Pitfalls in the diagnosis of haematological malignancies

Rishu Agarwal and Surender Juneja

Abstract

Increased knowledge of the immunophenotypic, cytogenetic and molecular heterogeneity of haematological diseases has challenged the traditional approach to disease classification based on morphology. In the current WHO classification, an integrated approach combining clinical details, morphology, immunophenotyping and genetic features is used for the diagnosis of haematological malignancies. An accurate diagnosis is essential for proper management of patients; moreover, there are novel drugs available which are designed to specifically target the underlying pathological abnormalities responsible for the development of the tumour. However, the risk of diagnostic errors remains high in diagnostic haematology and the main reason for this is lack of resources and expertise in small centres. There is a need for access to sub-specialised haematology services, to ensure access to specialist expertise and a full range of testing beyond traditional stains. In this review a range of potential pitfalls in the diagnosis of haematological malignancies is outlined, arising at all stages from specimen collection to reporting. Knowledge of such pitfalls, some of which are common while others are rare but of vital clinical importance, helps in avoiding potential sources of errors. Insight into the technical details has been provided which helps to avoid the errors related to sampling and processing of specimens. The final diagnosis of haematological malignancies should be based upon a combination of clinical details, good technical preparation, access to diagnostic resources, good communication and in depth knowledge and experience of the haematopathologist.

Key words: Haematological malignancies, pitfalls, diagnostic errors, centralization


The many stages of normal haemopoietic differentiation give rise to a number of biologically and clinically distinct cancers albeit with some overlapping features. Growing understanding of pathogenesis of haematological disorders by molecular and cytogenetic methods has significantly improved their sub-classification. However, given the overlapping features, an integrated approach combining clinical details, morphology, immunophenotyping and genetic features is now used for the diagnosis of haematological neoplasms (WHO 2008) (1). Apart from being important scientifically & biologically, an accurate diagnosis provides very useful guidance to clinicians for effective management of these patients. Despite the advancement in technology, the rate of diagnostic errors and the requirement for expert review remains high in haematopathology. One of the main reasons for this is the lack of access to adequate range of special investigations required to make a definitive diagnosis in most centers. In 2006, the National Institute for Health and Clinical Excellence (UK) issued improving outcomes guidance (IOG) for haematological cancers, with special emphasis on the crucial need for a central regionally based specialist review of haematopo-ncology diagnosis (2) and regular participation of laboratories in Quality Assurance Programmes. This component of the IOG is strongly influenced by audits, particularly the one conducted in Wales (3), showing rates of discordance of approximately 20% between initial non-specialist and subsequent specialist diagnosis of lymphomas.

In 8% of cases, the discrepancies required clinically significant changes in patient management following specialist diagnosis.

Sources of error in haematopathology can be of an administrative, clerical, technical or diagnostic nature, as in other areas of pathology. Administrative and clerical errors can occur at any stage starting from collection of specimen to the generation of final report. They are of great importance and a regular audit system should be in place to minimize their occurrence. Some of the most common pitfalls encountered in the daily practice of haematopathology are discussed in the six categories listed below:

- Inadequate clinical information
- Inadequate or inappropriate specimens
- Morphological diagnostic difficulties
- Errors related to immunohistochemistry
- Errors related to flow cytometry, cytogenetics/FISH and molecular studies
- Errors due to limited range of tests performed

Inadequate clinical information

Most of the haematological diseases require knowledge of clinical features—age, nodal versus extranodal presentation, specific anatomic site, and history of cytotoxic and other therapies to make the correct diagnosis. A detailed clinical history along with physical examination findings is very important to make an accurate diagnosis. Some examples where diagnostic difficulties may arise due to inadequate history and clinical details are:

- In WHO Classification, there is a subcategory of therapy related myeloid neoplasms and post transplant lymphoproliferative disorders (PTLD). Details of past treatment are essential to make this diagnosis.
- Rapidly progressive diseases like Burkitt lymphoma which is an aggressive neoplasm with rapid doubling time, requires detailed knowledge of presenting features for accurate diagnosis and timely treatment.
- While investigating for paraproteinemia, type of paraprotein should always be mentioned, as different flow cytometric immunophenotyping marker studies are required to be carried out.
- In the investigation for pancytopenia, drug history and information about organomegaly is important. For example, in rare diseases like hairy cell leukaemia, splenomegaly is a consistent clinical feature.

