for AFP

Germ Cell Tumor Risk Classifcation

Risk Group	Seminoma	Nonseminoma
Good	Any hCG Any LDH Nonpulmonary visceral metastases absent Any primary site	AFP <1,000 ng/mL hCG <5,000 mIU/mL LDH <1.5 × ULN Nonpulmonary visceral metastases absent Gonadal or retroperitoneal primary site
Intermediate	Nonpulmonary visceral metastases present Any hCG Any LDH Any primary site	AFP 1,000–10,000 ng/mL hCG 5,000–50,000 mIU/mL LDH 1.5–10.0 × ULN Nonpulmonary visceral metastases absent Gonadal or retroperitoneal primary site
Poor	Does not exist	Mediastinal primary site Nonpulmonary visceral metastases present (e.g., bone, liver, brain) AFP >10,000 ng/mL hCG >50,000 mIU/mL LDH >10 × ULN

hCG, human chorionic gonadotropin; LDH, lactate dehydrogenase; AFP, α-fetoprotein; ULN, upper limit of normal range.
Source: International Germ Cell Consensus Classifcation: a prognostic factor-based staging system for metastatic germ cell cancers. International Germ Cell Cancer Collaborative Group. *J Clin Oncol* 1997;15:594–603.

Elevated AFP has special significance in seminoma because it is only produced by NSGCT. Germ cell tumors that histologically appear to be pure seminoma with elevated serum AFP are given the clinical diagnosis of

NSGCT, and are treated as such. HCG can be elevated in either seminoma or NSGCT, but has prognostic significance only for NSCGT.

Total LDH concentration prior to chemotherapy functions as a prognostic factor in NSGCT and helps to determine stage. In seminoma with bulky metastases, LDH can be markedly elevated but has no prognostic significance. There are several potential causes of spurious elevation of tumor markers.

AFP is not cancer specific; it may be elevated in the presence of liver disease or as a nonspecific chronic elevation. Stability of AFP over time suggests a benign etiology. HCG is cancer specific in men, but it is not specific to germ cell tumors. It can be associated with other neoplasms and with exposure to cannabis products. Patients should be questioned about the use of marijuana. A positive hCG test can also occur as a laboratory artifact in patients with low serum testosterone, owing to the increased secretion of luteinizing hormone and its sequence similarity to hCG.

CLINICAL STAGING

The most commonly used methods for detecting metastatic disease are serum tumor markers and CT scan. Positron emission tomography (PET) scan can be helpful in the staging evaluation of a seminoma patient by distinguishing hypermetabolic lymph node metastases from reactive lymph nodes. PET is not as useful in NSGCT, where CT scan with oral and

intravenous contrast is the preferred technique for detecting retroperitoneal adenopathy.

PATHOLOGIC STAGING

The T classification is determined by pathology of the orchiectomy specimen. The presence of lymphovascular invasion (LVI) or invasion through the tunica albuginea with involvement of the tunica vaginalis are pT2, invasion of the spermatic cord is pT3, and involvement of the scrotum constitutes pT4 . Prophylactic retroperitoneal lymph node dissection (RPLND) is performed for surgical staging in some patients with clinical stage I NSGCT. In such cases, if no metastatic germ cell tumor is found, the stage is pathologic stage I; when disease is found, it is designated pathologic stage II

FACTORS AFFECTING OUTCOME

In clinical stage I NSGCT, the presence of LVI (pT2) is associated with an approximately 50% risk of recurrence with surveillance alone. A high percentage of embryonal carcinoma has also been associated with high risk in some series, but embryonal carcinoma is often seen together with LVI and has not been validated as an independent risk factor.

In clinical stage I seminoma, tumor size ≥4 cm is associated with approximately 30% risk of recurrence with surveillance alone. Involvement

of rete testis has not been validated as a risk factor, although it is often mentioned. The classification of patients with metastatic germ cell tumors as good, intermediate, or poor prognosis, based on serum tumor markers and extent of disease, was proposed in 1997 by The International Germ Cell Cancer Collaborative Group

In this system, the presence or absence of nonpulmonary, extranodal metastasis was validated as an independent prognostic factor for progression-free survival. For NSGCT, the degree of marker elevation and mediastinal primary (versus testis or retroperitoneal primary) were also validated as prognostic factors. The threshold values for tumor markers (hCG, AFP, and LDH) have been incorporated into the American Joint Commission on Cancer/ International Union Against Cancer staging system as the "S" classification. These prognostic groupings are used to make treatment decisions and are discussed in the following sections.

MANAGEMENT OF CLINICAL STAGE I DISEASE

Virtually all patients with clinical stage I germ cell tumors survive (cancer specific survival 98% to 99%).49 In general, patients managed with observation have the same life expectancy as those who receive adjuvant intervention. Treatment decisions must therefore be based on considerations of cost, burden of therapy, and patient preference.

SEMINOMA

Clinical stage I seminoma is grouped into high-risk and averagerisk based on tumor size, but similar options exist for all patients with stage I seminoma, regardless of risk. Stage I is a common presentation, accounting for approximately 70% of patients diagnosed with pure seminoma. Surveillance The average risk of recurrence with surveillance for stage I seminoma is 15% to 20%.

Recurrences are temporally distributed over a 10-year period. High-risk tumors have a recurrence probability of 30% to 35%, whereas tumors <4 cm without rete testis involvement may have a risk as low as 12%. Observation is heavily dependent on CT scanning because the region at highest risk is in the retroperitoneum, and most seminomas do not secrete tumor markers.

Adjuvant Chemotherapy Carboplatin is a simple and apparently safe form of adjuvant chemotherapy that is very similar in efficacy to prophylactic radiotherapy. In a randomized trial of carboplatin given as a single infusion (area under curve equals 7) versus radiotherapy to the para-aortic lymph nodes, there was no significant difference in progression-free survival (94.7% and 96%, respectively).

There was only one death from seminoma (N = 1,447) reported at a median follow-up of 6.5 years. Recurrences after adjuvant carboplatin were frequently retroperitoneal in location, meaning that follow-up CT scans are mandatory. There has been no reported evidence of second malignant neoplasms (SMN), and there have been fewer contralateral germ cell tumors reported in the patients treated with chemotherapy versus radiotherapy at medium-term follow-up.

Two courses of carboplatin have also been shown to be effective in the adjuvant setting, and in its 2014 guidelines the National Comprehensive Cancer Network (NCCN) endorsed one or two courses as a standard of care for clinical stage I seminoma, regardless of the estimated risk of recurrence.

Observation is also standard and is the preferred option. Prophylactic Radiotherapy Treatment of para-aortic lymph nodes to a dose of 20 Gy was associated with excellent local control approaching 100%. A randomized trial of 20 Gy versus 30 Gy showed no difference in rate of recurrence.

Omission of ipsilateral iliac lymph nodes from the treatment field resulted in less toxicity (infertility, gastrointestinal effects) and minimal loss of efficacy. The recurrence rate after prophylactic radiotherapy for clinical stage I seminoma is about 4%, and most of those patients survive with additional treatment (combination chemotherapy). Recurrences tend to occur

below the radiation field (pelvic lymph nodes) or above it (lungs). Radiotherapy is contraindicated for patients with horseshoe kidney or inflammatory bowel disease.

Radiotherapy was once popularized because it reduced the number of CT scans that were necessary for follow-up, with a net reduction in the cost of treatment.There is, however, a different type of cost with radiotherapy, and that is the risk of SMN. Studies of testicular cancer survivors 20 or more years after treatment have revealed an increase in midline cancers such as gastrointestinal and genitourinary malignancies.

This has brought about a reassessment of prophylactic radiotherapy, specifically, whether it is warranted when 80% of seminoma patients will be treated unnecessarily, there is a risk of SMN, and there is no survival benefit. While it continues to be offered, the use of prophylactic radiotherapy for seminoma is declining.

