Article published online: 2023-11-04

What Does an Adult Hemato-Oncology Physician Expect from a Hematopathologist?

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Ind J Med Paediatr Oncol 2023;44:452–459.

Abstract

Recent advances in the field of hemato-oncology have significantly improved outcomes for patients. However, these changes have also increased the complexity of investigations required at the time of diagnosis and during the follow-up of these patients. Close interaction and exchange of information between the pathologist and the clinician is important for sucessful management of patients. This article briefly discusses the advances in the field and the impact of these changes on the management of patients. A clinician's perspective of what is required from a hematopathologist while managing patients in the current era is presented. An attempt is made to classify the requirements as to what is expected in ideal as well as in resource-limited settings.

Keywords
- hematological malignancies
- cancer
- pathology
- expectations
- advances

Introduction

The last two to three decades have seen an explosion of research in hematological cancers, including biological and targeted therapies.[1–5] The classic example is the use of breakpoint cluster region-Abelson leukemia (BCL-ABL) tyrosine kinase inhibitors (TKIs) in chronic myeloid leukemia (CML).[5] The use of imatinib had converted a fatal malignancy into a chronic illness. Less dramatic, but equally significant are the targeted therapies now used in chronic lymphocytic leukemia (CLL; Bruton's tyrosine kinase [BTK] inhibitors), anti-CD20 monoclonal antibodies, several agents in multiple myeloma, and all-trans retinoic acid (ATRA) in promyelocytic leukemia.[6–8] We have also learned to risk-stratify cancers using biological markers. The intensity of chemotherapy can be tailored, and toxicities reduced with minimal residual disease (MRD) stratified treatment in acute lymphoblastic leukemia (ALL).[9,10] ►**Table 1** shows a few selected examples of how advances in the understanding of disease have affected diagnosis and management of hematological cancers.

Expanding knowledge has helped evolve the classification, prognostication, and treatment of hematological malignancies. The role of the hematopathologist has also grown in this period. Most pathologists in the 1970s to 1980s had access to hematoxylin and eosin (H&E) stained specimens and relied on their experience and expertise for the limited subclassification of lymphomas. For leukemias, additional tests like cytochemistry were used for basic differentiation. In the current era, a pathologist has a tremendous array of investigative tools available. These include immunohistochemical studies, conventional cytogenetics, flow cytometry (FCM), molecular techniques like polymerase chain reaction (PCR), in situ hybridization (ISH), and next-generation sequencing (NGS). The modern hematopathologist must wear many hats and plays a vital role in the optimal management of patients. In this article, the role of the hematopathologist in various cancers is discussed. The additional investigations currently available and often pursued also bring with them an enormous cost burden, which can be significant in the Indian context. To help clinicians and pathologists to optimize the use of expensive tests, these are classified as essential, optional, and optimal as per ►**Table 2**. This is similar to the classification used in the National Cancer Grid (NCG) guidelines for hematological cancers and is designed to help optimize patient assessment in Indian situations.[11]

DOI https://doi.org/10.1055/s-0043-1768567.
ISSN 0971-5851.

Thieme Medical and Scientific Publishers Pvt. Ltd., A-12, 2nd Floor, Sector 2, Noida-201301 UP, India

Table 1 Examples of how changes in understanding of the biology of hematological cancers affect management

Biology of cancer	Remarks/examples
Prognosis	● Minimal residual disease as a prognostic factor and as an endpoint for trials in ALL, myeloma, and CLL[40,44,45]
Identification of newer targets of treatment and development of tailored drugs	● *BCL-2* targeted by venetoclax has improved survival in AML and CLL[30,46] ● Anti-CD38 monoclonals in myeloma[47] ● Bispecific antibodies in acute lymphoid leukemia[48]
Classification of hematological cancers	● Regular review and revision by the WHO have resulted in identifying newer entities with different prognoses and treatments ● It has changed from a one-size-fits-all approach to specific treatment for individual entities[33,49]
Response adapted therapy	● Minimal residual disease-based treatment tailoring (intensification/allogenic transplant) in acute lymphoid leukemia[44] ● Interim PET-based response-adapted therapy in Hodgkin's lymphoma[50]

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; CLL, chronic lymphoblastic leukemia; PET, positron emission tomography; WHO, World Health Organization.

