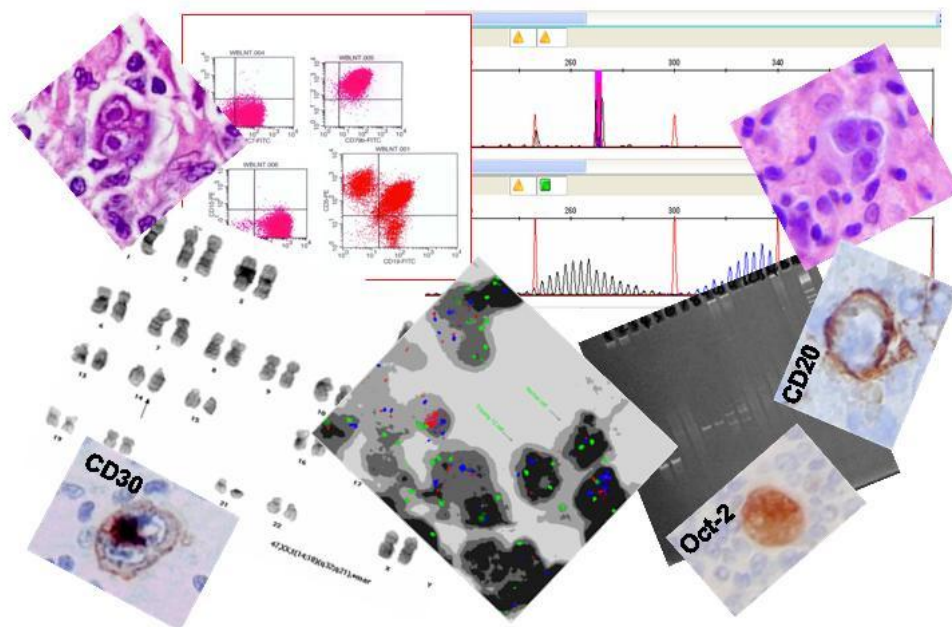


**AWLP Lymphoma Course and Workshop  
9-10 November 2011  
Hilton, Cardiff City Centre**

**PROGRAMME BOOK**



Sponsored by



This course and workshop are designed for more experienced trainees in Histopathology, Haematology and Oncology but also as an update for lymphoma pathology specialists. It is intended to provide an overview of the diagnostic principles, with a detailed display of reactive lymphoproliferations, lymphomas and leukaemias. Part of the course will address diagnostic methodology including immunohistochemistry, flow cytometry, conventional cytogenetics and molecular techniques. Special emphasis is paid to bone marrow assessment and cutaneous lymphoproliferations. A segment of the course will provide an update of the recent classification changes, newly emerged issues and contentious diagnostic areas. The workshop will illustrate the integrated laboratory approach to diagnosis and value of clinical liaison in the Multidisciplinary Team setting through a staged Multidisciplinary Laboratory Meeting with participation of clinicians. The histological and immunocytochemistry slides for the workshop will be available as online virtual slides and will be presented to the participants as a DVD teaching collection. Additional information and definitive programme at [www.awlp.org.uk](http://www.awlp.org.uk)

### **COURSE ORGANISER**

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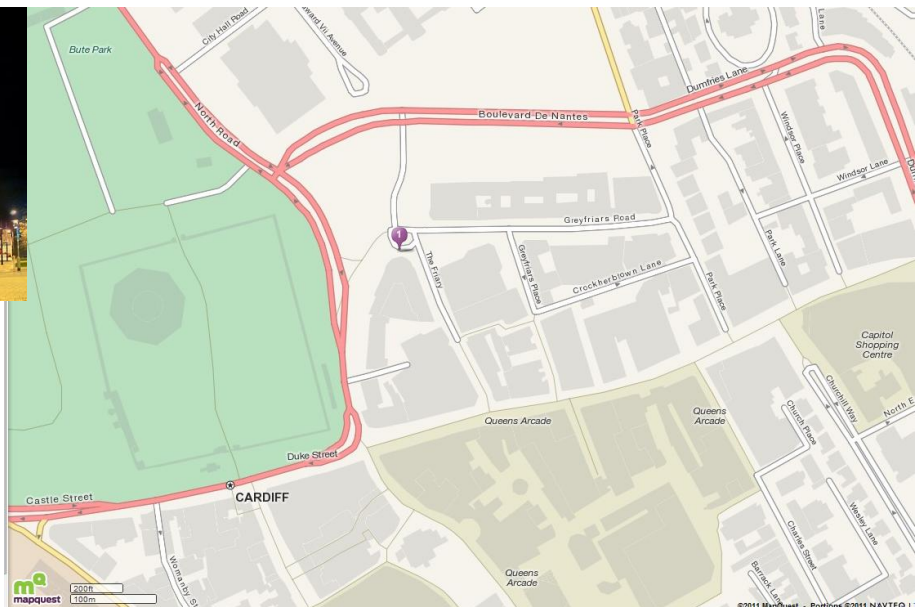
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**SPEAKERS**