NONSEMINOMATOUS GERM CELL TUMOR

Clinical stage I NSGCT is broadly categorized as high risk and average risk. Although there are many differences in presentation, including tumor size, histology, and tumor marker pattern, only LVI has been validated as a risk factor. NSGCT with LVI has a recurrence rate of approximately 50% with observation.

Most recurrences are seen within 2 to 3 years of the orchiectomy.The average risk is 30%, and most patients with LVI-negative (“good risk”) stage I NSGCT probably have a risk of recurrence <30%. The risk of recurrence for LVI-negative stage I NSGCT with predominantly embryonal carcinoma histology, however, may be higher (30% to 50%).

Surveillance Approximately two-thirds of relapses among patients on surveillance are in retroperitoneal lymph nodes and one-third occur in lungs or by marker elevation alone. Recurrences in nonpulmonary viscera are rare. Most relapses occur within 2 to 3 years of orchiectomy, and patients need to remain on follow-up for at least 5 years. The ability to cure systemic disease with cisplatin-based chemotherapy in those who relapse makes observation an attractive option. It avoids unnecessary therapy for about two-thirds of patients.

Criteria for selecting patients for observation have been suggested in the literature and in the NCCN guidelines. Selection based on whether the patient is “reliable” (i.e., likely to be compliant with follow-up) was once the prevalent view, but this has been largely replaced by a more objective risk-adapted approach.

Observation is the standard of care for patients with stage IA NSGCT. The alternative is nerve-sparing RPLND, which is an unnecessary procedure

for the majority of these patients. There is no subgroup of stage IA for whom RPLND is preferred, but patients who choose observation should agree to be compliant with follow-up and to receive chemotherapy in the event of recurrence. Patients with stage IA embryonal carcinoma–predominant tumors are probably the least likely to benefit from RPLND because of the tendency for hematogenous spread and associated risk of recurrence in lungs.

Most patients with LVI-negative, clinical stage I NSGCT are candidates for observation. There is less of a consensus on management of patients with pT2-pT4 tumors (including LVI-positive). A minority of patients with pT2 tumors may choose observation, but they must understand that the risk of recurrence is 50% and they should agree (as in stage IA) to comply with follow-up. The preferred treatment for LVI-positive or advanced T classification NSGCT is adjuvant chemotherapy.

ADJUVANT CHEMOTHERAPY

Primary chemotherapy for NSGCT consists of bleomycin, etoposide, and cisplatin (BEP) There are several published studies using either one or two courses of adjuvant BEP or similar regimens in patients with clinical stage I NSGCT.

A phase II study by the Medical Research Council found 98% relapse-free survival after two courses of bleomycin, vincristine, and cisplatin,

although chemotherapy-induced neurotoxicity (CIN) remained a problem. Two courses of BEP are similarly effective in preventing recurrence with less CIN; however, etoposide causes transient myelosuppression and is associated with a low but real risk of treatment-induced leukemia.

The toxicity of two courses of BEP makes it unacceptable for stage IA NSGCT, but reasonable for stage IB where the relapse rate on observation is 50%.One limitation of adjuvant chemotherapy is the continuing risk of growing teratoma syndrome. Only RPLND can remove foci of teratoma from the retroperitoneum, where they may persist and grow following chemotherapy.

One strategy for lowering the toxicity of adjuvant chemotherapy is by shortening treatment to one course of BEP. Two European studies provide data collected prospectively; in one study, patients were randomized to a single course of BEP or RPLND (none were observed), and in a second study, the patients with LVI-negative tumors were offered surveillance or one course of BEP, while patients with LVI-positive tumors were offered one or two courses of adjuvant BEP.

Both of these studies showed a <5% recurrence rate after one course of BEP and an acceptably low rate of growing teratoma. The 2014 NCCN

guideline endorses either one or two courses of adjuvant BEP for stage IB NSGCT.

Chemotherapy Regimens for Stage II/III Germ Cell Tumor	
Previously Untreated—Good Risk[a]	
Etoposide Cisplatin	100 mg/m² IV daily × 5 days 20 mg/m² IV daily × 5 days; *Four cycles administered at 21-day intervals*
Etoposide Cisplatin Bleomycin	100 mg/m² IV daily × 5 days 20 mg/m² IV daily × 5 days 30 units IV weekly (e.g., days 1, 8, 15); *Three cycles administered at 21-day intervals*
Previously Untreated—Intermediate and Poor Risk	
Etoposide Cisplatin Bleomycin	100 mg/m² IV daily × 5 days 20 mg/m² IV daily × 5 days 30 units IV weekly (e.g., days 1, 8, 15); *four cycles administered at 21-day intervals*
Previously Treated—1st-Line Salvage Therapy	
Ifosfamide Mesna Cisplatin Vinblastine	1.2 g/m² IV daily × 5 days 400 mg/m² IV every 8 h × 5 days 20 mg/m² IV daily × 5 days 0.11 mg/kg IV days 1 and 2; *Four cycles administered at 21-day intervals*
Paclitaxel Ifosfamide Cisplatin Mesna	250 mg/m² IV by continuous infusion over 24 h on day 1 1.5 g/m² IV daily on days 2–5 25 mg/m² IV daily on days 2–5 500 mg/m² IV every 8 h on days 2–5; *Four cycles administered at 21-day intervals*

LONG-TERM FOLLOW-UP

The mandatory duration of follow-up for detection and management of recurrence of germ cell tumors is 5 years for NSGCT and 10 years for seminoma. There is wide consensus, however, that survivors of testicular cancer should have lifelong follow-up, whether it is at the primary treatment center or with a general internist who is knowledgeable about survivorship issues.

Long-term follow-up is necessary for detection of late recurrences, second testicular primaries, and SMN. Survivors require management of cardiovascular effects and symptoms of neurotoxicity. Special care may be required for maintenance of sexual health, fertility issues, and psychosocial functioning.

CHEMOTHERAPY-INDUCED PULMONARY TOXICITY

The list of chemotherapeutic agents reported to cause cytotoxic drug-induced lung disease continues to grow. The true incidence of toxicity is unknown, but the reported frequency of pulmonary toxicity ranges from 10% to 30%. True toxicity must be distinguished from other factors that affect the lung in patients with cancer including cardiac-induced pulmonary edema, infection, lymphangitic carcinomatosis, and diffuse alveolar hemorrhage.

With the development of targeted monoclonal antibodies used in cancer treatment, new toxicities are continuously being reported. Classes of drugs added to the list include tyrosine kinase inhibitors, anti-VEGF agents (such as bevacizumab), rituximab, granulocyte– colony-stimulating factor (G-CSF), and the mammalian target of rapamycin inhibitors.

MECHANISMS OF PULMONARY INJURY AND HISTOLOGY

Except for a few chemotherapeutic agents (e.g., bleomycin), the details of the pathophysiology of lung injury are unknown. Various

mechanisms of pulmonary toxicity have been proposed based on the mechanisms of action of different classes of therapeutic agents. These include a direct toxic effect on alveolar epithelial cells, the induction of an inflammatory immunologic response and endothelial cell injury, or activation causing capillary leak syndrome.

These events can result in a variety of clinical presentations including nonspecific interstitial pneumonitis, hypersensitivity reaction syndrome, noncardiogenic pulmonary edema, infusion reaction, capillary leak syndrome, eosinophilic pneumonia, bronchospasm, acute lung injury, diffuse alveolar damage, or fibrosis. The histopathologic changes of drug-induced pulmonary toxicity show common features. Similar to radiation-induced damage, abnormalities are seen in endothelial and epithelial cells.