Table 2 Classification of usefulness and impact of investigations in current practice

Classification	Explanation
Essential	They are considered mandatory for current practice. Even with resource constraints, efforts must be undertaken to get this information. All pathology laboratories must endeavor to establish these tests in-house
Optimal	This would be considered a basic standard in most of the tertiary centers currently managing hematological cancers in India. It would be preferable to have this established in-house in most centers
Optional	These investigations are ideal and may affect a particular patient or situation. Still, for the most part, they may not be considered part of routine care or have a prohibitive cost, which is not justifiable in the Indian context. It may be done rarely. Sample numbers may not be sufficient to justify in-house establishment of these facilities

Acute Lymphoblastic Leukemia

Recent Advances

Pediatric ALL survival has increased to 80 to 90% due to optimized chemotherapy, better supportive care, risk-adapted treatment (especially with MRD assessment), and baseline molecular characterization.

Adult ALL: Survival gains are modest except in the adolescent and young adult (AYA) population.[12] More intense therapies based on pediatric protocols are preferred in the AYA population. About 15 to 25% of adults would be Ph+, where targeted therapy with TKIs is incorporated. Molecular characterization has identified early T precursor ALL (ETP-ALL), and Philadelphia-*like* (Ph-like) ALL, with worse prognosis.[13] Identification of cell surface antigens helps decide targeted therapy, like anti-CD20 (rituximab), anti-CD19 (blinatumomab) and anti-CD22 (inotuzumab ozogamicin) monoclonal antibodies. Anti-CD38 antibody daratumumab has shown efficacy in relapsed T-cell ALL (T-ALL). Hence, the pathologist must have a close liaison with the treating team to make markers relevant and tailored to the requirement. MRD assessment is crucial in determining survival and decisions for allogeneic consolidation transplants. Thus, there is a demand for more complex investigations during workup, which have had varying degrees of impact on the outcomes (►**Table 3**).

Expectations from Hematopathologist

- FCM for diagnosis and subtyping is currently considered standard work up for ALL. In addition, most current protocols expect stratification based on MRD assessment. The most popular and established method for MRD assessment would be multiparameter FCM.
- Conventional cytogenetics is a well-established technique for the characterization of leukemia. Alternatively, fluorescence in situ hybridization (FISH) and PCR may help look for specific abnormalities like the *BCR::ABL1* translocation.
- During therapy, there are situations where there are cytopenias, and the clinician is unsure about the cause (persistent disease vs. chemotherapy effect). Bone marrow examinations done during these conditions need careful interpretations with close interaction between the pathologist and clinician (information on the duration of cytopenias, diluted aspirates, and the use of growth factors).
- Assessment of MRD using NGS shows high concordance with quantitative PCR while being more specific and is a potential alternative for frontline MRD evaluation.[14]

Acute Myeloid Leukemia

Recent Advances

The initial treatment of AML depends entirely on the risk category (National Comprehensive Cancer Network [NCCN], European LeukemiaNet [ELN] guidelines).[15,16] The primary investigations needed for this would be conventional cytogenetics and molecular studies to establish the status of *NPM*, *FLT3*, and other molecular markers. Patients classified as high risk would be eligible for considering allogeneic transplants in the first remission. FCM-based MRD is not as well established in AML as in ALL, but data is emerging on its usefulness