<b>Dr Stefan Dojcinov</b>	Consultant Pathologist Department of Pathology University Hospital of Wales Cardiff CF14 4XW
<b>Dr Ciaran O'Brien</b>	Consultant Pathologist Department of Pathology Morriston Hospital Swansea SA6 6NL
<b>Dr Anurag Joshi</b>	Consultant Pathologist Department of Pathology Llandough Hospital Penarth CF64 2XX
<b>Dr Bridget Wilkins</b>	Consultant Pathologist Cellular Pathology Department Second Floor, North Wing St Thomas' Hospital Westminster Bridge Road London SE1 7EH
<b>Professor Rein Willemze</b>	Professor and Chair Department of Dermatology Leiden University Medical Centre Leiden The Netherlands
<b>Dr Eve Gallop-Evans</b>	Consultant Clinical Oncologist Velindre Hospital Cardiff CF14 2TL
<b>Dr Clare Rowntree</b>	Consultant Haematologist Department of Haematology University Hospital of Wales Cardiff CF14 4XN
<b>Mr Steven Couzens</b>	Head of Immunophenotyping Department of Haematology University Hospital of Wales Cardiff CF14 4XW
<b>Mrs Sandra Birdsall</b>	Principal Clinical Cytogeneticist Head of Haematology Cytogenetics Laboratory University Hospital of Wales Cardiff CF14 4XW
<b>Mr Stephen Austin</b>	Clinical Scientist Molecular Diagnostics Histopathology Department University Hospital of Wales Cardiff CF14 4XW
<b>Ms Sian Meyrick</b>	Clinical Scientist Molecular Diagnostics Histopathology Department University Hospital of Wales Cardiff CF14 4XW

## **FINAL PROGRAMME**

### **Day 1**

**9 November 2011**

**08.00 – 08.30 REGISTRATION**

**08.30 - 08.45**

Opening address

**PART 1:**

**BACKGROUND**

**08.45 - 09.30** Dr Ciaran O'Brien

Anatomy of lymphoid tissue and organs  
Background immunology  
Molecular events in the germinal centre

**09.30 -10.15** Dr Anurag Joshi

An approach to lymph node assessment

**10.15 -10.45**

Tea/Coffee break

**10.45 -11.30** Dr Stefan Dojcinov

Basics of flow cytometry PCR, karyotyping and  
FISH analysis - Concept of integrated laboratory  
diagnosis

**PART 2: NEOPLASIA**

**11.30 -12.15** Dr Ciaran O'Brien

Clinically non-aggressive B-cell neoplasms

**12.15 -14.00**

Lunch / Sponsors' time

**14.00 -14.45** Dr Ciaran O'Brien

Clinically aggressive B-cell neoplasms

**14.45 -15.30** Dr Stefan Dojcinov

Peripheral T-cell lymphomas

**15.30 -16.15** Dr Stefan Dojcinov

Hodgkin lymphoma

**16.15 -16.30**

Tea/Coffee break

**16.30 -17.15** Prof. Rein Willemze

Cutaneous lymphomas

**17.15 - 17.45**

Discussion, questions, sponsors time

**Day 2**

**10 November 2011**

<b>08.00 -08.45</b>	Dr Stefan Dojcinov	WHO and beyond: Classification update and emerging issues in lymphoma diagnosis
<b>8.45 – 9.30</b>	Dr Bridget Wilkins	An approach to the interpretation of bone marrow histology
<b>09.30 -10.00</b>		Tea/Coffee break
<b>10.00 -10.45</b>	Dr Bridget Wilkins	Histological features of myelodysplasia and myeloproliferative neoplasms
<b>10.45 -11.30</b>	Dr Eve Gallop-Evans	Lymphoma diagnosis and treatment: Clinician's view

**PART 3: MULTIDISCIPLINARY TEAM MEETING STYLE WORKSHOP**

<b>Moderators:</b>	Dr Stefan Dojcinov, Dr Bridget Wilkins, Prof. Rein Willemze
<b>Flow Cytometry:</b>	Mr Steven Couzens
<b>Cytogenetics:</b>	Mrs Sandra Birdsall
<b>Molecular diagnosis:</b>	Ms Sian Meryck, Mr Stephen Austin
<b>Clinical aspect:</b>	Dr Eve Gallop-Evans, Dr Clare Rowntree

<b>11.30-11.45</b>	Introduction	<b>15.00-16.00</b>	Session 3
<b>11.45-12.45</b>	Session 1	<b>16.00-16.15</b>	Tea/Coffee break
<b>12.45-14.00</b>	Lunch / Sponsors' time	<b>16.15-16.30</b>	Session 4
<b>14.00-15.00</b>	Session 2	<b>16.30-17.00</b>	Summary

## **LECTURE HIGHLIGHTS**

Day 1, 08.45-09.30 Dr Ciaran O'Brien

### **ANATOMY OF LYMPHOID TISSUE AND ORGANS, BACKGROUND IMMUNOLOGY, AND MOLECULAR EVENTS IN THE GERMINAL CENTRE**

All lymphoid cells originate from bone marrow stem cells. Maturation of T-cells in the thymus includes a process of positive and negative selection ("thymic education") which results in cells that weakly react with self Major Histocompatibility Complex (MHC) antigens and which eliminates strongly auto reactive clones. Early B-cell maturation occurs in the bone marrow.

The T-cell receptor (TCR) is a transmembrane molecule consisting of Alpha/Beta or Gamma/Delta heterodimers linked to the CD3 complex. The B-cell receptor (BCR) consists of an antibody molecule linked to a hydrophobic transmembrane region anchoring it to the cell membrane. Both immunoglobulin and TCR genes have similar organisation with V, D, J and C segments and each V, D and J locus containing multiple genes. Each lymphocyte uses a different combination of these gene segments to form the genetic code of its antigen receptor. The recombination process together with splicing inaccuracies and the insertion of additional nucleotides by the terminal deoxynucleotidyl transferase (TdT) enzyme, generates a unique receptor for each T or B cell clone and thereby immense receptor diversity.