The vascular damage is characterized by endothelial swelling with exudation of fluid into the interstitium and the intra-alveolar spaces. Destruction and desquamation of type I pneumocytes occurs with delamellation and proliferation of type II pneumocytes. Mononuclear cell infiltration and fibroblast proliferation with fibrosis are common findings; the character of the inflammatory cellular infiltrate may be a feature that distinguishes the toxicity of one drug from another.

Pathways involved in chemotherapy-induced lung disease include apoptosis, impaired repair response, and angiogenesis. Apoptosis pathways include the death receptor pathway and the Fas ligand as well as the mitochondrial pathway and Bcl-2 protein. Both of these lead to activation of caspases, which activate degradative enzymes within the cell. Cells affected can be immune cells, endothelial cells, or epithelial and other parenchymal cells.

Impaired pathways involving epidermal growth factor and TGF may contribute to alterations in repair mechanisms; both factors are expressed in type II pneumocytes and epithelial cells. The older chemotherapy agents are the best described in their mechanism of lung injury. It is thought that a major contributor to the toxicity from these agents results from ROS including superoxide anions, hydrogen peroxide, and hydroxyl radicals, primarily from activated neutrophils.

Bleomycin induces reactive oxygen radicals by forming a complex with Fe3+. Consistent with a direct pathologic role for this mechanism, iron chelators ameliorate the pulmonary toxicity of bleomycin in animal models. ROS formed after bleomycin therapy can produce direct toxicity through participation in oxidation-reduction reactions and subsequent fatty acid oxidation, which leads to membrane instability.

Oxidants also cause other inflammatory reactions within the lung. For example, the oxidation of arachidonic acid is an initial step in the metabolic cascade that produces active mediators, including prostaglandins and leukotrienes.Because ROS are so highly tied to bleomycin toxicity, high-dose oxygen can exacerbate its effects, and toxicity can be seen years after its administration.

In addition to ROS direct toxicity, cytokines such as IL-1, macrophage inflammatory protein-1, monocyte chemoattractant protein-1, and TGF-β are released from alveolar macrophages in animal models of bleomycin toxicity resulting in fibrosis.

Through modulation of fibroblast proliferation, bleomycin can cause excessive interstitial and intra-alveolar collagen deposition leading to pulmonary fibrosis. Bleomycin directly upregulates collagen synthesis in fibroblasts by stimulating transcription. There is some evidence to support a role for a mast cell/fibroblast interaction in the generation of this fibrosis as well.One of the potential determinants of bleomycin toxicity is the bleomycin hydrolase, which is the major enzyme responsible for metabolizing bleomycin to a nontoxic molecule. Interestingly, the two organs that are the most common targets for bleomycin toxicity (lung and skin) have the lowest levels of the enzyme. Studies are needed to determine if genetic variability

of this enzyme accounts for individual susceptibility or immunity to bleomycin pulmonary toxicity in humans, as it does in animals.

Likewise, with linkage and association studies, genetic susceptibility to bleomycin-induced pulmonary fibrosis has been investigated in mice. Polymorphisms in Cep55, a gene encoding proteins involved in autophagy, and Masp2, a gene encoding complement pathway proteins, as well as several others, have been identified as potentially increasing toxicity susceptibility.

However, these associations have not been replicated in other studies and are currently not being used clinically. Because the lung is exposed to many substances that can activate its immune system, there appears to be a pulmonary immune tolerance state to avoid overreactions. This tolerance state in part may be a result of an effector and suppressor cell balance.

Cytotoxic drugs can alter the normal balance, which may cause tissue damage. For example, lymphocytic alveolitis is a consistent finding in methotrexate pneumonitis, with an imbalance of the CD4-to-CD8 ratio. Bronchoalveolar lavage studies in patients with methotrexate pulmonary toxicity have shown the presence of a T-lymphocytic alveolitis, whereas studies on some patients with bleomycin toxicity have revealed a polymorphonuclear alveolitis.

G-CSF is also implicated in pulmonary toxicity through its effects on neutrophil activation. G-CSF toxicity is thought to be secondary to activation of inflammatory pathways and ROS within the neutrophils, causing pulmonary edema and acute respiratory distress syndrome. IL-2, used to treat metastatic melanoma and renal cell cancer, stimulates the immune system by stimulating CD4+ T cells and increasing natural killer cell activity.

These effects likely contribute to a generalized inflammatory state, contributing to the capillary leak syndrome and noncardiogenic pulmonary edema associated with this drug. IL-2 has been shown to directly increase endothelial permeability and indirectly increases vascular leak by increasing cytokine levels, particularly tumor necrosis factor (TNF)-α, leukocyte adhesion, and inflammation, and altering the extracellular matrix.

CLINICAL FEATURES

Several distinct pulmonary syndromes have been linked to the use of bleomycin, including bronchiolitis obliterans with organizing pneumonia (BOOP), eosinophilic hypersensitivity, and, most commonly, interstitial pneumonitis, which may ultimately progress to fibrosis The latter, bleomycin-induced pneumonitis (BIP), occurs in 0 to 46% of patients treated with bleomycin-containing chemotherapy, depending on the diagnostic criteria used .A reasonable estimate of BIP incidence is 10%. The mortality

of patients with BIP has been reported to be approximately 10–20% in patients who develop bleomycin-induced lung injury (2-3% of all patients treated with bleomycin. To our knowledge, there are no reported cases of BIP secondary to the use of intralesional bleomycin for the treatment of vascular anomalies.

While BIP normally develops gradually during treatment, the development of BIP up to two years after discontinuation of bleomycin therapy has also been reported. The clinical diagnosis of BIP is difficult and sometimes delayed by its similarity to other conditions that are often encountered in cancer patients, such as respiratory tract infections, pulmonary metastasis, and lymphangitic carcinoma. Bleomycin-induced hypersensitivity pneumonitis may present with more rapidly progressive symptoms.

The most common symptoms are exertional dyspnea and nonproductive cough. With progressive pneumonitis, dyspnea at rest, tachypnea, and cyanosis may occur. Physical examination of the lungs may be normal or may reveal end-inspiratory bibasilar crepitations or rhonchi. Pleural rubbing and finger clubbing are unusual.

Because of the resemblance of the symptoms of BIP with other diseases, the diagnosis of BIP is often one of exclusion. Infectious diseases are often excluded by culture and Gram-staining of sputum, polymerase

chain reaction analysis of pathogens such as viruses, serology, or identification of antigens of pneumonia-causing pathogens. Patients have often been treated unsuccessfully with antibiotics because the suspicion of pneumonia before the diagnosis is established. Pneumocystis jiroveci pneumonia (PJP) should always be investigated. The clinical and radiological features of PJP (dyspnea, dry cough, bilateral infiltrates, and ground-glass opacities) may resemble those of bleomycin-induce pneumonitis. PJP incidence is increased in patients with non-Hodgkin's lymphoma and those receiving long-term steroids. Empirical treatment of PJP is recommended in cases of clinical suspicion .Patients who survive an episode of BIP almost always recover completely, with disappearance of symptoms, signs, and disturbances of pulmonary function.

PULMONARY FUNCTION TESTS

The most common abnormalities associated with chemotherapyinduced pulmonary toxicity are a reduced diffusing capacity for carbon monoxide and a restrictive ventilatory defect. Isolated gas transport abnormalities manifested by a decrease in the diffusing capacity and/or arterial hypoxemia, especially with exercise, have been seen.

Screening pulmonary function tests to predict which patients receiving chemotherapy are likely to develop toxicity would be helpful but

have not been established. In bleomycin toxicity, changes in the diffusing capacity may be transient, whereas decreases in total lung capacity seem to correlate better with radiographic abnormalities.