These are just the few examples; in reality there are very few diagnoses that can be made accurately or completely without good communication between pathologists, haematologists, oncologists and radiologists. Therefore, an integrated approach in haematology should be encouraged combining clinical details with the diagnostic modalities. Regular clinico-pathologic meetings and well developed IT infrastructure are essential to achieve this goal.
1. Failure to obtain adequate sample can compromise accurate diagnosis. Bone marrow aspirate and trephine biopsy should be performed by an experienced operator. The preferred anatomic site is posterior iliac crest. However, in certain conditions like the patient having received previous radiotherapy to the pelvis or there is a dry tap, sternal aspiration may be carried out. Marrow sampling from previously irradiated area can result in false negative results because the marrow may be aplastic at the irradiated site.

2. Sternal aspiration should always be performed with extreme caution because of the risk of potential damage to vital tissues including cardiac tamponade associated with it. Sternal puncture should be avoided in patients with suspected plasma cell myeloma or other disorders associated with bone resorption.

3. Failure to collect samples for flow cytometry, cytogenetics and molecular studies with bone marrow aspirates can give insufficient diagnostic material. Samples for ancillary studies should be collected routinely at the time of bone marrow aspiration and may be discarded later if the investigations are considered to be unnecessary after the bone marrow slides have been reviewed.

4. Failure to make touch imprints with aspirate may prevent cytologic examination of Giemsa stained smears in cases of dry tap.

5. Obtaining an adequate length of bone marrow biopsy is very important. The length of the core from an adult should be at least 15mm. The biopsy shrinks by approximately 20% after processing. However, the larger the amount of tissue biopsied, the greater is the likelihood of a focal lesion (e.g. lymphoma, metastatic tumour, granuloma) being detected. Bilateral trephine biopsies may be performed to increase the yield of detecting focal lesions. The recommended thickness of biopsy sections is two to three microns. Thicker sections may mask subtle details of chromatin density and distribution, mitotic figures and apoptotic bodies. It is useful to examine multiple levels (three or more) of bone marrow biopsy for lymphoma staging as failure to do this may result in lower incidence of marrow involvement with lymphoma.

6. Fixation method can significantly affect morphology, cytological details and immunoreactivity. Failure to understand the use of proper fixatives can result in unsatisfactory diagnostic samples. Mercuric chloride based fixative (BS) is frequently used for bone marrow biopsies. It gives excellent cytological details but requires immediate processing and cannot be used later for molecular studies or cytogenetics. Neutral buffered formalin with ethylene-diamine tetra-acetic acid (EDTA) decalcification provides adequate morphology, preserves antigens for immunohistochemistry and nucleic acids for molecular studies. So, the choice of fixative used should be determined by the type of studies which need to be done on the specimen.

7. Decalcification of bone marrow biopsies should be carried out by reagents which allow the biopsy specimen to be used for further studies, if required. EDTA decalcification allows a wide range of immunostains with current antigen retrieval techniques. It also preserves good quality DNA for PCR studies, and FISH for lymphoma associated translocations is entirely feasible using intact EDTA-decalcified bone marrow trephine sections. Premature exposure to EDTA before adequate fixation, or exposure to poorly buffered EDTA (excessively alkaline), causes severe loss of morphological definition; it also destroys many antigens and degrades nucleic acids (5). Hence, adequate fixation and decalcification of bone marrow biopsies is very essential for accurate diagnosis.

Lymph node preparation
For the diagnosis of lymphoma, the ideal specimen is an excised intact lymph node, transported rapidly to the laboratory without previous fixation. This allows immediate sampling of the material on receipt to make imprint preparations (for rapid cytological assessment and FISH), disperse cells into medium for flow cytometry, and freeze small pieces for subsequent nucleic acid studies by polymerase chain reaction (PCR) (6). However, this ideal is rarely achievable and when fixed specimen is received, it cannot be used for flow cytometry and FISH studies. Whether received fresh or in fixative solution, lymph node specimens should be inspected and sliced as soon as possible, after recording measurements and a brief description of the intact specimen, to aid fixation.

Fine needle aspiration cytology (FNAC)
Fine needle aspiration cytology is increasingly done to minimise patient discomfort and increase the speed of diagnosis. However, FNAC only plays a limited role in screening of lymph nodes for infections, granulomas and metastatic solid tumour deposits and an excisional biopsy should be performed for the diagnosis of lymphoma.