Naive T and B cells circulate continuously to secondary lymphoid tissues (lymph nodes, spleen, mucosa associated lymphoid tissue (MALT)). Migration is directed by specific adhesion molecule expression on lymphoid high endothelial venules, except in the spleen where lymphoid cells leave the circulation from marginal zone vessels.

On contact with specific antigen processed by and linked to MHC molecules, T-cells undergo clonal expansion and activation, homing to sites of inflammation to interact with other cells of the inflammatory response or orchestrating humoral immunity by co-operating with activated B-cells in lymphoid tissue.

The B-cell zones of activated secondary lymphoid organs are characterised by the presence of lymphoid follicles with germinal centres (GC). GC formation is initiated by the binding of antigen-antibody complexes to Follicular Dendritic Cells with consequent B-cell proliferation, somatic hypermutation and class switching and the production of memory B cells and plasma cell precursors.

Day 1, 09.30-10.15 Dr Anurag Joshi

### **AN APPROACH TO LYMPH NODE ASSESSMENT**

Lymph nodes are a common histological specimen in the general histopathology lab. Proper assessment of a lymph node begins with appropriate handling when fresh. Histological evaluation of lymph nodes can be extremely challenging- but a systematic approach utilising evaluation of nodal architecture and cellular composition helps provide differential diagnoses that are confirmed using IHC and other ancillary testing.

This presentation seeks to outline an approach to lymph node handling in the lab and a simple schema for histological assessment is provided. Formulation of differential diagnoses is explained and some challenging reactive conditions will be explored.

Day 1, 10.45-11.30 Dr Stefan Dojcinov

### **BASICS OF FLOW CYTOMETRY, PCR, KARYOTYPING AND FISH ANALYSIS; CONCEPT OF INTEGRATED LABORATORY DIAGNOSIS OF LYMPHOMA**

Classification of lymphoid malignancies is based on morphological, clinical, immunophenotypic and genetic characteristics. Practical application of the classification, in addition to the clinical and morphological features, requires detailed interrogation of the immunophenotype and genotype. Moreover, the numerous lymphoma entities often morphologically closely resemble reactive lymphoproliferations. In these circumstances assessment of clonality becomes pivotal in reaching the right diagnosis. Accurate diagnosis of lymphoproliferative disorders is facilitated by integrated application of a number of complex laboratory techniques. These include immunohistochemistry, flow cytometry, conventional cytogenetics (karyotyping), interphase FISH and polymerase chain reaction (PCR). Each of these techniques provides valuable information but is not without limitations. Immunohistochemistry can assess expression of an innumerable number of antigens but in routine practice only one at a time. Quality and interpretation of immunostains may be affected by variations in fixation and tissue processing. Immunophenotyping on flow cytometry provides rapid turnaround time, simultaneous assessment of co-expression of a number of antigens and provides information on clonality, cell size, ploidy and proliferation. However, it requires fresh tissue, expensive equipment and does not provide morphological correlation. Conventional cytogenetics gives detailed information on numerical and structural chromosomal abnormalities but need fresh tissue, it has a slow turnaround time and is highly labour intensive. Interphase FISH has become the gold standard for identification of numerical and structural chromosomal aberrations, performing equally well on fresh and formalin fixed material. However, valid interpretation often requires morphological correlation. Finally, clonality and mutational genetic analysis could be assessed by PCR but the results are best on fresh material and the technique is highly dependent on DNA quality. All the above mentioned techniques provide best results if applied in a structured way so that the individual tests are contributory between each other. This is possible in the “Integrated, Multidisciplinary Laboratory” for lymphoma diagnosis where the diagnostic process from the point of entry of the tissue sample is precisely structured. The diagnostic conclusions are based on the joint expertise of all the participants in the process.

Day 1, 11.30-12.15 Dr Ciaran O'Brien

### CLINICALLY NON-AGGRESSIVE B-CELL NEOPLASMS

The WHO classification of haematological malignancies stratifies neoplasms primarily according to lineage: myeloid, lymphoid etc. Within each category, distinct diseases are defined according to a combination of morphology, immunophenotype, genetic and clinical features.

Mature B-cell neoplasms are clonal proliferations of B-cells at various stages of differentiation ranging from naive B-cells to plasma cells. To a certain extent, they can be categorised by comparison with the corresponding normal B-cell stage. Within the B-cell category, two major groups are recognised – precursor neoplasms corresponding to the earliest stages of differentiation and peripheral or mature neoplasms corresponding to more differentiated or mature neoplasms.

Though the WHO classification specifically avoids sub classification based on prognosis or clinical behaviour, this lecture will concentrate on the more indolent of the mature B-cell neoplasms specifically, B-cell Chronic Lymphocytic Leukaemia / Small Lymphocytic Lymphoma (CLL/SLL), Hairy cell Leukaemia (HCL), Follicular Lymphoma (FL), Mantle cell Lymphoma (MCL), Lymphoplasmacytic Lymphoma (LPL) and the Marginal Zone Lymphomas (MZL). These entities present as predominantly disseminated leukaemia/lymphoma (CLL, HCL), predominantly extranodal (MZL of MALT-type, extranodal MZL) or predominantly nodal (FL and MCL) diseases. All are composed predominantly of small lymphoid cells admixed with variable numbers of transformed cells. Several of the entities can have a nodular architectural pattern, and most can undergo terminal blast transformation. Accurate classification is possible with a combination of morphology and immunophenotype with molecular genetic analysis, cytogenetics and FISH providing important additional information.