DIAGNOSIS

Lung biopsy is usually necessary for a definitive diagnosis and to rule out other causes of lung injury including infection, tumor progression, or diffuse alveolar hemorrhage. Acute respiratory distress syndrome is suggested by radiographic and physical examination, and lymphangitic spread is suggested by malignant cells within BAL fluid

Carcinogenic pulmonary edema is suggested by echocardiography as well as brain natriuretic peptide values and resolution with diuresis. Through the use of BAL, several studies reported the presence of a characteristic or predominant cell associated with particular drugs.Although these data might be of value in understanding the pathogenesis of drug-induced lung disorders, their usefulness in diagnosing drug toxicity is limited.

To date, biomarkers for chemotherapy-induced lung injury are research tools more than clinically applicable tests. No serum marker for drug-induced pulmonary toxicity currently exists. KL-6 may be helpful in diagnosis as it is elevated in 53% of patients with drug-induced pneumonitis.It will not be elevated in patients With bacterial pneumonia,

pulmonary aspergillosis, asthma, bronchiectasis, emphysema, eosinophilic pneumonia, or organizing pneumonia.

It lacks specificity, however, as it can also be elevated in patients with radiation pneumonitis, viral pneumonia, and Pneumocystis pneumonia in this patient population. Though elevated levels of TGF-β in plasma after high-dose chemotherapy for breast cancer predicted an increased risk of pulmonary toxicity after autologous bone marrow transplantation, its clinical applicability has been limited.

TREATMENT

The most effective way to manage pulmonary toxicity associated with chemotherapeutic agents is to prevent it. G-CSF should be discontinued as soon as leukocytes reach over 1,000 cell/μL. Hypersensitivity reactions can be mitigated with the administration of steroids and H2 blockers prior to dosing. If toxicity occurs, withdrawal of the offending agent is the cornerstone of therapy.

Although no controlled studies in humans have systematically examined the efficacy of corticosteroids, a trial of these agents is probably warranted in most cases. The optimal dose and duration of therapy are not known; however, 1 mg/kg per day is usually initiated with a slow and careful

tapering schedule because clinical deterioration after tapering has been reported.

The use of lung transplant in the treatment of advanced druginduced pulmonary fibrosis should be considered in appropriate patients. One report described the case of a 23-year-old male patient who underwent a single lung transplant because of presumed drug-induced pulmonary fibrosis 12 years after undergoing chemotherapy for acute lymphocytic leukemia

BLEOMYCIN

MOLECULAR PHARMACOLOGY

Bleomycin sulphates are water-soluble glycopeptide products of the actinobacterium Streptomyces verticillus. Drug formulations consist of a mixture of bleomycin analogues that differ in their cationic C-terminal amine. The chemical formulas used are primarily bleomycin A2 and B2 . The crystal structures of bleomycin B2 and A2 reveal important interactions with DNA and cellular proteins.The most active chemotherapeutical forms are bleomycin A2 and B2.15 The effect of bleomycin is cell cycle specific, because its main effects are mediated in the G2 and M phases of the cell cycle.

The exact mechanism for DNA strand scission has been suggested to be due to bleomycin's chelating of metal ions (primarily iron) and producing a pseudoenzyme that reacts with oxygen to produce superoxide- and hydroxide-free radicals, thus cleaving DNA. Alternatively, bleomycin may bind at specific sites in the DNA strand and induce scission by abstracting the hydrogen atom from the base, resulting in strand cleavage as the base undergoes a Criegee-type rearrangement, or bleomycin may form an alkali-labile lesion.

Bleomycin is used in the treatment of Hodgkin lymphoma (as a component of the ABVD and BEACOPP regimen), squamous cell carcinomas, and testicular cancer; in the treatment of plantar warts,18 as a means of effecting pleurodesis,19 as well as an intralesional agent with electrochemotherapy in the management of cutaneous malignancies

Cytotoxic activity of bleomycin is through oxidation of deoxyribose of thymidylate and other nucleotides, which produce single-strand and double-strand breaks in DNA, chromosomal aberrations, gaps, fragments and translocations. Bleomycin is deactivated by bleomycin hydrolase (BLMH), which is found predominantly in the liver, spleen, bone marrow, and intestine, but is poorly expressed in skin and lung, which relates to cutaneous effects and lung injury of bleomycin.

Resistance of tumours to bleomycin is due to high levels of hydrolase activity , but increased protein binding, decreased cellular uptake, drug inactivation by thiols and adaptation to oxidative stress and enhanced capacity to DNA repair may also participate

CLINICAL PHARMACOLOGY

Bleomycin has poor oral bioavailability. On intravenous administration it has a terminal half-life of approximately 90 min. In patients with a normal renal function approximately 65% of an administered dose is excreted in the urine as active bleomycin within the first 24 h while renal dysfunction leads to signifi- cantly increased drug exposure. Bleomycin poorly crosses the blood–brain barrier, however it does cross the placenta.

PATHOPHYSIOLOGY OF BLEOMYCIN-INDUCED LUNG INJURY

Cytokines and free radicals are key effectors of BILI and are related to low levels of BLMH in lung, particularly in type II pneumocytes. This feature renders lung cells vulnerable to toxic effects of bleomycin at mitosis. On molecular level, bleomycin binds to DNA using iron ions as cofactor, and in the presence of oxygen generates hydroxyl radicals, which causes DNA breaks and leads to cell apoptosis of both epithelial and endothelial cells in lung. Acute inflammation is central to development of BILI. Inflammatory exudate consists mainly of mononuclear macrophages, lymphocytes and neutrophils.

Bleomycin stimulates alveolar macrophages to secrete inflammatory cytokines such as tumour necrosis factor (TNF), interleukin (IL)-1, IL-18 [20], IL-22 and IL-17a [21] and endothelial cells to secrete IL-6 [22,23]. Cytokines activate lymphocytes and upregulate the expression of adhesion molecules on endothelial cellsfacilitating inflammatory cells to adhere to the endothelium, influx into the interstitium and damage endothelial cells through the Fas–FasL pathway.

Fibroblasts are activated early in BILI through stimulation of fibronectin, which is produced by damaged endothelial cells or stimulation by cytokines, such as TNF, platelet derived growth factor (PDGF) and transforming growth factor (TGF). Continued exposure of lung to bleomycin can lead to increasing collagen synthesis and deposition of various matrix proteins including collagens, elastin, and proteoglycans .

Moreover, bleomycin-activated alveolar macrophages stimulate the synthesis of hyaluronan, a connective tissue molecule that is seen in fibrotic lungs. T lymphocytes are also involved in the inflammation driven lung damage. They release cytokines secreted during Th1 inflammation (e.g., IFN-) or Th2 inflammation (e.g., IL-13) that modulate the expression of growth factor activity through the STAT family of transcription factors.

INCIDENCE AND RISK FACTORS

Pulmonary toxicity is reported to occur in up to 46% of patients treated with bleomycin-containing chemotherapy with a mortality rate approaching 1–3%. A number of risk factors have been identified in several clinical studies. Those are cumulative dose,

- old age,
- reduced renal function,
- supplemental oxygen exposure,
- cigarette smoking
- route of drug delivery (intravenous or intramuscular administration)

More recently, pharmacogenetic studies have associated single nucleotide polymorphisms in the bleomycin hydrolase gene with drug activity and toxicity. The incidence of BILI increases from 3% in patients receiving a cumulative dose of 500 IU. However large inter-patient variability has been noted. Fatal BILI has been described in patients treated with 500 IU did not develop any pulmonary toxicity. In another study no difference in the cumulative dose of bleomycin was found between patients who died of BILI and those who did not.

Generally, cumulative doses above 400 IU are associated with increased risk for BILI, although significant lung injury can occur with lower doses . Patients' age is also an established risk factor for the development of BILI. Patients older than 70 years have an increased susceptibility to develop BILI .

MATERIALS AND METHODS:

STUDY POPULATION:

SOURCE OF DATA:

The study will be conducted on 30 patients admitted to Government Rajaji Hospital & Madurai Medical College during the study period from March 2018 to August 2018.