Needle core biopsy
Needle core biopsy of inaccessible masses suspected of harbouring lymphoma is also an invaluable tool. However needle biopsies should only be used for inaccessible sites under radiological guidance and should never replace excisional biopsies because of the limited amount of tissue they provide for diagnosis.

Specimen limitations that lead to error
- Inadequate specimen size for morphology
- Inadequate sample for flow cytometry, cytogenetics or molecular studies
- Sample in wrong preservative
- Poor fixation
- Dry tap
- Crushed, necrotic or otherwise distorted tissue

Morphological diagnostic difficulties
Accurate diagnosis of haematological malignancies depends on the knowledge and expertise of the pathologist as well as availability of modern diagnostic techniques. It is very important to keep ourselves updated with the latest literature and diagnostic pitfalls. Use of ancillary techniques is advisable for definitive diagnosis. Below are some examples which cause diagnostic dilemmas on morphology:

1. Haematogones and lymphoblasts
Bone marrow haematogones often cause problems in diagnosis because of their morphological and immunophenotypic similarities to leukaemic lymphoblasts. They occur in large numbers in some healthy infants and young children and in a variety of diseases in both children and adults. Haematogones may be particularly prominent in the regeneration phase following chemotherapy and bone marrow transplantation and in patients with autoimmune and congenital cytopenias, neoplasms and acquired immunodeficiency syndromes. In some instances they constitute 5% to more than 50% of all nucleated cells (7). They pose important diagnostic challenge when evaluating acute lymphoblastic leukemia (ALL) bone marrow post chemo therapy marrow. They are often expanded in regenerating marrow and can potentially be mistaken for residual disease. While morphologic and immunophenotypic overlap exists between haematogones and leukemic lymphoblasts, a careful morphological review combined with evaluation of immunophenotypic characteristics by flow cytometry and immunohistochemistry as well as architectural distribution in the bone marrow can help distinguish haematogones from residual leukemic blasts.
Haematogones show continuous and complete maturation spectrum on immunophenotyping, lack aberrant or asynchromous antigen expression while lymphoblasts from ALL deviate from the normal B-lineage maturation spectrum by exhibiting maturation arrest, over or under antigenic expression, asynchromous antigen expression or expression of myeloid associated antigens.

2. Metastatic mimickers of acute leukemias
Metastatic involvement of the bone marrow by malignant small round cell tumour is not uncommonly encountered in paediatric patients, and these tumours include neuroblastoma, Ewing's sarcoma and rhabdomyosarcoma. In some patients, bone marrow disease may be the primary manifestation, thus mimicking acute lymphoblastic leukemia. Cases of rhabdomyosarcoma mimicking acute leukemia have been described in the literature (8). Similarly, a case of metastatic small cell carcinoma of the lung has been reported mimicking acute leukemia (9). We should therefore be vigilant while making a diagnosis of acute leukemia especially in those cases which on immunophenotyping show abnormal cells neither to be of B or T cell origin and do not show reactivity for myeloid antigens. In addition to considering the diagnosis of acute undifferentiated leukemias, possibility of metastatic tumours should always be kept in mind. A detailed work-up of such cases including detailed history and radiological findings, bone marrow biopsy examination, extended panel of antibodies by immunohistochemistry and relevant cytogenetic analysis should always be carried out to make a correct diagnosis.

3. Hypocellular AML
Rare cases of AML present with moderate to markedly hypocellular marrow. Causes of this reduced cellularity are unknown. The key challenge in these cases is to document that blasts exceed the 20% threshold. Distinction from hypocellular myelodysplastic syndrome (MDS), hypocellular hairy cell leukemia and aplastic anemia can usually be achieved by integration of morphology, immunohistochemistry, immunophenotyping, cytogenetics and molecular studies.

4. AML with fibrosis
Fibrosis is highly characteristic of acute megakaryoblastic leukemia and therapy related myeloid neoplasms. Because of the difficulty in obtaining bone marrow aspirate in these fibrotic bone marrows, definitive diagnosis of AML often relies upon immunohistochemical assessment of bone marrow biopsy sections to estimate the number of blasts and myeloid expression by them.