Table 3 Investigations in adult ALL and how they impact management

Investigation	Implications	Usefulness/ application
Flow cytometry for phenotyping and subclassification	Accurate diagnosis of acute leukemia subtype; helpful information for future MRD studies (not mandatory). In addition, entities like ETP may be identified.[51] FCM can also assess ploidy	Essential
BCR::ABL1 assessment by FISH and PCR	Survival improvement by 20% with the addition of TKI. The decision of transplant in CR1[52]	Essential
MLL status (break apart probe *KMT2A*) A mixed phenotype is suggested by morphology and immunophenotyping, and require molecular studies for confirmation[53]	The presence of MPAL has variable implications on prognosis but is generally considered to have poor outcomes Demonstrated 11q23 abnormality is a definite poor prognostic factor. Many protocols recommend CR1 allogenic transplant in these patients	Optimal
Minimal residual disease assessment by FCM (most common) or molecular studies	Risk-stratified therapy. Intensifying therapy in MRD-positive patients may improve outcomes. Persistent MRD positivity may indicate allogeneic transplantation	Optimal
Ph-like ALL assessment	May have a poorer prognosis. It may be represented in up to 25–30% of patients in AYA age groups. However, testing is complicated and expensive, and treatment implications are few at this point[13]	Optional
Conventional cytogenetics	It may help detect the Ph chromosome. Ploidy may be assessed and may have a prognostic impact (less in adults than in the pediatric age group). More than five chromosomal anomalies may have a poor prognosis	Optimal
CSF cytology	Involvement of CSF needs additional CNS-directed treatment. This is the standard workup for all ALL patients. The sensitivity of assessment may be increased by adding FCM	By spin and cytology: essential By FCM: optimal

Abbreviations: ALL, acute lymphoblastic leukemia; AYA, adolescent and young adult population; BCR-ABL, breakpoint cluster region-Abelson leukemia; CNS, central nervous system; CR1, complement receptor type 1; CSF, cerebrospinal fluid; ETP, early T-cell precursor; FCM, flow cytometry; FISH, fluorescence in situ hybridization; MLL, mixed lineage leukemia; MPAL, mixed-phenotype acute leukemia; MRD, minimal residual disease; PCR, polymerase chain reaction; TKI, tyrosine kinase inhibitor.

(►**Table 4**). Mutation profiling using NGS provides information regarding the type and number of gene mutations, variant allele frequency, and amenability to targeted therapeutics. In addition, it helps us to understand the ontogeny of the disease, underlying germline predisposition, clonal hematopoiesis, and resistance-causing mutations.[17]

Expectations from Hematopathologist

- FCM for diagnosis and subtyping is standard.
- Conventional cytogenetics is mandatory in AML. It remains the cornerstone of risk stratification in AML, classifying the disease into favorable, unfavorable, and

Table 4 Investigations in adult AML and how they impact management

Investigation	Implications	Usefulness/ application
Flow cytometry for diagnosis	Diagnosis of acute leukemia and subclassification	Essential
FCM for MRD assessment	Prognostic as of now. No therapy indications established[54]	Optional
Conventional karyotyping	Risk stratification and treatment. Can pick complex anomalies missed by FISH or PCR	Essential
Molecular studies: *NPM* mutations and *FLT3* mutations/duplications	To help in risk stratification	Essential
Other molecular tests (*CEBPA, c-kit, p53*)	These also have prognostic, and treatment implications but are rarer	Optimal
FISH/PCR for *PML::RARA* translocation	Important implications: either technique must be available in suspected patients to confirm diagnosis	Essential
NGS	MRD assessment	Optimal

Abbreviations: FCM, flow cytometry; FISH, fluorescence in situ hybridization; MRD, minimal residual disease; NGS, next-generation sequencing; PCR, polymerase chain reaction; PML, promyelocytic leukemia; RARA, retinoic acid receptor alpha.

intermediate-risk categories.[18] FISH and PCR may help look for specific abnormalities and further refine prognostication, especially in those with intermediate cytogenetics.