Inclusion criteria:

Newly diagnosed Hodgkins lymphoma and germ cell tumour patients with age more than 18 years confirmed by histopathological examination and immunohistochemistry.

Exclusion criteria:

1. Underlying lung disease

COLLOBORATING DEPARTMENTS

- Department of Medical oncology
- Department of Thoracic medicine

METHOD OF DATA COLLECTION:

Hodgkin's lymphoma and germ cell tumour patients of varying age and sex were selected their consent was taken. The history was elicited. Age, height weight were recorded. Thorough clinical examination were carried out. The performance of baseline PFT was carried out. In all the patients relevant information will be collected in a predesigned proforma.

The patients are selected based on clinical examinations, histopathological examination and immunohistochemistry. The patients are followed over a period of 6 months with pulmonary function test and HRCT at regular intervals.

LABORATORY INVESTIGATIONS

a) Complete blood count
b) Liver function test
c) Renal function test
d) Sputum AFB
e) Sputum Culture and sensitivity
f) HPE
g) IHC
h) Chest X ray

i) PFT

j) HRCT

DESIGN OF STUDY:

Prospective and observational study.

PERIOD OF STUDY:

6 months (March 2018 to August 2018)

STATISTICAL ANALYSIS

TABLE – 1

AGE	No Of Cases	Percentage
< 20	3	10.0
21 - 30	6	20.0
31 - 40	10	33.3
41 - 50	9	30.0
> 50	2	6.7
Total	30	100.0

AGE DISTRIBUTION

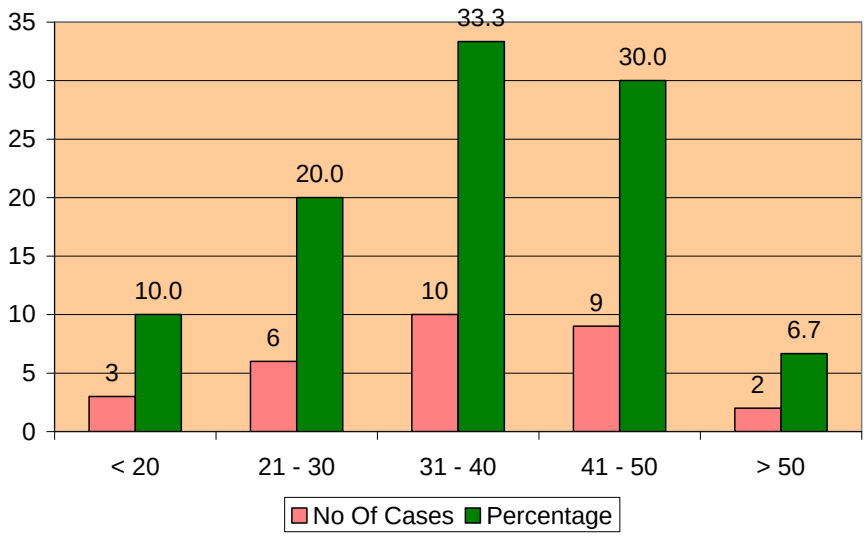


TABLE – 2

SEX	No Of Cases	Percentage
MALE	18	60.0
FEMALE	12	40.0
Total	30	100.0

GENDER DISTRIBUTION

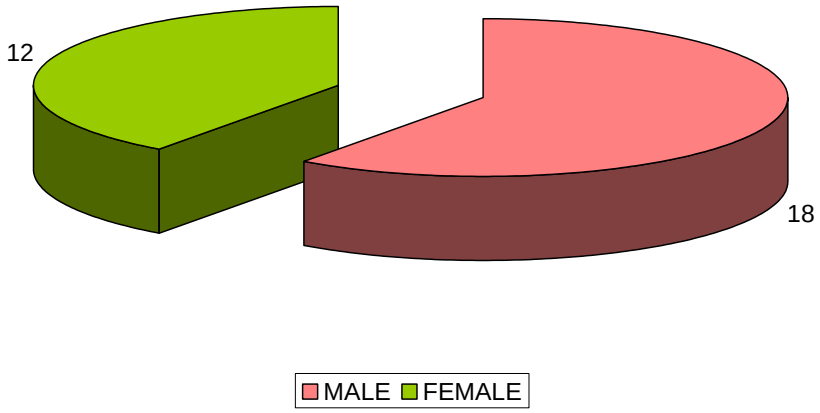


TABLE – 3

TYPES OF DISEASE	No Of Cases	Percentage
HL	18	60.0
Germ all tun	12	40.0
Total	30	100.0