5. Reactive vs neoplastic conditions:
Misdiagnosis of reactive lesion as neoplastic or vice versa can be obviously detrimental for the patients as well as have medicolegal implications for the pathologist. Therefore, all effort should be made by the diagnostic team to avoid these errors. Some of the common benign conditions which mimic malignancy are:
- Kikuchi lymphadenitis- a self limited disease of young women, frequently misdiagnosed as lymphoma due to many histiocytes and many activated T cells in lymph nodes.
- Infectious mononucleosis and other viral infections, reaction to vaccines and drug hypersensitivity, often cause diagnostic confusion due to reactive immunoblastic proliferation and large activated lymphoid cells. Reed-Sternberg like cells can also be seen which causes one to consider differential diagnosis of Hodgkin lymphoma.
- Castleman disease- especially plasma cell variant which in small biopsies can be misdiagnosed as an extraskeletal plasmacytoma or marginal zone lymphoma.
- Autoimmune lymphoproliferative syndromes: This is due to non functional or dysfunctional apoptosis of lymphocytes. Because of impaired apoptosis, lymphoid cells that have proliferated in response to antigens or infections persist after the challenge is over, causing persistent enlargement of the reticulo-endothelial system, manifesting as generalised lymphadenopathy and hepatosplenomegaly. This often causes resemblance to peripheral T cell lymphoma (PTCL); however, diagnosis of PTCL should be made with caution in young children and after thorough investigations.

6. Low grade vs high grade lymphoma
Different types of lymphomas tend to have very varied clinical course including survival rates and therefore an accurate diagnosis is critical. Failure to appreciate the higher biological grade of some lymphomas composed of small cells can be a cause of misdiagnosis. Mantle cell lymphoma, lymphoblastic lymphoma and even Burkitt lymphoma can fall into this category. In thick sections, intercellular nuclear membrane staining may mask subtle details of chromatin density and distribution that normally assist in the distinction between small lymphocytes, lymphoblastic and Burkitt type cells. It may also make mitotic and apoptotic bodies difficult to recognise against the tissue background. Immunostaining and other diagnostic investigations like MYC translocations for Burkitt lymphoma should always be carried out.

7. Hodgkin lymphoma vs non Hodgkin lymphoma
It is important to distinguish Hodgkin lymphoma (HL) from non-Hodgkin lymphoma (NHL) because of different treatments and clinical outcomes. Histological criteria for diagnosing HL are well defined and few years ago it was considered so straightforward that no immunostaining or specialist opinion was considered important. However, the distinction between HL & NHL can be difficult in some cases; in fact, a specific entity B cell lymphoma, unclassifiable, with features intermediate between DLBCL and HL is defined in the 2008 WHO classification. These lymphomas generally have a more aggressive clinical course and poorer outcome than classical Hodgkin lymphoma or diffuse large B cell lymphomas. Hence, when no regular immunostaining is used in HL, there could be potential problem of failure to recognize atypical cases that might instead be NHL. Reed-Sternberg cells are considered as diagnostic hallmark for HL. However, they can be found in many reactive conditions like infectious mononucleosis, after drug therapy and some NHL.

8. DLBCL vs Burkitt lymphoma
BL is a highly aggressive neoplasm with extremely short doubling time and correct identification is important due to the need for higher intensity treatment as compared with other lymphomas. Misdiagnosis as DLBCL may lead to undertreatment of patients who might otherwise be cured. There can be considerable overlap between BL and DLBCL and morphology alone should never be the basis for distinguishing these two entities. Immunophenotyping is essential and can be very helpful (the neoplastic B cells of BL are typically CD10 positive and BCL2 negative) but variant immunophenotype can be seen in BL. Ki-67 is another helpful marker and in BL it is close to 100%. However, some DLBCL also approach this value and their true biological nature is being questioned. Demonstration of c-MYC translocation by FISH is considered as gold standard for the diagnosis of BL. However, this is complicated by the occurrence of MYC abnormalities in some DLBCL. Cases of DLBCL with MYC abnormalities are invariably aggressive and require higher intensity treatment in comparison to other DLBCL. To fit these grey zone cases, category of B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL has been added in WHO classification.

9. Other important grey areas
Some lymphoproliferative disorders like CD23 negative chronic lymphocytic leukemia’s, CD5 negative mantle cell lymphomas, CD5 positive follicular lymphomas, CD10 negative follicular lymphomas and CD5 positive splenic marginal zone lymphomas also exist and should always be considered in differential diagnosis in appropriate cases.
10. Post Rituximab changes
Treatment with Rituximab often causes downregulation of CD20 expression. So, in a patient previously treated with Rituximab, CD20 antibody should not be used for B lineage assessment. Instead, other markers like PAX, CD79a, OCT-2 & BOB.1, should be used. PAX5 is a nuclear stain which gives excellent staining and is very helpful in identifying B cells in Rituximab treated cases.