	CD5	CD10	CD23	bcl 2	bcl 6	Cyclin D1	Annexin-A1
BCLL/SLL	+	-	+	+	-	-	-
HCL	-	-	-	+	-	+/-	+
FL	-	+	-	+	+	-	-
MCL	+	-	-	+	-	+	-
LPL	-	-	-	+	-	-	-
MZL	-	-	-	+	-	-	-

Day 1, 14.00-14.45 Dr Ciaran O'Brien

### CLINICALLY AGGRESSIVE B-CELL NEOPLASMS

Diffuse Large B-cell Lymphoma (DLBCL) is the commonest lymphoma worldwide, accounting for 30% of all cases. It may involve nodal or extranodal sites. A number of clinical subtypes are recognised, including T/Histiocyte Rich Large B-cell Lymphoma, Mediastinal Large B-cell Lymphoma, EBV+ DLBL of the Elderly, Primary Effusion Lymphoma and Intravascular Lymphoma. In contrast, morphological subtypes, emphasised in previous classifications, have proven to be poorly reproducible by diagnostic pathologists, and their clinical relevance remains controversial. Gene expression profiling has more recently provided prognostically useful sub-

categorisation of DLBCL. Most cases respond to R-CHOP-type chemotherapy, and approximately 50% will be cured by primary therapy.

Burkitt lymphoma (BL) is a highly aggressive tumour of medium sized, rapidly proliferating B-cells occurring in endemic, non-endemic and HIV associated situations. BL occurs at a younger age than DLBCL, with a high incidence of intra-abdominal, CNS and bone marrow disease. BL cells have a germinal centre-type phenotype, with 100% of cells proliferating and c-myc gene rearrangement in the absence of other chromosomal translocations or complex karyotypes.

Precursor B lymphoblastic leukaemia (B-ALL)/lymphoblastic lymphoma (B-LBL) is a neoplasm of B-lymphoblasts involving blood/bone marrow and occasionally presenting with primary involvement of nodal or extranodal sites. B-ALL is predominantly a disease of children (75% less than 6yrs); Median age of B-LBL is 20yrs. Lymphoblasts have B-cell immunophenotype and are TdT positive. Karyotypic features are complex, but prognostically important. Prognosis with modern therapy is good.

Day 1, 14.45-15.30 Dr Stefan Dojcinov

## **PERIPHERAL T-CELL LYMPHOMAS**

This is a heterogeneous group of malignancies of T-cells and NK-cells. The precursor T-cell and NK-cell leukaemia / lymphoma (T-cell acute lymphoblastic leukaemia) are mentioned here for completeness. The “mature” (post-thymic) peripheral T-cell lymphomas account for 10% of Non-Hodgkin lymphomas (All Wales Lymphoma Panel file), showing variable prevalence of types in different parts of the world. The primary cutaneous types are discussed separately.

Clinical presentation is variable. A leukaemic picture is seen in T-cell prolymphocytic leukaemia, T-cell large granular lymphocytic leukaemia (LGL), aggressive NK-cell leukaemia and adult T-cell lymphoma / leukaemia. Other types are characterised by extranodal presentation (NK/T-cell lymphoma of nasal type, enteropathy type T-cell lymphoma, hepatosplenic T-cell lymphoma and subcutaneous panniculitic T-cell lymphoma). The types presenting as primary nodal disease include those most commonly encountered by histopathologists including peripheral T-cell lymphoma - unspecified, angioimmunoblastic T-cell lymphoma (AIL) and anaplastic large cell lymphoma (ALCL).

The phenotypes are variable. T-cell lineage could be confirmed by a battery of markers including CD2, CD4, CD5, CD7 and CD8. Particularly the extranodal types are characterised by cytotoxic phenotypes which could be assessed by the expression of TIA1, granzyme, perforin, and CD56, a phenotype also shared with NK cells which in addition show cytoplasmic expression of the epsilon chain of CD3, CD57 and CD16. Origin of some T-cell lymphomas from specific T-cell subsets is now better understood. Utilising a range of novel markers it is for example possible to identify regulatory T-cell differentiation as in adult T-cell leukaemia/lymphoma or germinal centre specific T-cell differentiation as in angioimmunoblastic T-cell lymphoma. Some of the types are most likely aetiologically linked to EBV which could be demonstrated by *in situ* hybridisation for EBER. This is a diagnostic requirement for NK/T-cell lymphoma of nasal type and angioimmunoblastic T-cell lymphoma in which the “bystander” B-cell blast also express this marker. Clonality of T-cells is assessed by polymerase chain reaction using primers against beta and gamma chain genes of the T-cell receptor, occasionally requiring assessment of delta gene rearrangements in very primitive tumours. Restriction to expression of certain families of beta or

delta chains within the T-cell receptor could be assessed by flow cytometry and immunocytochemistry. Specific genetic markers are generally lacking with the exception of ALK1 rearrangement characterising a subgroup of ALCL.

Most of the extracutaneous peripheral T-cell lymphomas are clinically highly aggressive with poor outcomes. The exception to this rule is a subgroup of LGL and ALK1 positive ALCL.

Morphological diversity of T-cell lymphomas is such that Hodgkin lymphoma and non-haematological tumours (carcinoma, sarcoma or germ cell tumours) are often in the differential diagnosis.

Day 1, 15.30-16.15 Dr Stefan Dojcinov

## **HODGKIN LYMPHOMA**

Hodgkin lymphoma (HL) is classified into two broad categories: classical Hodgkin lymphoma (comprising nodular sclerosis, mixed cellularity, lymphocyte-rich and lymphocyte depleted); and nodular lymphocyte predominant Hodgkin lymphoma (NLPHL).