- As in ALL, cytopenias need careful evaluation and interaction between the clinician and hematopathologist during therapy. Hematogones are normal precursors in the bone marrow, recognized by their characteristic morphological features and immunophenotype. The pathologist must help distinguish the residual disease from hematogones.
- In cases of suspected acute promyelocytic leukemia (APML), urgency is required in the diagnosis. Early treatment in these patients can improve survival to >90 to 95%. It is also essential to detect variant translocations in APML as they have therapeutic implications. For example, *NPM1::RARA* [t5,17 (q35;q21.1)] and *NuMA::RARA* are responsive to retinoids [t11,17(q13;q21.1)], while *PLZF::RARA* [t11,17(q23;q21.1)] is resistant to retinoids.
- Bone marrow examination is done to assess response to AML induction therapy. The initial response to therapy is assessed using a bone marrow examination done on day 14 of treatment. However, the D14 marrow is not always practiced due to inherent challenges in its interpretation. Specific centers prefer to repeat induction or to escalate therapy for persistent disease. Another bone marrow examination is performed on day 28 of induction or after white blood cell (WBC) and platelet count recovery to assess achievement of remission.
- The role of MRD assessment in AML is established as a potent prognostic factor. However, the impact of treatment modification based on MRD is unclear. The commonly employed techniques for MRD assessment are PCR and multiparameter flow cytometry (MFC) and, less typically, NGS. NGS has been used for MRD assessment in patients with *RUNX1, NPM1, FLT3* mutations, and other novel targets.[19] However, what is unclear is the best modality to adopt and how to incorporate the findings in therapy alterations. Each center would do its best to evolve its own MRD technique and standardize these until more data develop in this space. Leukemia-associated aberrant immunophenotypes (LAIPs) present on the blasts are identified at diagnosis using MFC and are later used to assess MRD. MFC can be performed in 80% of AML patients and has a sensitivity of one blast cell per 10^3 to 10^4 nucleated cells. MRD assessment of core binding factor AML (t(8,21) or inversion 16) using quantitative reverse transcription PCR (RT-PCR) allows the identification of patients at high risk of relapse. It is now being studied to predict the role of risk-directed/preemptive therapy.

Chronic Myeloid Leukemia

Recent Advances

The advent of imatinib in the last two decades and subsequent generations of TKIs have made CML a chronic disease. A chronic phase CML patient can expect a life span similar to an age-matched person.[20] However, optimum treatment needs close monitoring of *BCR-ABL* by quantitative PCR.[21] When patients develop resistance, mutation analysis helps decide the subsequent line of TKIs.

Expectations from Hematopathologist

- *At diagnosis:* From bone marrow studies, we need to understand the blast percentage to know the risk group and the phase of CML. Conventional karyotyping helps diagnose additional chromosomal abnormalities and variant translocations and understand clonal evolution. Additional cytogenetic abnormalities, including trisomy 8, an additional Ph translocation, isochromosome 17q, and trisomy 19, are seen in the bone marrow aspirate of 3% of patients, which may have prognostic implications.[22]
- Up to 15% of CML patients may be negative on cytogenetic analysis and can be diagnosed by FISH testing. These are referred to as Philadelphia chromosome (Ph)-negative CML. FISH also helps diagnose patients with complex (e.g., Chr 9;14;22) and cryptic translocations. Commonly used RT-PCR assays will only detect the p210 and p190 variants of *BCR::ABL1*. Hence, if a patient with suspected CML is negative for *BCR::ABL1* transcripts by RT-PCR, it is essential to rule out *BCR::ABL1* fusion using FISH testing.[23] Bone marrow fibrosis is less relevant in the TKI era. However, this is valuable information and may predict cytopenias during early treatment.[24]
- For a patient presenting with blast phase CML, knowing the type of blast crisis is helpful—lymphoid versus myeloid. In lymphoid blast crises, we can use vincristine, steroids, and TKIs. In myeloid crises, TKIs are often used with or without cytarabine-based chemotherapy.
- *Molecular studies:* At baseline, we need to confirm the diagnosis by demonstrating the *BCR::ABL1* transcript. Qualitative PCR is preferred at baseline to know whether the transcript is p190, p210, or others like p230, which are rare. It is essential to see if it will be a quantifiable transcript during follow-up (as most of the qPCR assays are kit based and can detect only known transcripts). A qPCR may be appropriate at baseline as the follow-up evaluations are normalized based on the International Standard (IS).[21]
- During follow-up: Current follow-up standard for CML is quantitative PCR for *BCR::ABL1* transcripts. This is important to define treatment landmarks and to change therapy in those without optimal response or treatment failure. Well-defined cutoffs are available from the ELN guidelines that most centers worldwide follow. The availability of cheaper generic second-generation TKIs have made these results relevant in India. For patients wanting to attempt treatment-free remission (TFR), qPCR needs to be done once every 4 to 6 weeks, and the turnaround time should be less than 2 weeks. The laboratory needs to mention the sensitivity of the testing, the *ABL* copies, and the sensitivity of the PCR machine itself in these reports. The reporting must conform to standard guidelines.[25] The kinetics of *BCR::ABL1* transcripts predict treatment response and overall prognosis. It has been found that the time taken

for the *BCR::ABL1*value to halve was a strong independent predictor of sustained TFR. A quicker *BCR::ABL1* decline increased the likelihood of achieving TFR eligibility.[26]