Errors related to immunohistochemistry (IHC)
Immunohistochemistry is an essential component in the diagnosis of haematological neoplasms. We should therefore be familiar with technical and interpretive pitfalls, since many factors may influence the technical preparation of the immunoreactions and a wide variety of causes can result in incorrect interpretations.

Fixatives
Length and type of fixation may influence immunohistochemical staining. Specimens for immunohistochemistry should be fixed immediately as drying can result in non-specific binding. 10% neutral buffered formalin is the most commonly used fixative for histology specimens. Mercury based fixatives such as B5 and Zenkers are also used in bone marrow trephine biopsies as they provide excellent nuclear details. However, they induce molecular cross linking and may hamper reactivity of a number of important antigens, for example CD4, CD5, CD10, CD23 and sometimes CD30 (10-12). On the contrary, kappa and lambda light chain staining appears better in B5 than in formalin fixed tissues (10). The length of fixation also affects the results of immunostaining as underfixation often produces a reduced immunostaining in the central region of the tissue block with stronger immunoreaction in the marginal area of the section while overfixation generates the opposite effect (13).

Decalcification
Decalcification by strong acid on bone and bone marrow samples can have a negative effect on detection of CD markers. Good quality immunohistochemistry has been reported using formalin fixation with ethylene-diamine tetra-acetic acid (EDTA) decalcification.

Antigen retrieval
Different methods for antigen retrieval are used such as enzyme or protease induced antigen retrieval or heat induced antigen retrieval. Successful retrieval depends upon factors like fixation time, temperature, pH and molarity of the solution. A number of methods to achieve antigen retrieval are used such as enzyme or protease induced antigen retrieval or heat induced antigen retrieval. Successful retrieval depends upon factors like fixation time, temperature, pH and molarity of the solution. A standard protocol should be developed by individual laboratories and should be strictly followed.

Inappropriate controls
For the validation of IHC results, relevant positive and negative controls should always be run. Maintaining supplies of relevant positive control tissue can be problematic, but is essential. For bone marrow biopsy, as far as possible, controls should be applied on specimens which are fixed and decalcified in the same way as the tissue for diagnosis.

Knowledge of normal expression
It is also essential to know what internal control reactivities to expect in normal/ reactive as well as neoplastic lymphoid tissues. Internal controls are elements of diagnostic tissue itself that help provide reassurance that immunostaining has worked. Normal reactivities should not be misinterpreted as pathological, for example, endothelial cell expression of CD34 and expression of cyclin D1 by occasional bone marrow stromal cells.

It is crucial to know the normal distribution of antigens and their anticipated variation in neoplasia. A key example is bcl2 expression in follicular lymphoma, for example CD45, CD10, CD23 and CD20. There is no bcl2 expression in reactive germinal center B cells but as a result of t (14; 18) they are expressed in germinal center B cells. However, bcl2 expression is normal in T lymphocytes which can sometimes be prominent in germinal centers and peripheral B cells. So the interpretation of bcl2 expression should be made with caution by an experienced observer. In summary, in IHC, every brown cell is not positive and positivity should be interpreted with the detailed knowledge of antibody expression in normal and malignant cells.

Errors related to flow cytometry
Flow cytometry is a very useful tool in diagnostic haematology. The introduction of multicolour cytometry has enabled the rapid diagnosis of haematological neoplasms to be routinely achieved. The advantages of flow cytometry are rapid turnaround time, small specimen volume, identification of multiple antigens simultaneously, demonstration of monoclonality and detection of weakly expressed antigens. However, flow cytometry requires a high degree of expertise and a thorough understanding of technical details for a meaningful analysis. Some of the important issues which one should address before interpreting flow cytometry data are:

- Characteristics of fluorochromes: The fluorochromes chosen should be biologically inert i.e. they should not bind normal antigens resulting in background staining, they should be stable and readily conjugate to the monoclonal antibody of choice without inducing conformational changes to the surface antigens through mechanisms such as charge interactions. Ideally they should also produce a bright fluorescent signal resulting in a high signal to noise ratio.