The diagnosis of classical Hodgkin lymphoma is pathologically determined by the identification of scattered large mononucleated and multinucleated tumour cells (Hodgkin and Reed-Sternberg cells respectively) in a polymorphous mixture of non-neoplastic inflammatory cells. Whilst the presence of classical Reed-Sternberg cells and variant forms defines the disease entity, subtyping is governed by the background fibro-inflammatory milieu.

Nodular sclerosis is the commonest type of classical Hodgkin lymphoma and is characterised by the features of nodularity and banded fibrosis. Lacunar cells (in which cytoplasmic retraction is seen in formalin fixed tissue) are variant forms of Reed-Sternberg cells common in this subtype. Two grades of nodular sclerosing Hodgkin lymphoma have been recognised historically: BNLI grade 1 (70%) and grade 2 (30%). Pathologically grade 2 disease is characterised by either disease nodules showing lymphocyte depletion; fibrohistiocytic stromal reaction in a lymphocyte deplete background; or numerous bizarre anaplastic Reed-Sternberg cells without lymphocyte depletion.

In comparison all other subtypes including: Mixed cellularity (characterised by scattered, often more numerous Reed-Sternberg cells and variant forms in the diffuse polymorphous inflammatory cell background); Lymphocyte-rich classical Hodgkin lymphoma (characterised by sparse Reed-Sternberg cells in a lymphocyte-rich stroma with / without retention of background lymph node architecture); and Lymphocyte deplete classical Hodgkin (with numerous Reed-Sternberg cells and pleomorphic variants associated with a variable cellular / fibrohistiocytic stromal infiltrate) are rare.

The differential diagnosis of classical Hodgkin lymphoma is largely dependent on the subtype but includes a variety of reactive and neoplastic lymphoid proliferations. In rare cases Hodgkin lymphoma may mimic amelanotic melanoma and undifferentiated carcinoma. Immunohistochemistry has a central role in the identification of classical Reed-Sternberg cells and variant forms and is shown below.

Nodular lymphocyte predominant Hodgkin lymphoma is pathologically characterised by a nodular and/or diffuse proliferation of scattered large multilobated nucleated cells termed “popcorn” or “L&H” cells admixed in a polymorphous inflammatory milieu. Small localised clusters of epithelioid cells may be associated with these nodules.

The differential diagnoses are wide and include: non-Hodgkin lymphomas with a follicular growth pattern; lymphocyte-rich classical lymphoma; diffuse large B-cell lymphoma and the reactive condition progressive transformation of germinal centres

Immunohistochemistry has a central role in the identification of the popcorn/L&H cell and is shown below.

It is now established that both classical and lymphocyte predominant Hodgkin lymphoma represent B-cell neoplasms.

Immunophenotype of Hodgkin lymphoma

	CD20 / CD79a	CD45	CD30	CD15	EBV
Classical HL	Weak -/+	-	+	+	-/+
NLPHL	+	+	-	-	-

	OCT2	BOB1	MUM1	PAX5	Bcl6	CD10
Classical HL	-	-	+	+	-	-
NLPHL	+	+	-	+	+	-

Day 1, 15.30-16.15 Dr Stefan Dojcinov

**PRIMARY CUTANEOUS LYMPHOMAS**

Primary cutaneous lymphomas (PCL) are defined as non-Hodgkin lymphomas that present in the skin with no evidence of extracutaneous disease at the time of diagnosis. After the gastrointestinal lymphomas, PCL are the second most common group of extranodal non-Hodgkin lymphomas with an estimated annual incidence of 1/100,000. These PCL must be distinguished from nodal or systemic malignant lymphomas involving the skin secondarily, which often have another clinical behaviour, have a different prognosis and require a different therapeutic approach. In recent lymphoma classifications (WHO-EORTC; WHO 2008) the different types of primary cutaneous T-cell lymphomas (CTCL) and primary cutaneous B-cell lymphomas (CBCL) are therefore included as separate entities. In the western world, CTCL constitute about 75-80% of all primary cutaneous lymphomas and CBCL 20-25%, but different distributions have been observed in other parts of the world.

Primary cutaneous lymphomas are special for two reasons. First, different types of CTCL (and CBCL) with different clinical behaviours and different therapeutic requirements may have an identical histologic appearance. This implies that pathologists with only (immunostained) sections, but no adequate clinical data at their disposal, often can make only a differential diagnosis. Second, a major advantage of cutaneous lymphomas, compared with lymphomas arising at other sites, is that they can be seen with the naked eye and can be biopsied easily. Since

the different types of CTCL and CBCL have distinctive clinicopathologic features, a definite diagnosis (classification) can be easily made in most cases, when clinical data are combined with histological and immunophenotypical data. Thus, clinicopathologic correlation and close collaboration between pathologist and clinician are mandatory and the best guarantee for correct diagnosis and adequate treatment of patients with primary cutaneous lymphomas.

Day 2, 08.00-08.45 Dr Stefan Dojcinov

## **WHO AND BEYOND: CLASSIFICATION UPDATE AND EMERGING ISSUES IN LYMPHOMA DIAGNOSIS**

The recent update of the WHO classification retained the entity based approach, still providing clinically relevant diagnostic categories. The update introduces a number of new entities and attempts to resolve the contentious issues regarding overlapping categories. Many definitions and diagnostic criteria have been clarified and improved.

New entities have been included which represent the earliest phases of lymphoma genesis and for some the term “lymphoma in situ” has been utilised. Monoclonal B-cell lymphocytosis, follicular and mantle cell lymphoma in situ are recognised. The information gathered around these entities provides insight into understanding of lymphoma genesis. These conditions run an indolent course and should be managed differently from their systemic counterparts.

The significance of anatomical site in determining biological features of lymphomas has been reiterated. A number of entities are included the behaviour of which is at least in part determined by the specific site. Thus, cutaneous lymphomas remain classified separately. In addition intestinal follicular lymphoma has emerged as a new indolent entity with specific pathological and molecular features.

A new significant parameter governing classification is age. At both ends of the age spectrum there are newly recognised entities. Paediatric follicular and marginal zone lymphomas have unique pathological, biological and some molecular features. EBV positive T-cell lymphoproliferative disorder of childhood has been included as a separate category. At the other end of the age range, the EBV positive B-cell lymphoproliferative disorders of the elderly have been characterised as a spectrum of indolent and aggressive conditions.

A new diagnostic category has been established for lymphomas showing pathological and biological overlaps between classical Hodgkin lymphoma and mediastinal large B-cell lymphoma. This “grey zone” appears to have unique biological and gene expression features. Similarly, a new category is dedicated for cases showing overlapping features between Burkitt lymphoma and diffuse large B-cell lymphoma. This category still remains contentious and poses diagnostic and classification difficulties. A new provisional entity of splenic diffuse red pulp B-cell lymphoma has been introduced and hairy cell variant has been moved in the classification table to make a clear separation from hairy cell leukaemia.

While significantly contributing to the better understanding of lymphoma biology, gene expression profiling has not been adopted as a classification platform. This will undoubtedly change in the future as it provides valuable information for targeted, personalised management based on recognition of specific malfunctions in cellular molecular pathways. Management based on gene expression profiles is being introduced through clinical trials. It is likely that future classification updates will further clarify the overlaps and difficult diagnostic grey zones such as one between Burkitt lymphoma and diffuse large B-cell lymphoma. Clinical experience, detailed genetic interrogation of each individual case and gene expression data will help subdivide these cases into more clinically meaningful categories. Additional insights likely to affect future classification and management are in the area of tumour-environment interactions. Exciting new data is emerging on the antigen presentation and cellular mimicry in Hodgkin lymphoma.

## WHO Lymphoma Classification - Update 2008

### PRECURSOR LYMPHOID NEOPLASMS

B-cell lymphoblastic leukaemia/lymphoma  
T-cell lymphoblastic leukaemia/lymphoma

### MATURE B-CELL NEOPLASMS

Chronic lymphocytic leukaemia /Small lymphocytic lymphoma

Monoclonal B-cell lymphocytosis

B-cell prolymphocytic leukaemia  
Splenic B-cell marginal zone lymphoma  
Hairy cell leukaemia

Splenic B-cell lymphoma/leukaemia, unclassifiable  
Splenic diffuse red pulp B-cell lymphoma  
Hairy cell leukaemia variant

Lymphoplasmacytic lymphoma

Heavy chain diseases

Alpha heavy chain disease  
Gamma heavy chain disease  
Mu heavy chain disease

Plasma cell neoplasms

MGUS  
Plasma cell myeloma  
Solitary plasmacytoma of bone  
Extrasosseous plasmacytoma  
Monoclonal immunoglobulin deposition diseases

Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)

Nodal marginal zone lymphoma

Paediatric MZL

Follicular lymphoma

Paediatric follicular lymphoma  
Primary intestinal follicular lymphoma  
Intrafollicular neoplasia / "in situ" follicular lymphoma