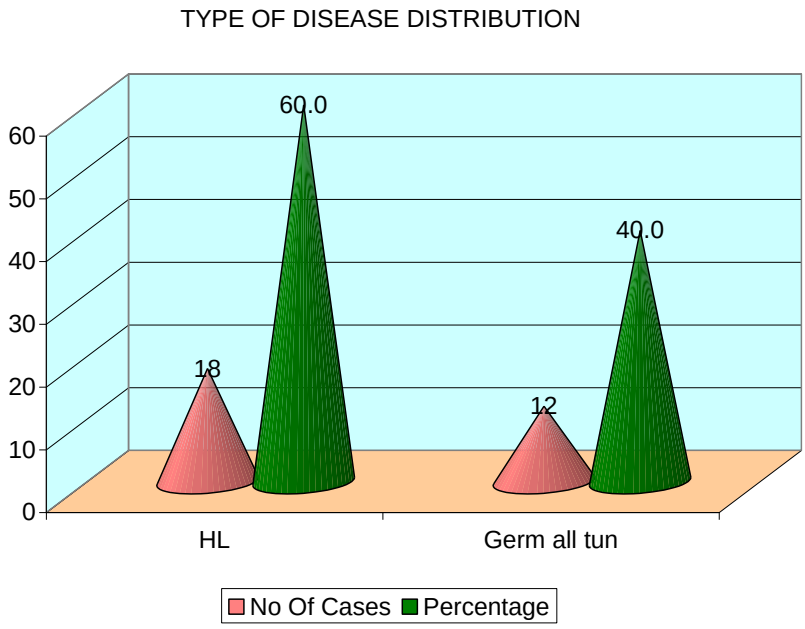


TABLE - 4

Severity of Disease	No Of Cases	Percentage
Stage 1	0	0.0
Stage 2	15	50.0

Stage 3	10	33.3
Stage 4	5	16.7
Total	30	100.0

SEVERITY OF DISEASE

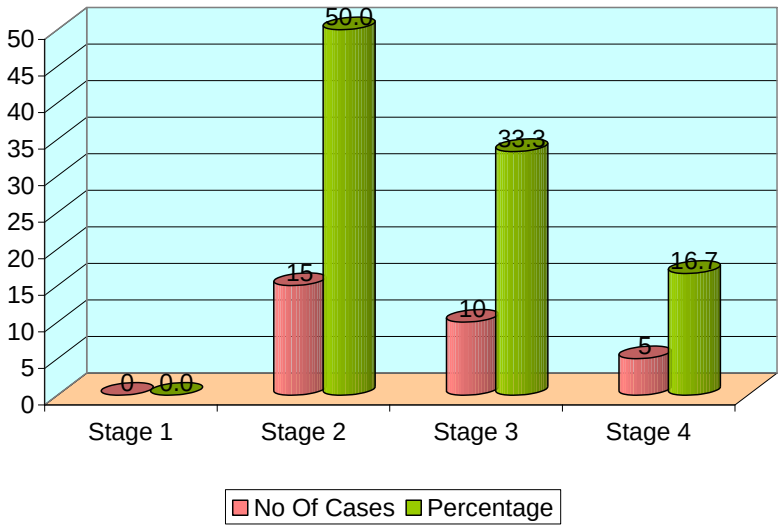


TABLE - 5

Respiratory symptoms	No of Cases	Percentage
NP Cough	9	30.0
Dyspnoea	3	10.0

Nil	18	60.0
Total	30	100.0

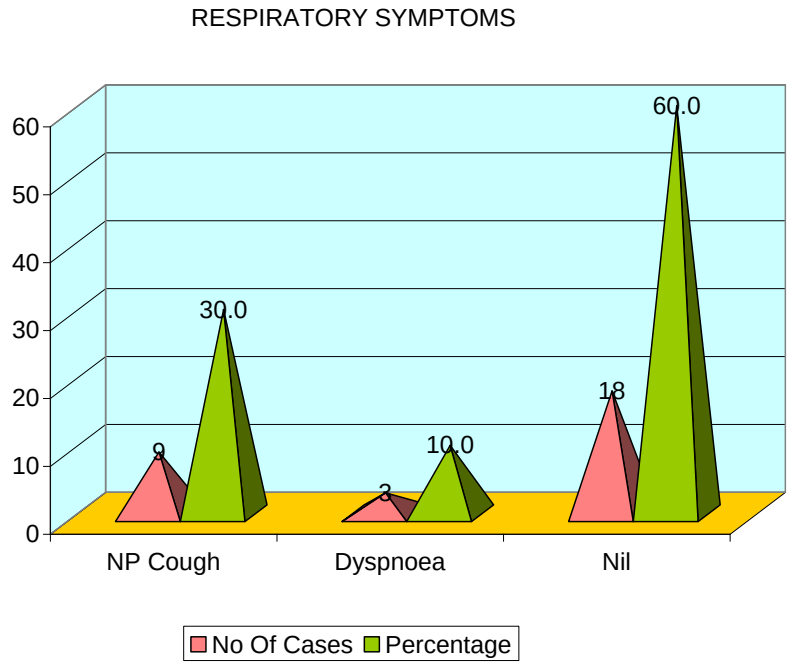


TABLE - 6

PFT	No Of Cases	Percentage
Abnormal	9	30.0

Mixed	1	3.3
Obstructive	5	16.7
Restrictive	3	10.0
Nil	12	40.0
Total	30	100.0

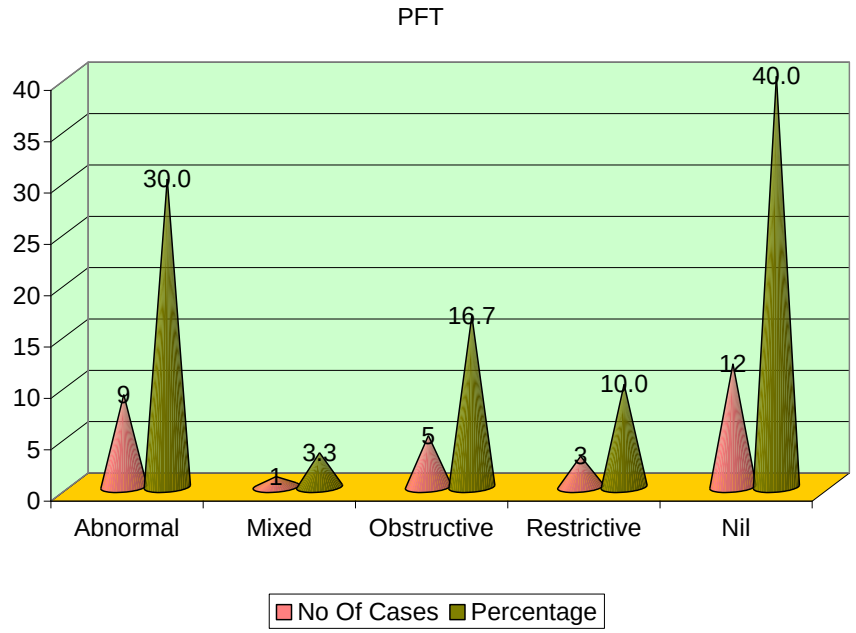


Table - 7

AGE vs PFT	Abnormal	Mixed	Obstructive	Restrictive

< 20	1	0	0	0
21 - 30	1	1	1	1
31 - 40	5	0	2	1
41 - 50	1	0	1	1
> 50	1	0	1	0
Total	9	1	5	3

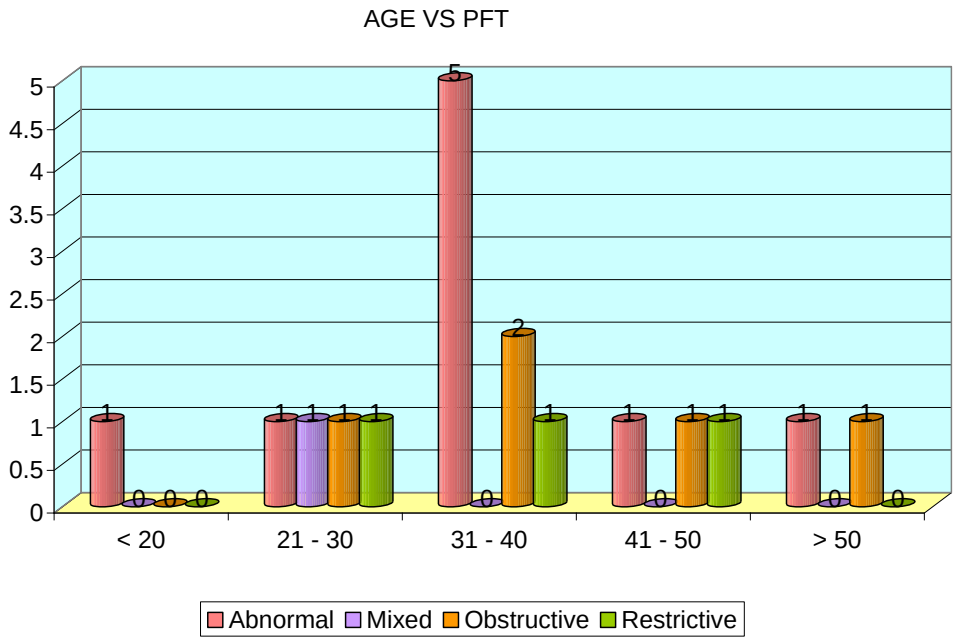


Table - 8

AGE vs PFT	Abnormal	Mixed	Obstructive	Restrictive
MALE	4	1	2	2
FEMALE	5	0	3	1
Total	9	1	5	3