- Analysis for kinase domain (KD) mutations is necessary for patients who progress to accelerated and blast phases. PCR and Sanger's sequencing are the commonly employed techniques. Sanger's sequencing has limited sensitivity and has limited ability in diagnosing polyclonal and compound mutations. NGS is a promising technique for KD mutation analysis.[27] The advantages of NGS are its higher sensitivity, ability to scan the entire KD for any mutation, and the ability for clonal analysis in case of multiple compound and polyclonal mutations falling within the same sequence reads.[28]

Chronic Lymphoid Leukemia

Recent Advances

From conventional treatment with rituximab and chemotherapy (fludarabine, cyclophosphamide, rituximab [FCR] or bendamustine, rituximab [BR]), the current standard of care in CLL management are BTK inhibitors, and venetoclax.[29,30] In India, BTK inhibitors like ibrutinib and acalabrutinib are available as generics at lower costs. Although these are expensive, access has increased in the last 2 to 3 years.

Expectations from Hematopathologist

- Apart from establishing the diagnosis, which is straightforward in the majority of the cases (lymphocytosis with typical morphology with FCM demonstration of CD5+ expressing B cells, which are negative for cyclin D1 and positive for CD23), pathologists would need additional tests to confirm the diagnosis in situations where the typical markers are not expressed. These include differentiating rare cyclin D1 negative mantle cell lymphoma (MCL) cases, CD23 negative CLL, etc. Additional markers that help in these situations are available.
- Though BTK inhibitors can be considered superior to chemoimmunotherapy for all patients with CLL, they are still expensive and require lifelong therapy. Hence, many Indian hemato-oncology physicians prefer chemoimmunotherapy in their patients and reserve the BTK inhibitors for later lines. However, it becomes crucial to check for 17p deletion. FISH can usually do this from the peripheral blood. While detecting *TP53* mutation using NGS increases sensitivity, it may also slightly increase the false-positivey rate.[31] 17p deleted patients do poorly with BR or FCR and should be treated with BTK inhibitors. FISH can also detect other cytogenetic abnormalities, which correlate with prognosis, namely, deletion 13q (favorable) and deletion 11q (adverse).
- Multiple markers like molecular studies for 17p, β-2 microglobulin, and various FCM-based markers are available for prognostication. The mutational status of the variable region of the immunoglobulin heavy chain (*IGHV*) gene using PCR is a powerful predictor of response duration and overall survival.[32] Although not standard, increased expression of zeta-associated protein 70 (ZAP-70) and CD38 are imperfect surrogates for *IGHV* unmutated status, which correlates with higher clinical stage, increased chances of disease progression, and poorer overall survival. Although it would be helpful to have this information, these are not mandatory for patient care. The decision on whether to start treatment in a given patient can be made based on simple clinical parameters and the Rai stage.[29]
- In patients with anemia, a Coombs test would be needed to look for hemolysis.
- A bone marrow aspirate and biopsy is not routinely recommended for CLL diagnosis. The only indications for bone marrow examination in the contemporary era is to look for factors that might contribute to cytopenias other than leukemia cell infiltration. It can also be helpful in patients who have persistent cytopenias after treatment to establish disease versus therapy-related causes.
- Acquired Bruton tyrosine kinase/phospholipase C gamma 2 (PLCG2) mutations have been associated with resistance to BTK inhibitors resulting in CLL progression and Richter transformation. Longitudinal, targeted, deep multigene sequencing is an emerging modality to diagnose these mutations. As the usage of BTK inhibitors expands in India due to the availability of generic molecules, these tests will become relevant very soon, and laboratories will benefit from developing these additional capabilities.