Primary cutaneous follicle centre lymphoma

Mantle cell lymphoma

Diffuse large B-cell lymphoma (DLBCL), not otherwise specified

T cell/histiocyte rich large B-cell lymphoma

Primary DLBCL of the CNS

Primary cutaneous DLBCL, leg type

EBV positive DLBCL of the elderly

DLBCL associated with chronic inflammation

Lymphomatoid granulomatosis

Primary mediastinal (thymic) large B-cell lymphoma

Intravascular large B-cell lymphoma

ALK positive large B-cell lymphoma

Plasmablastic lymphoma

Large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease

Primary effusion lymphoma

Burkitt lymphoma

B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL

B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma

### MATURE T- AND NK-CELL NEOPLASMS

T-cell prolymphocytic leukaemia

T-cell large granular lymphocytic leukaemia

Chronic lymphoproliferative disorder of NK-cell

Aggressive NK cell leukaemia

EBV+ T-cell LPD of childhood

Systemic EBV positive T-cell

lymphoproliferative disorder of childhood

Hydroa vacciniforme-like lymphoma

Adult T-cell leukaemia/lymphoma

Extranodal NK/T cell lymphoma, nasal type

Enteropathy-associated T-cell lymphoma

Hepatosplenic T-cell lymphoma

Subcutaneous panniculitis-like T-cell lymphoma

Mycosis fungoides

Sezary syndrome

Primary cutaneous CD30-positive T-cell

lymphoproliferative disorder

Primary cutaneous anaplastic large cell

lymphoma

Lymphomatoid papulosis

Primary cutaneous peripheral T-cell lymphomas, rare subtypes

Cutaneous gamma-delta T-cell lymphoma

Primary cutaneous CD8 positive aggressive

epidermotropic cytotoxic T-cell lymphoma

Primary cutaneous small/medium CD4 positive

T-cell lymphoma

Peripheral T-cell lymphoma, not otherwise specified

Angioimmunoblastic T-cell lymphoma

Anaplastic large cell lymphoma (ALCL), ALK positive

Anaplastic large cell lymphoma (ALCL), ALK negative

### HODGKIN LYMPHOMA

Nodular lymphocyte predominant Hodgkin lymphoma

Classical Hodgkin lymphoma

Nodular sclerosis classical Hodgkin lymphoma

Lymphocyte-rich classical Hodgkin lymphoma

Mixed cellularity classical Hodgkin lymphoma

Lymphocyte depleted classical Hodgkin

lymphoma

### IMMUNODEFFICIENCY-ASSOCIATED LYMPHOPROLIFERATIVE DISORDERS

Lymphoproliferative disorders associated with primary immune disorders

Lymphomas associated with HIV infection

Post-transplant lymphoproliferative disorder

Other iatrogenic immunodeficiency-associated lymphoproliferative disorders

### HISTIOCYTIC AND DENDRITIC CELL NEOPLASMS

Histiocytic sarcoma

Tumours derived from Langerhans cells

Langerhans cell histiocytosis

Langerhans cell sarcoma

Interdigitating dendritic cell sarcoma

Follicular dendritic cell sarcoma

Other rare dendritic cell tumours

Fibroblastic reticular cell tumour

Intermediate dendritic cell tumour

Disseminated juvenile xanthogranuloma

## **AN APPROACH TO THE INTERPRETATION OF BONE MARROW HISTOLOGY**

Bone marrow is a complex tissue that repays a consistent and systematic approach to histological interpretation, rather similar to that used with renal or liver core biopsy specimens. An adequate specimen with adequate clinical details is an essential starting point. There is no other tissue for which interpretation is routinely undertaken parallel for paired cytological and histological samples, often by different individuals (haematologists and histopathologists) in different departments. Consequently, collaboration is essential. Moreover, integration of bone marrow histology into an overall haemato-oncological diagnostic assessment is mandated by NICE Improving Outcomes Guidance for haematological cancers.

Bone and stromal reticulin fibres should be assessed and the overall level of cellularity interpreted according to the patient's age. It is essential to recognise the different maturational stages of each of the three major haemopoietic cell lineages (erythrocytes, granulocytes, megakaryocytes), and know their normal distribution, so that features of myelodysplasia and myeloproliferative neoplasms are not missed. Additional features such as lymphoid nodules and granulomas should prompt investigations to determine their reactive or neoplastic origin and/or seek causative micro-organisms.

Most lymphomas can be characterised fully by immunophenotyping, FISH and PCR clonality analyses if bone marrow is the only diagnostic tissue. Abnormal plasma cell infiltrates can also be assessed, even at minimal levels of interstitial involvement, by immunophenotyping plus demonstration of kappa and lambda light chains. In many labs, mRNA ISH for kappa and lambda can provide more readily interpretable results than immunohistochemistry. Metastatic deposits originating from malignant solid tumours of unknown origin can usefully be immunophenotyped in bone marrow trephine sections, as at other sites.

## **HISTOLOGICAL FEATURES OF MYELOYDYSPLASIA AND MYELOPROLIFERATIVE NEOPLASMS**

'Myelodysplasia' describes abnormal maturation of haemopoietic cells, which may be neoplastic or reactive. It is reflected in varying patterns of spatial and cytological abnormalities. The term 'myelodysplastic syndrome' (MDS) more specifically describes one of several clinicopathological entities arising from neoplastic haemopoietic stem cells. The usual clinical presentation of myelodysplastic haemopoiesis is with cytopenias in peripheral blood. Myelodysplasia of reactive or toxic origin ('inflammatory myelopathy') typically has stromal reactive changes in parallel, such as oedema, red cell leakage, plasmacytosis, increased macrophage activity and/or fibrosis.

Spatial disturbance in myelodysplasia reflects displacement of proliferative stages of granulopoiesis from their normal paratrabecular location. Erythroid nests are often larger and more irregular than normal; megakaryocytes (MK) may be clustered. Cytological abnormalities in granulocytes are better appreciated in blood or aspirated bone marrow. Erythroid cells often show an abnormal degree of synchrony within a single cluster and adjacent clusters are

synchronised at different stages. There is a strong tendency for overall erythroid left shift and abnormal, megaloblast-like maturation. MK tend to be smaller than normal, with reduced nuclear lobation. True micro-megakaryocytes (MK in the same size range as promyelocytes) are relatively specific to MDS and are typically absent in inflammatory myelopathies. Precise WHO classification of MDS is not possible from histology alone but low grade patterns, with varying dysplasia but predominantly retained maturation, and high grade variants with increased immature and blast cells can generally be distinguished. The count of CD34+ve early haemopoietic cells provides a surrogate blast cell count in MDS and is of some prognostic value. Fibrosis develops in a minority of patients and there may be overlap with myeloproliferative neoplasms. Juvenile and chronic myelomonocytic leukaemias are distinct entities with overlapping MDS/MPN features.

Of the MPN, bone marrow is rarely sampled by trephine biopsy for assessment of chronic myeloid leukaemia (CML); peripheral blood morphology and demonstration of BCR-ABL1 rearrangement usually suffice. The same is becoming true for polycythaemia vera (PV) and JAK2-V617F although baseline assessment by trephine biopsy is recommended in order to monitor subsequent development of fibrosis or accelerated phase features. Essential thrombocythaemia (ET) and primary myelofibrosis (PMF) are less closely associated with JAK2-V617F, show marked clinical overlap with reactive conditions and can be difficult to separate from each other in early stages, so that histological assessment is recommended.