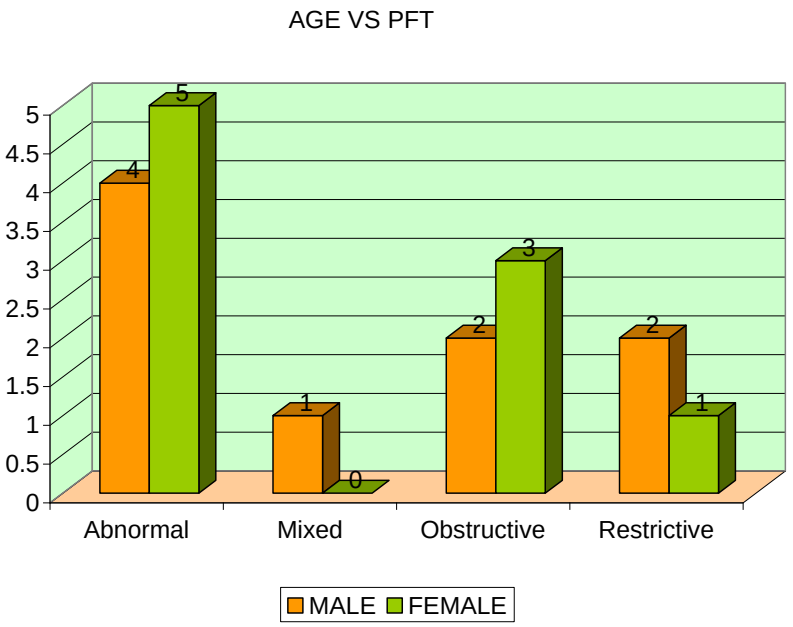


TABLE – 9

PFT vs Bleomycin	Mean Bleomycin
Abnormal	172.8
Obstructive	173.2
Restrictive	168

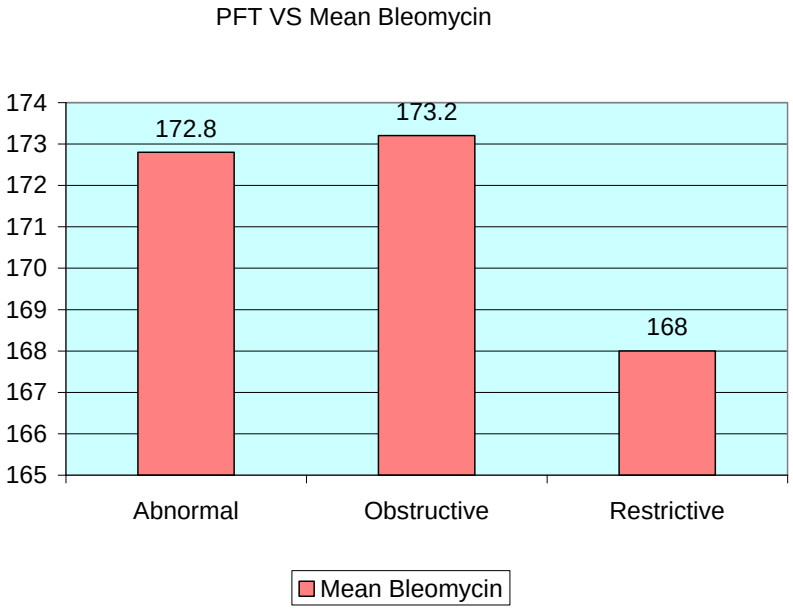
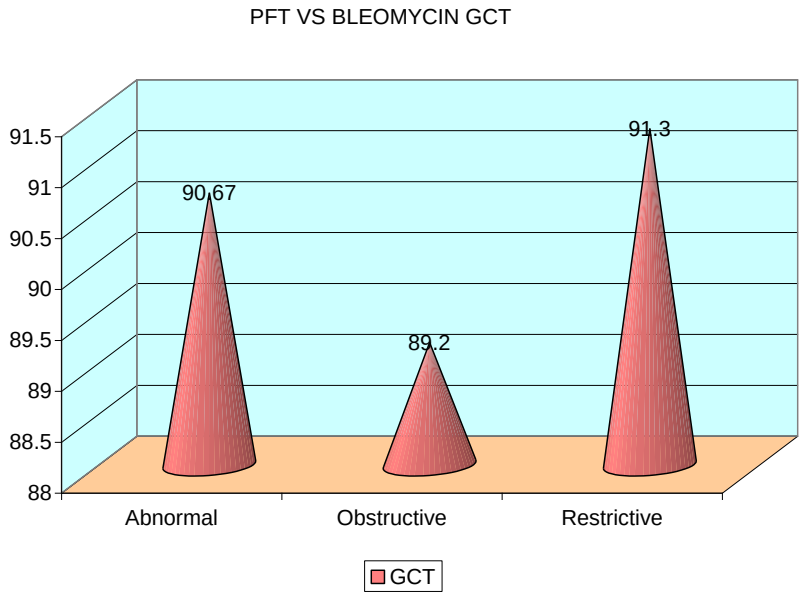


TABLE – 10

PFT vs Bleomycin	GCT
Abnormal	90.67
Obstructive	89.2
Restrictive	91.3



DISCUSSION

The mechanism of bleomycin-induced lung injury is not entirely clear but likely involves oxidative damage, relative deficiency of the deactivating enzyme bleomycin hydrolase, genetic susceptibility, and elaboration of inflammatory cytokines.

Bleomycin induces the generation of reactive oxygen radicals by forming a complex with . Consistent with a direct pathologic role for this mechanism, iron chelators ameliorate the pulmonary toxicity of bleomycin in animal models. Reactive oxygen species can produce direct toxicity through participation in redox reactions and subsequent fatty acid oxidation, which leads to membrane instability. Oxidants can cause inflammatory reactions within the lung. For example, the oxidation of arachidonic acid is the initial step in the metabolic cascade that produces active mediators including prostaglandins and leukotrienes. Cytokines such as interleukin-1, macrophage inflammatory protein-1, platelet-derived growth factor (PDGF), and transforming growth factor (TGF)- are released from alveolar macrophages in animal models of bleomycin toxicity, resulting in fibrosis. Damage and activation of alveolar epithelial cells may result in the release of

cytokines and growth factors that stimulate proliferation of myofibroblasts and secretion of a pathologic extracellular matrix, leading to fibrosis.

Specifically, TGF-, PDGF receptor- (PDGFR-), and tumor necrosis factor- (TNF-) are believed to stimulate the transformation, proliferation, and accumulation of fibroblasts, which leads to the deposition of extracellular matrix. The progressive accumulation of this collagen matrix causes distortion and destruction of alveolar structures and, eventually, loss of lung function. In animal models, it has been demonstrated that PDGFR- expression is increased in BIP. PDGFR- has also been shown to be increased in epithelial cells and alveolar macrophages in the lungs of patients with idiopathic pulmonary fibrosis. Recent evidence obtained using a bleomycin-induced lung fibrosis model indicates that some fibroblasts in fibrosis may be formed from bone marrow progenitors, as well as from epithelial cells through epithelial-mesenchymal transition Cytotoxic drugs may also affect the local immune system. Because the lung is exposed to numerous substances that can activate its immune system, there appears to be pulmonary immune tolerance, which avoids overreactions. This tolerance may, in part, be the result of an effector and suppressor cell balance. Cytotoxic drugs can alter the normal balance, leading to tissue damage.

Other homeostatic systems within the lung can also be affected, such as the balance between collagen formation and collagenolysis. Bleomycin

may upregulate collagen synthesis by modulating fibroblast proliferation through a TGF- response. Excessive collagen deposition may result in severe, irreversible pulmonary fibrosis. Bleomycin also has profound effects on the fibrinolytic system, altering the balance between fibrin deposition and fibrinolysis on the alveolar surface, thereby leading to fibrin deposition.

The alveolar macrophage is thought to play a central role in the development of bleomycin-induced lung injury due to its ability to induce the release of a number of effector molecules (e.g., cytokines, lipid metabolites, and oxygen radicals). The mechanism by which alveolar macrophages are activated is unknown. Bleomycin receptors have been identified on the surfaces of rat alveolar macrophages, suggesting that macrophage activation may occur via a second messenger.

Bleomycin-containing regimens remain the standard of care for HL and for patients with intermediate and poorrisk GCTs. Those patients need close medical monitoring for early diagnosis of lung toxicity to prevent morbidity and mortality. Although no category 1 recommendations exist, the following are generally accepted guidelines in this setting:

- consider all risk factors for BILI when treating patients with HL or GCT;
- maintain dose-intensity/density in parallel with efforts to minimize potentially lethal BILI;

- carefully assess for symptoms or signs suggestive of pulmonary toxicity, perform DLCO/FVC tests;
- discontinue bleomycin in case of clinical or radiographic signs of pulmonary toxicity and/or if significant declines in DLCO;
- restrict the total bleomycin dose to less than 400 IU;
- consider omitting bleomycin at first cycle in the rare case of high volume choriocarcinoma, extensive lung metastases, hemoptysis or hypoxemia;
- limit as much as possible the inhaled oxygen concentration
- cease smoking (lifelong);
- monitor the fluid balances to minimize the risk for clinically significant lung toxicity
- considersubstituting 4EP for 3BEP in good-risk metastatic GCTs for patients with higher risk for bleomycin toxicity (i.e., smokers, advanced age, renal insufficiency not amenable to correction before initiation of treatment, prior radiation therapy);
- efforts are currently undertaken to develop pharmacogenomic predictors of bleomycin toxicity by studying variants of the gene that encodes bleomycin hydrolase.