Lymphomas

Recent Advances

In the last two decades, there have been multiple studies on diffuse large B-cell lymphoma (DLBCL) biology. Prominent ones are the cell of origin classification (including gene expression–based study and later histochemistry-based substitution), and the use of FISH to subclassify high-grade B lymphomas (HGBL) into DLBCL/HGBL with MYC and BCL2.[33] The subclassification has led to better prognostication, but the impact on treatment has been limited. One exception is the double-hit lymphoma, where more aggressive regimens may be warranted but not proven to have benefits beyond the R-CHOP (Rituximab, Cyclophosphamide, Vincristine, Doxorubicin, and Prednisolone) regimen.[34] Despite several studies, most patients with DLBCL continue to be treated with R-CHOP as other regimens have failed to demonstrate superiority.[35]

Expectations from Hematopathologist

- The World Health Organization (WHO) classification in 2016 brought a clear pathway for distinguishing various entities called high-grade B-cell lymphomas.[36] This differentiation is essential in the clinic as the treatment varies. So, a specific classification can help the clinician, especially when FISH testing needs to be optimized because of cost constraints.
- **Testing for DHL by FISH:** DLBCLs that show rearrangements of the *C-MYC* and *BCL2* (DHL) and/or *BCL-6* respond poorly to the R-CHOP regimen. Hence, it is advisable to check for these aberrations in patients with germinal center

B (GCB) subtype with advanced clinical stage, extranodal involvement, bone marrow and central nervous system (CNS) involvement, and high International Prognostic Index (IPI) score. Since this entity constitutes only 5 to 10% of all DLBCLs, routine testing for all cases is not financially viable. Hence, it would be preferable to have an algorithmic approach to decide when to go for FISH testing.[37]

- There are entities within the B-cell lymphoma category that are CD20 negative, like ALK + DLCBL and plasmablastic lymphomas. These need to be flagged as they are not eligible for rituximab.
- In situations where tissue availability is limited (sick patient, only fine-needle aspiration cytology [FNAC] is available, or a small needle biopsy material), the crucial information required from the pathologist is whether it is high grade or low grade and whether CD20 is expressed or not. This will help in important treatment decisions. The other important information is whether a high-grade lymphoma has a blastic morphology. This can be very challenging in situations with limited tissue, and markers like TdT may help out. Cell blocks prepared from the FNAC specimen can be used for immunohistochemistry and molecular testing.
- WHO classification of lymphoid malignancies: Arguably, this is one area of hematological cancers with maximum changes and subdivision of entities. Again, it is helpful for pathologists to consider subtyping as much as possible. Although everything may not change treatment immediately, the information about newer subtypes may inform future understanding.
- Regarding Hodgkin's lymphomas (HLs), tissue diagnosis is crucial. Subtyping into individual entities has become less relevant from treatment and prognostic points of view with modern therapies. R-CHOP is considered the preferred treatment for the entity of nodular lymphocyte predominant HL.[33]
- Identifying surface molecules has helped in the evolution of targeted therapy in lymphomas. A few examples include anti-CD20 (rituximab, obinutuzumab, ofatumumab, and ibritumomab in B-NHLs), anti-CD22 (moxetumomab pasudotox in hairy cell leukemia), anti-CD30 (brentuximab vedotin in HL, anaplastic large cell lymphoma, and CD30+ T cell lymphomas), anti-CD 79b (polatuzumab vedotin in DLBCL), and crizotinib in ALK + ALCL.
- In patients with marginal zone lymphomas, detection of t(11,18) using RT-PCR helps identify those who show resistance to *Helicobacter pylori*–based antibiotic therapy.
- Analysis of T-cell receptor (TCR) gene rearrangements using PCR helps differentiate between lymphomas and benign pathologies like reactive lymphoid hyperplasia. This is particularly useful in samples where small quantities of DNA are available, such as skin biopsies in cutaneous T-cell lymphomas or cell suspensions[38]