CML is an orderly and predominantly granulocytic proliferation with retained maturation; eosinophilia is common and there are increased basophils but the latter cannot be appreciated in fixed tissue sections. Erythropoiesis is typically reduced and MK, which maybe abundant, have distinctive appearances (small with reduced, often single, nuclear lobes). In PV there is panmyelosis within which erythropoiesis may or may not predominate. Granulopoiesis is distributed in a disorderly fashion but retains normal maturation while MK are pleomorphic and include large variants with poorly lobated, 'cloud-like' nuclei. Histology in ET is typically normocellular with selectively increased MK that form a mixed population of normal cells plus large variants that are currently regarded as hypermature rather than dysplastic. These hypermature cells have hyperlobated ('staghorn') nuclei and voluminous cytoplasm; they are unevenly distributed and show a modest tendency to cluster. Pre- and early fibrotic stages of PMF are recognised separately from ET by the WHO classification and remain controversial clinically. There is considerable overlap with ET, although the defining histological features as stated by the WHO are clear. They involve increased granulopoiesis and MK production with marked spatial disturbance, MK clustering and pleomorphism. A range of overtly atypical large and small MK can be appreciated with few or absent 'staghorn' nuclei. Advanced stages of PMF show progressive increase in stromal fibrosis in tandem with reduction in haemopoiesis, often with increasing MK atypia and nuclear hyperchromatism. There may be new bone formation, which is usually appositional in type.

Several grading schemes have been developed to score the extent of reticulin and collagen fibrosis in bone marrow trephine specimens. It is important to have high quality and consistent reticulin staining of BMT specimens and to become familiar with using one or more of these grading systems. Reports must be unequivocal, whichever grading scheme is preferred locally, because the assigned values will differ.

'Accelerated phase' features may develop in any of the MPN, with emergence of myelodysplasia or an increasing number of blast cells. Immunostaining for CD34 may help identify the latter but

so far only in CML has this approach been validated by comparison with blood, marrow aspirate and clinical features.

Day 2, 10.45-11.30 Dr Eve Gallop—Evans

### **TREATMENT OF LYMPHOMA – THE ONCOLOGIST’S PERSPECTIVE**

The lymphomas are a diverse group diseases ranging from the very indolent, requiring only surveillance, to the highly aggressive and rapidly fatal. Patients should be managed by multidisciplinary teams, which bring together the appropriate expertise of haematologists, oncologists, radiologists, pathologists and specialist nurses. Surgery has an important role in making a tissue diagnosis but does not usually contribute to definitive treatment. The development of curative treatment protocols has to date been based on chemotherapy and radiotherapy and more recently immunological and biological therapies. Research strategies are increasingly focusing on maximising the chances of cure while minimising late effects of treatment, for example by using PET scans for response adapted therapy. The lecture will use a case-based approach to discuss the principles of management of the most common types of lymphoma, emphasizing the importance of clinico-pathological correlation in management decisions.

## **WORKSHOP**

The workshop is intended to closely mimic the routine case discussions in the AWLP multidisciplinary laboratory meeting. For each case it would commence with the relevant clinical findings followed by brief display of histological and immunophenotypic features together with the results of other ancillary tests (flow cytometry, cytogenetics, PCR). Using all the available results the definitive diagnosis is formulated followed by discussion of relevant pathological and clinical features. The cases are thematically grouped and a summary discussion would follow after each set of cases.

## **ONLINE ACCESS FOR WORKSHOP CASES**

The link to the workshop virtual slides is on the AWLP web site:

<http://dental.uwcm.ac.uk:82/AWLP%20Course%202011/view.apml>

Click on directory "AWLP Course 2011". Each case is placed in a separate directory containing multiple scans. By clicking on a thumbnail the case could be viewed through the internet browser.

The Workshop cases are on the DVD set included with the Course materials. Disk 4 contains the "ImageScope" software required to view the digital scans.

## **WORKSHOP CASES**

- Case 1:** Male, 19. Two year history of unilateral neck lymphadenopathy. Lymph node excised.
- Case 2:** Male, 61. Peripheral blood lymphocytosis (6.3). No lymphadenopathy or organomegaly. Lymph node excision biopsy of a clinically unremarkable neck lymph node submitted. Previous needle biopsy inconclusive.
- Case 3:** Male, 76. Right retroperitoneal and perinephric mass. Trucut needle biopsy with aspirate.
- Case 4:** Female, 18. Mediastinal and cervical lymphadenopathy. Lymph node excision biopsy.
- Case 5:** Female, 8. Widespread lymphadenopathy (cervical, intraabdominal, mediastinal) and splenomegaly. Hypercalcaemic. Cervical lymph node removed.
- Case 6:** Female, 80. Patient undergoing investigations for anaemia (Hb 9.3 g/dl). Normal white blood cell and platelet counts. Found to have an IgM paraprotein of 25g/l. Bone marrow trephine biopsy enclosed.
- Case 7:** Male, 75. Patient presented with pancytopenia (Hb 9.7g/dl, WBC 2.9 x 10<sup>9</sup>/l with neutrophils 0.9 x 10<sup>9</sup>/l, platelets 87 x 10<sup>9</sup>/l) and splenomegaly. Bone marrow trephine biopsy enclosed.
- Case 8:** Male, 13. Presented with infiltrated plaques on the face and arms. Punch biopsy enclosed.
- Case 9:** Male, 75. Infiltrated plaque on upper back. Punch biopsy enclosed.
- Case 10:** Female 63. Left cervical lymphadenopathy. Marginal zone B-cell lymphoma suspected. Lymph node excision biopsy.
- Case 11:** Male 63. Gradually developing left side abdominal discomfort over a period of 5-6 months. Pancytopenia with splenomegaly. Splenectomy performed.
- Case 12:** Female, 60. History of rheumatoid arthritis. Non healing ulcer in mouth suspicious of malignancy. Biopsy of lesion on lower lip.

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