SUMMARY

Totally 30 patients among which 18 hodgkin's lymphoma and 12 germ cell tumour patients admitted in the Govt Rajaji Hospital were studied for bleomycin induced lung toxicity during march 2018 to August 2018.

Age of the subject varied from 18 to 60 years of age. Mean value of age is 42 years

Male were 18 female were 12.

Most of the HL and GCT patients were in the stage 3 disease.

Selected patients were free from previous lung disease as screened by PFT and HRCT.

After completing the course of chemotherapy for HL and GCT patients they were assessed. 12 patients developed symptoms among which 9 had dry cough and 3 had dyspnoea.

All patients were subjected to PFT at the end of the treatment. 9 of them developed abnormal PFT in the form of obstruction in 5 patients, restrictive pattern in 3 patients and mixed form in 1 patient.

When HRCT was repeated for these patients they did not show any abnormality.

Since bleomycin toxicity is not only dose dependent these patients have to be further followed up from earlier course of treatment to a minimum of 2 years for earlier identification of bleomycin induced toxicity.

LIMITATIONS OF THE STUDY

- The major limitation of the study is the small sample sizeof the included patients
- Lack of control group
- Period of study is of short duration and hence mortality benefit could not be assessed

CONCLUSION

Bleomycin-induced lung injury is a major pulmonary toxicity. The mortality of this complication is high, ranging from 10 to 20%, and significantly impacts quality of life and five-year overall survival. The diagnosis of interstitial lung disease and BIP is particularly challenging and often depends on clinical, radiological, and cytological findings. Progress in understanding the mechanisms behind the therapeutic efficacy and unwanted toxicity of bleomycin, as well as elucidation of its biosynthetic pathway, may lead to the development of agents capable of preventing or treating BIP. Until then, physicians administrating bleomycin should be aware of potential lung toxicity, especially in the presence of risk factors.

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PROFORMA

PROFORMA

BLEOMYCIN INDUCED PULMONARY TOXICITY CASE REPORT FORM

Name:

Age : Yrs SEX: M F

Occupation:

Socio Economic Status:

ADDRESS

MOBILE

ANTHROPOMETRY

HT cm Wt kg

Presenting complaints:

Clinical Findings:

Past History:

HT DM CAD

LUNG DISEASE Y N

If yes

Personal History:

Smoking

Alcoholic

Investigations

Biopsy Report

Date/No

IMMUNOHISTO CHEMISTRY

OTHER REPORTS

DIAGNOSIS

	BASELINE	INTERMEDIATE	POST
CHEMO			
TC			
DC	P L M E	P L M E	P L M E
ESR	mm/hr	mm/hr	mm/hr

LFT
T.Billirubin
D
T
SGOT
SGPT
S.ALBUMIN
GLOBUMIN

RFT

S.UREA
S.CREATININE
RBS

SPUTUM AFB

GENE XPERT

CHEST xray

HRCT

PFT

Brochoscopy

Surgery if done

Radiotherapy

Response of primary disease to the Treatment

MASTER CHART

S.No	Name	I.P No	Age	Sex	Disease	Severity of Disease	Respiratory symptoms	PFT	Urea	Creatinine	Bleomycin dose	GCT
1	selvi	29068	33	F	Germ cell tumour	Stage 2	NP Cough	Restrictive	24	0.6	162	86
2	muniyandi	4796	25	M	HL	Stage 3	NP Cough	Mixed	26	0.8	164	88
3	murugan	31021	27	M	HL	Stage 2		Abnormal	32	0.8	166	90
4	visweswaran	31407	43	M	HL	Stage 4			34	0.9	168	92
5	ramayee	6098	17	F	Germ cell tumour	Stage 3	NP Cough		35	1.1	170	94
6	selvakumar	35949	46	M	HL	Stage 2	NP Cough	Restrictive	22	1.3	176	96
7	perumayee	32672	38	F	Germ cell tumour	Stage 2	Dyspnoea		20	0.6	180	92
8	chellasamy	38863	54	M	HL	Stage 4		Abnormal	24	0.8	176	90
9	boominathan	39807	26	M	HL	Stage 2	NP Cough		28	0.7	170	94
10	Lakshmi	8665	23	F	Germ cell tumour	Stage 3		Obstructive	29	1	172	96
11	Chellammal	11760	19	F	HL	Stage 2		Abnormal	26	1.1	174	92
12	subramani	47294	36	M	HL	Stage 3			24	1.2	176	94
13	Saraswathy	48023	48	F	Germ cell tumour	Stage 2	NP Cough	Abnormal	32	0.8	178	88
14	rengaiah	3214	55	M	Germ cell tumour	Stage 3		Obstructive	30	0.9	174	84
15	Usha	10921	37	F	HL	Stage 2	Dyspnoea	Obstructive	34	1.2	178	86
16	muthu	18311	19	M	HL	Stage 2			26	0.9	172	83
17	mariyammal	16606	32	F	Germ cell tumour	Stage 3	NP Cough	Abnormal	22	0.8	170	84
18	mohan	15334	44	M	HL	Stage 4			20	0.9	172	86
19	kannan	13090	22	M	Germ cell tumour	Stage 3		Restrictive	24	1.1	166	92
20	babu	2263	45	M	HL	Stage 2			26	1.3	162	98
21	rajamani	7587	34	F	HL	Stage 3	NP Cough	Abnormal	26	0.6	164	100
22	mariyammal	56874	47	M	Germ cell tumour	Stage 2		Obstructive	28	0.8	176	92
23	raja	23548	38	M	HL	Stage 2		Abnormal	24	0.7	178	82
24	Gangadevi	1258	29	F	HL	Stage 3	Dyspnoea		26	1	174	83
25	rajakannan	16486	32	M	Germ cell tumour	Stage 2		Abnormal	28	1.1	178	96
26	Roja	25463	37	F	HL	Stage 4		Abnormal	24	1.2	172	94
27	Kumaran	26548	43	M	HL	Stage 2	NP Cough		30	1.3	170	85
28	Mohangandhi	65841	48	M	HL	Stage 3			32	0.6	172	84
29	Rathika	25487	35	F	HL	Stage 2		Obstructive	34	0.7	166	88
30	Rajagopal	23654	49	M	HL	Stage 4			27	1.1	162	82

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ETHICS COMMITTEE CERTIFICATE

Name of the Candidate	:	Dr.R.Rajakumari,
Course	:	PG in MD., General Medicine
Period of Study	:	2016-2019
College	:	MADURAI MEDICAL COLLEGE
Research Topic	:	A prospective study of earlier prediction of lung toxicity in patients receiving bleomycin for hodgkin's lymphoma & Germcell tumour
Ethical Committee as on	:	31.03.2018

The Ethics Committee, Madurai Medical College has decided to inform that your Research proposal is accepted.

Member Secretary | Chairman | Dean / Convenor

Prof Dr V Nagaraajan
M.D., MNAMS, D.M., Dsc.,(Neuro), Dsc (Hon)
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This is to certify that this dissertation work titled **"**A PROSPECTIVE STUDY OF EARLIER PREDICTION OF LUNG TOXICITY IN PATIENTS RECEIVING BLEOMYCIN FOR HODGKIN'S LYMPHOMA & GERMCELL TUMOURS of the candidate **Dr.R.Rajakumari** with registration number **201611114** for the award of **M.D Degree** in the branch of **GENERAL MEDICINE.** I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file contains introduction to conclusion pages and result shows 9 percentage of plagiarism in the dissertation.

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