- Compensation: Compensation is the process by which the spectral overlap between different fluorochromes is mathematically eliminated. Compensation adjustments are routinely applied in all flow cytometry experiments, but in many instances the proper application of this vital step is poorly understood. In order to properly analyse experimental data, users must be aware of the effects of compensation and understand how to apply it correctly and recognize when data are not properly compensated.

- Choosing the right fluorochrome: As mentioned above, fluorochromes differ in the relative brightness of the signal they produce. Conjugation of various fluorochromes to the same antibody, and staining of the same population of cells, can therefore result in large differences in resolution of positive and negative events. This difference may be largely inconsequential for highly expressed markers but is of great importance for surface markers that exhibit low levels of expression. The general rule of thumb therefore is that the lower the level of antigen expression, for a given marker, the more important it is to use a bright fluorochrome in order to generate superior results.

- Proper use of isotype controls: Isotype controls have historically been used to assess the background staining level and set criteria to differentiate between positive and negative expression of antigens. This can be problematic when the isotype control does not precisely match the analytical antibody in terms of antibody class, protein concentration and fluorescein to protein ratio. Thus, over reliance on isotype controls carries a risk of misinterpretation and in many instances the use of “internal controls” provides a more robust understanding of the antigen expression of a given cell population.

- Use of appropriate gating strategies: In flow cytometric analysis of a heterogeneous population of cells it is usual to apply electronic “gating” so that only the subset of cells of interest is displayed for interpretation. An example is the use of a CD45 vs side scatter gating strategy in the diagnosis of leukemias and lymphomas to delineate the population of interest. There are several alternative approaches including forward scatter vs side scatter that may be used to differentiate cells of different lineages, and “lineage” gating – where a lineage specific monoclonal antibody is used to select cells of interest. Irrespective of the strategy used, if the cellular population of interest is excluded from the gate, then erroneous results will be obtained.
Molecular studies. Fixatives (e.g., Bouin's solution for bone marrow biopsies) are sometimes used due to a solenotic or markedly hypercellular marrow, sufficient sample cannot be obtained for analysis. Careful correlation with clinical history and other diagnostic details is necessary in such situations to ensure accurate diagnosis.

Cytogenetics/FISH studies

Cytogenetics and FISH studies play an important role in the diagnosis of haematological neoplasms. They also help in deciding management of patients as well as to predict prognosis, response to treatment and disease progression. An example is demonstration of MYC translocation for the diagnosis of Burkitt lymphoma.

Conventional cytogenetics are helpful in identifying chromosomal abnormalities but are limited due to the poor quality of metaphases and low mitotic index associated with many diseases. As a result, a significant proportion of bone marrow karyotypes are reported as normal by cytogenetic studies. FISH helps to overcome this problem by using non dividing cells as target (interphase FISH), and allowing for the identification of both numerical and structural abnormalities in a large number of cells. This has considerable advantage for some haemopoietic malignancies, where the proliferative activity is low, or when dividing cells do not represent the neoplastic clone. For example, in the cytogenetic studies of B-CLL with interphase FISH, a much higher incidence of trisomy 12 is found as compared to conventional cytogenetics. However, FISH is a more targeted approach and generally requires prior knowledge of anomaly of interest, so cannot be used as a screening tool. Also, the number of commercially available probes is limited and interpretation can be challenging with FISH when analysing suboptimal sampling (especially background fluorescence with formalin fixed paraffin embedded tissue). Hence, one should understand the limitations of these techniques and also should keep in mind that they are adjuncts and should not form the sole basis for a diagnosis.

Conventional cytogenetics requires fresh tissue, so the lymph nodes tissue should be sent to the laboratory fresh before formalin fixation. For blood and bone marrow, samples should be collected in heparin as per laboratory guidelines and sent immediately to cytogenetic laboratory. FISH can be performed on fresh, frozen or paraffin embedded tissue and can provide results when there is insufficient tissue for conventional cytogenetics.

It should also be remembered that cytogenetics and FISH studies cannot be performed on tissue fixed in mercury based fixatives (e.g., Bouin's solution for bone marrow biopsies), so sample should be collected in appropriate medium for these studies.