Multiple Myeloma and Plasma Cell Disorders

Recent Advances

The diagnostic criteria for myeloma have been updated, and the high serum-free light chain (SFLC) ratio and high plasma cell proportion in the bone marrow (>60%) are considered myeloma defining (even without symptoms).[39] Besides multiple myelomas, the SFLC assay helps diagnose and monitor oligo-secretory and nonsecretory myelomas, light chain myelomas, and light chain amyloidosis. An abnormal SFLC kappa/lambda ratio has prognostic significance for monoclonal gammopathy of unknown significance, smoldering myeloma, and solitary plasmacytomas. MRD assessment has been recognized as an essential prognostic factor in myeloma.[40] Posttransplant D-100 is the preferred timepoint for assessment of MRD. Although clinical trials have assessed MRD as an endpoint, it is yet to find favor as a clinically relevant endpoint. It would be good to have this information, especially from academic large-volume centers treating myeloma. The methodology for assessing the MRD status for myeloma has been standardized, and guidelines are available. The most common techniques used for MRD assessment in myeloma are multiparameter FCM and NGS. In centers with limited resources for MRD assessment, a stringent complete response is taken as a treatment endpoint, as demonstrated by the absence of clonal cells by immunohistochemistry or immunofluorescence in the bone marrow. In terms of treatment, several new drugs have been approved for treating myeloma in the last few years.[4] These do not change the diagnostic requirement for the most part. However, there are unique challenges in interpreting reports, as detailed below.

Expectations from Hematopathologist

- In situations of monoclonal gammopathy of undetermined significance (MGUS)/smoldering myeloma, when there are minimal plasma cells in the bone marrow, it becomes critical to know whether they are clonal. Appropriate IHC would be required for the final decision. When the flow laboratory is well developed, FCM-based assays can also demonstrate clonality. These are unique situations and may not be routinely required in practice.
- Current classification recognizes >60% plasma cells in the marrow as myeloma-defining criteria.
- FISH studies from the bone marrow for del 17p, t(4,14) t(14,16). These are the commonest cytogenetic abnormalities in myeloma and are part of current prognostic scores. Gain or amplification of chromosome 1q is a poor prognostic factor and has been included in the second revision of the international staging system (R2-ISS). These are desirable to have in all patients. Certain treatment decisions, like the continuation of proteasome inhibitors in maintenance, may be considered in patients with high-risk disease. Plasma cell enrichment of the bone marrow specimen improves FISH sensitivity.
- Hyperdiploidy, which occurs due to trisomy of odd-numbered chromosomes, can be detected using cytogenetics. It is a favorable prognostic factor associated with improved overall survival.
- There are challenges in interpreting monoclonal spikes when treating with monoclonal antibodies like daratumumab. The clinician must convey this information to the pathologist. Similarly, challenges exist while blood

grouping these patients, which the clinician and hematopathologist must be aware of.

- The percentage of circulating plasma cells using peripheral smear helps diagnose plasma cell leukemia and is a prognostic factor in multiple myeloma. A study showed that the median overall survival of patients with <5% plasma cells was 50 months compared with 6 months in those with ≥5% circulating plasma cells.[41]
- Identifying solitary/multiple plasmacytomas using immunohistochemistry on tissue samples for CD138 and CD38 and establishing monoclonality by kappa/lambda light chain restriction helps identify cases that could have been labeled as nonmyeloma as per bone marrow examination showing the limited number of plasma cells.[41]

Myeloproliferative Neoplasms

Myeloproliferative neoplasms (MPNs) other than CML (polycythemia vera, myelofibrosis, and essential thrombocytosis being the three common ones) have been recognized by specific gene alterations such as the *JAK2* mutation. The WHO classification specifies diagnostic criteria for each of these entities.[42] Besides standard histomorphology, molecular testing for mutations (such as *JAK2)* is essential. However, since these are usually rare conditions, with most institutions seeing 5 to 10 cases per year, it may not be financially viable to develop in-house molecular testing capabilities in all except the few very high-volume centers. Cytogenetics is vital for the prognostication of primary myelofibrosis.[43]

Conclusions

- Several advances in understanding biology and newer therapeutic agents have led to significant alterations in the diagnosis, classification, and treatment of hematological cancers.
- A modern unit treating hematological cancers as well as the supporting hematopathology laboratory needs to adapt to the rapid changes to stay updated with the best treatment practices.
- However, many of the advances are expensive and may not be available in all centers. Hence, a balance needs to be struck between what is practical and achievable and what is desirable. These requirements need to be tailored toward individual center requirements and capabilities.
- A close interaction between the treating physician and the hematopathologist is ideal to realize the best utilization of resources and ensure optimal outcomes.

Funding
None.

Conflict of Interest
None declared.

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