Molecular haematology

Molecular techniques can contribute to establishing the correct diagnosis, prognostic stratification and predicting and assessing response to treatment in haematological malignancy. For example, presence of JAK2 mutations confirms a suspected diagnosis of chronic myeloid leukemia (APML), and BCR-ABL1 confirms chronic myeloid leukemia (CML) and FIP1L1-PDGFRα confirms chronic eosinophilic leukemia. Examples of targeted therapies based on molecular markers are all-transretinoid acid in APML and imatinib in CML. Success of molecular haematology analysis largely depends upon tissue type, sample quality and sensitivity of assay used. The sample required is usually peripheral blood or bone marrow in EDTA which should be sent to molecular laboratory as soon as possible.

A major problem in diagnosing neoplasia by molecular methods is the background of normal cells which are invariably present in tumour specimen. For example, in systemic mastocytosis, the KIT mutation (D816V) may not be reliably detected if there are insufficient numbers of mast cells (14). Enrichment techniques are usually applied which improves sensitivity of the techniques.

Contamination is also a major problem with molecular techniques and can result in false positive results. Care should be taken to minimize contamination, including setting up reactions in a dedicated PCR cabinet, keeping pre-PCR and post-PCR areas separate, decontaminating equipment regularly and rigidly adopting good laboratory practice at all times.

In some cases, when the level of positivity is low as in MRD or some rare genetic abnormality is identified, it is always advisable to run the test in duplicate and correlate with other test results.

False negative results are often due to poor sample quality. Tests based on RNA analysis are highly sensitive because RNA is labile and prone to rapid degradation by RNAase enzymes which are abundant in the environment. Samples for RNA analysis must therefore be processed within 48-72 hrs. Sometimes, false negative results also occur if wrong molecular target is monitored. This may occur if fusion break points at diagnosis are not established, and an assumption made that the patient has a common fusion type that can be detected with standard primers. One should keep these limitations in mind and should have adequate experience in molecular techniques in order to get accurate results.

Errors arising due to limited range of tests done

Haemotoxylin and eosin staining alone is insufficient in making the diagnosis in many cases of haematopathology and an extended panel of antibodies as well as genetic tests is required to reach accurate final diagnosis. Sometimes, due to limitations in the availability of tests, only limited investigations are performed which may lead to inaccurate diagnosis. For example, leaving a diffuse small lymphoid cell infiltrate categorised as small B cell lymphoma without investigating CD5, CD23, cyclin D1, and Ki-67 expression to differentiate chronic lymphocytic leukemia/ small lymphocytic lymphoma, mantle cell lymphoma or marginal zone lymphoma is incorrect as these diseases are often treated with different protocols. Mantle cell lymphoma is an aggressive disease as compared to chronic lymphocytic leukemia, although both are small lymphocytic proliferations.

Along with the diagnosis, there is an increasing need to evaluate prognostic markers like CD10, BCL2, BCL6 and MUM1 to subclassify DLBCL into Germinal Centre (GC) type or Activated B Cell (ABC) type as they have different survival rates.

Several additional algorithms involving antibody combinations based on gene expression profiling have been proposed for improved prognostication in aggressive B cell lymphomas. It is also understandable that all these diagnostic modalities are not available in every centre, so a centralized referral haematology department should ideally be established in each region which is well equipped with modern diagnostic facilities and experienced staff and it should serve as the referral centre for all cases.

Conclusion

The final diagnosis in haematological malignancies should be based upon a combination of the following:

- Clinical details: Adequate history as well as results of additional tests (in particular haematological/ biochemical investigations and imaging).
• Technical preparation: Sufficient sample in proper preservative analysed by experienced scientific officer/pathologist.

• Resources: Facilities for immunohistochemistry, flow cytometry and genetic studies.

• Knowledge: Familiarity with WHO Classification, access to guidelines and references, experience in interpretation of flow cytometry and genetic results.

• Experience: In differentiating reactive or benign and malignant conditions as well as proper application of ancillary techniques in diagnosing them. And finally the ability to refer difficult cases to a centralized laboratory which is well equipped and well staffed by experts.

• Deficiency of one or more of the above has the potential to compromise accurate diagnosis of haematological malignancies.

Author information
Rishu Agarwal, MBBS MD, Fellow in Haematology
Surender Juneja, MBBS MD FRCPA, Head and Associate Professor Diagnostic Haematology, Melbourne Health Pathology, Royal Melbourne Hospital, Parkville, Melbourne, Australia

References


Copyright: © 2013 The authors. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

The New Zealand Institute of Medical Laboratory Science (Inc) would like to thank the following companies for their sponsorship of the NZIMLS ASM 2013: