the Pathologist

SPECIAL SERIES:

Hematology and Hematopathology

EDITORIAL

Magic

Join us on a journey through hematopathology and hematology-oncology

Magic. There is no other word to describe it. We clearly remember the wide-eyed wonder with which we looked up to our hematopathology mentors as they converted bits and pieces of complex data into comprehensive hematopathology reports. Interpretation of subtle morphologic findings and unusual (often unheard-of) cytogenetics and molecular genetics – these were all "simple" tricks of their trade. We watched in awe as they spoke with their hematology-oncology colleagues, assuring them with mutual respect. Even now, as practicing hematopathologists ourselves, we marvel at our good fortune in having stumbled across this subspecialty and the incredible ancillary toolbox it brings with it.

It's difficult to believe that it was only around 50 years ago that Janet Rowley discovered the chromosomal translocation underlying chronic myeloid leukemia and changed our understanding of cancer biology forever. The field of hematopathology grew exponentially thanks to significant changes to the way we approach and classify hematopoietic disorders. Within the pathology subspecialties, hematology led the way in using phenotypic, cytogenetic, and molecular genetic classification schemes – some of which even trump morphologic manifestations of disease. The literature is burgeoning with evolving data that further strengthen the classification of hematologic malignancies, continually improving our ability to care for our patients.

As hematopathologists, one of our most important responsibilities is to apply literature-based, objective criteria when establishing a diagnosis and communicating our findings with our hematologist-oncologist colleagues so that they can offer the best possible care to our patients. Precise subclassification is particularly essential for accurate risk stratification and appropriate treatment selection. The WHO classification scheme for hematopoietic neoplasms is an incredible resource that has rendered this task feasible for the vast majority of patients we encounter in daily practice – but, even so, it is essential to realize that no classification system is perfect.

"Some of the world's most renowned experts in our field summarize the historical context of hematopathology classification and discuss contemporary issues in diagnosing and treating hematopoietic disorders."

We are excited to present this "hematology/hematopathology takeover" issue of The Pathologist. In it, some of the world's most renowned experts in our field summarize the historical context of hematopathology classification and discuss contemporary issues in diagnosing and treating hematopoietic disorders. We have selected cases that present challenges in the categorization and therapeutic approach of bone marrow and lymph node malignancies – and it's our hope that they will help guide you in understanding how to approach these diseases from diagnostic and therapeutic angles.

We are grateful to Michael Schubert, Editor of The Pathologist, for allowing us space and intellectual liberty to present to you hematopathology at the intersection of history, morphology, phenotype, molecular analysis, and therapeutics. For the entire readership – health sciences students, medical laboratory scientists, pathology trainees, pathology faculty, and our patient-facing hematology colleagues – we hope that you find these cases interesting, educational, and inspiring, and that you leave with a sense of renewed wonder and intrigue about disorders of the hematopoietic system.

Sanam Loghavi is Assistant Professor of Hematopathology, Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA.

Kamran Mirza is Associate Professor and Vice Chair of Education in the Department of Pathology and Laboratory Medicine, Loyola University Chicago Stritch School of Medicine, Maywood, USA.

INTRODUCTION

Myeloid Neoplasms

An introduction and brief historical perspective

Prior to the 1970s, we lacked a uniform system for classification and nomenclature of the leukemic diseases. In fact, most of the terminology used to describe diseases represented the work of one or a few individuals. An international effort led to the French-American-British (FAB) classification of acute leukemias originally published in 1976 (1, 2, 3, 4), which became the first universally adopted classification of these neoplasms. Additionally, the FAB group published several other papers over the next quarter-century providing guidelines for the classification of both acute and chronic hematologic neoplasms (5, 6, 7). FAB schemes were based largely on the morphologic characteristics used to distinguish various myeloid neoplasms.

The 2001 WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues reflected a paradigm shift in our approach. Classification was based on consensus among many hematopathologists from around the world, as well as input from a clinical advisory committee of international expert hematologists and oncologists. For the first time, genetic information was incorporated into the diagnostic algorithms provided. Subsequent editions and revisions (8, 9, 10)incorporated new genetic data that have become available since the publication of the third edition.

The revised fourth edition follows the philosophy of its predecessors – integrating clinical features, morphology, immunophenotyping, cytogenetics, and molecular genetics to define disease entities of clinical significance. The most recent revision explicitly acknowledges that recurrent genetic abnormalities not only provide objective criteria for recognition of specific entities, but are also vital for the identification of abnormal gene products and pathways that can be used as therapeutic targets. Several disease subgroups and sets of defining criteria now include the presence of gene mutations with or without a cytogenetic correlate. However, the importance of a careful clinical, morphological, and immunophenotypic characterization of every myeloid neoplasm – and correlation with genetic findings – cannot be overemphasized.

Myeloproliferative neoplasms (MPN)

Since the 1980s, chronic myeloid leukemia (CML) has been recognized as a molecularly defined entity based on the presence of the *BCR-ABL1* gene fusion. The discoveries of activating *JAK2* mutations and mutations in *CALR*, *MPL*, and *CSF3R* have revolutionized the diagnostic approach to myeloproliferative neoplasms (11, 12, 13, 14, 15, 16). However, these mutations are not specific to any single clinical or morphological MPN phenotype and some are also reported in certain cases of myelodysplastic syndromes (MDS), MDS/MPN, and acute myeloid leukemia (AML). Therefore, we need an integrated multimodality for the classification of these myeloid neoplasms. Early MPN can be difficult to identify. Polycythemia vera, for instance, is often missed by relying only on CBC data; bone marrow morphology represents a critical criterion for diagnosis. Essential thrombocythemia must be distinguished from prefibrotic or early primary myelofibrosis (pre-PMF) – achieved by applying standardized morphologic criteria (10).

Myelodysplastic/myeloproliferative neoplasms (MDS/MPN) and myeloid/lymphoid neoplasms with eosinophilia

The MDS/MPN neoplasms category was introduced in 2002 to include myeloid neoplasms with clinical, laboratory, and morphologic features that overlap between MDS and MPN. Chronic myelomonocytic leukemia (CMML) – considered a variant of MDS by the FAB system – became a separate entity within this new disease group. In 2016, based on accumulated scientific evidence, MDS/MPN with ring sideroblasts and thrombocytosis was moved from provisional to full status (9, 10). The approach for diagnosing MDS/MPN is strictly multiparametric. An important point is the separation of CMML from PMF with monocytosis in patients with *JAK2* V617F mutation, which requires careful consideration of clinical and genetic results (17).

The presence of specific gene rearrangements is key to the classification of myeloid/lymphoid neoplasms with eosinophilia, a group of truly molecularly defined diseases. In 2016, the myeloid neoplasm with t(8;9)(p22;p24.1);*PCM1-JAK2* became a new provisional entity (18).

Myelodysplastic syndromes (MDS)

Persistent cytopenia is required for diagnosing a myelodysplastic syndrome (MDS). The cytopenia levels that should trigger an investigation were redefined in 2017 (19). MDS classification still incorporates morphologic elements of the FAB classification originally proposed in 1982 (3). Cytogenetics was added in 2001 and expanded in 2008. Targeted sequencing of myeloid neoplasm-associated genes can detect mutations in a vast majority of MDS patients (20, 21) and selected gene mutations are integrated into several diagnostic algorithms. *SF3B1* mutation is now a defining criterion for MDS with single or multilineage dysplasia and ring sideroblasts in cases with less than 15 percent ring sideroblasts. Evaluation for *TP53* mutation, a negative prognostic marker, is particularly relevant in cases of MDS with isolated del(5q), which identifies a clinically adverse subgroup. Importantly, acquired clonal mutations identical to those seen in MDS can occur in the hematopoietic cells of apparently healthy older individuals (22, 23) – so-called "clonal hematopoiesis of indeterminate potential" (CHIP). Although a minority of patients with CHIP subsequently develop MDS, the presence of MDS-associated somatic mutations alone is not considered diagnostic of MDS.

Acute myeloid leukemias (AML)

The WHO continues to define specific AML disease entities by focusing on significant cytogenetic and molecular genetic subgroups. Many recurring, balanced cytogenetic abnormalities are recognized in AML; most that are not formally recognized by the classification are rare (9, 10, 24), often occurring in pediatric patients. Although important to recognize, they do not represent separate disease categories. The realization that the improved prognosis seen in AML with mutated *CEBPA* is only associated with the presence of a biallelic mutation of the gene has modified the disease definition. Additionally, the presence of *NPM1* or biallelic *CEBPA* mutation now supersedes the presence of multilineage dysplasia in the classification. Finally, a provisional category of AML with mutated *RUNX1* has been added for cases of de novo AML with *RAS* mutation that are not associated with MDS-related cytogenetic abnormalities.

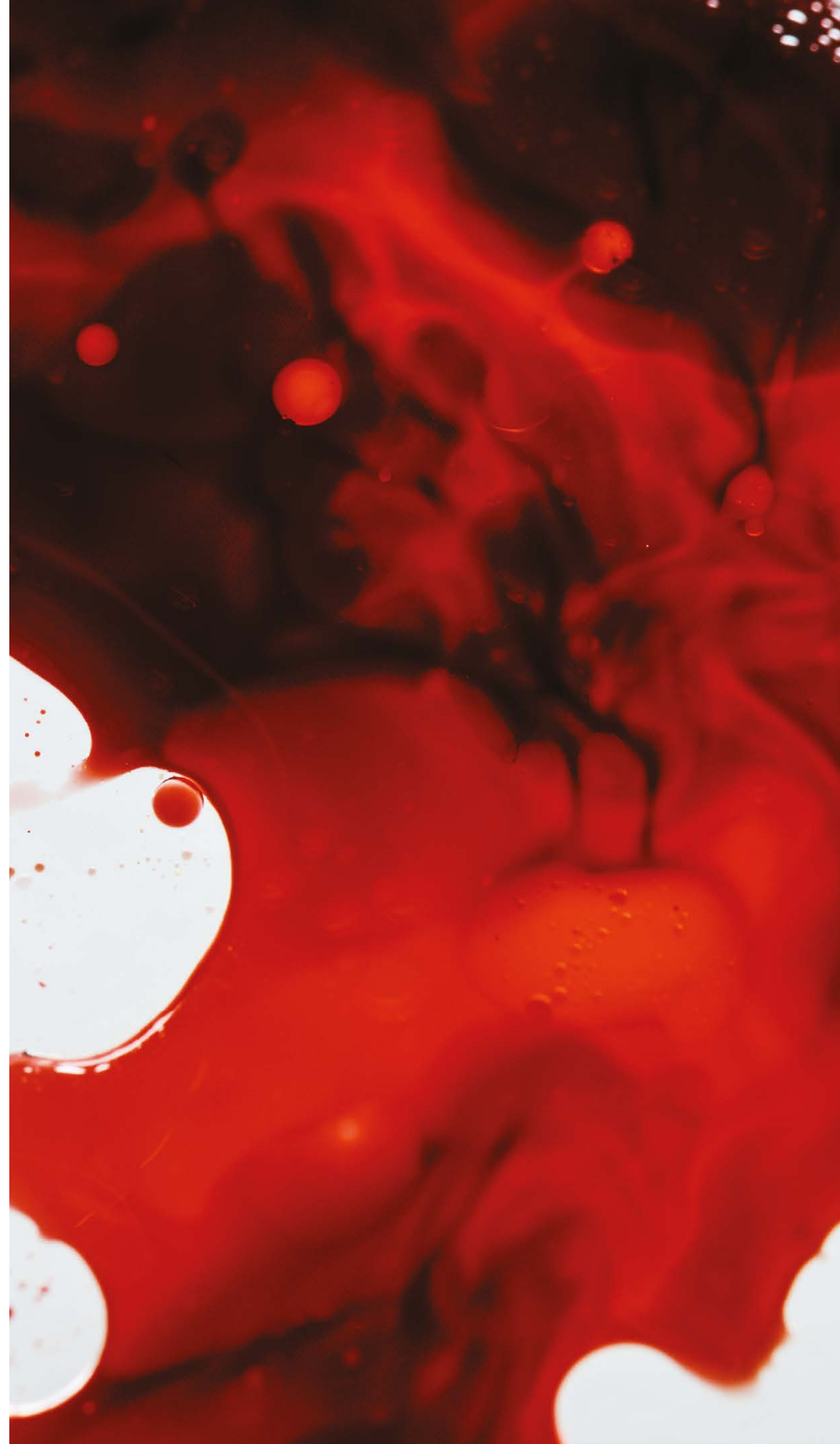
Myeloid neoplasms with germline predisposition

Cases of MDS or acute leukemia can be associated with germline mutations. A major change to the 2016 revision of the WHO classification was the addition of a new section on myeloid neoplasms with germline predisposition, which includes cases of MDS, MDS/ MPN, and AML that occur on the background of those mutations (9, 10). The presence of germline genetic aberrations should indicate a need to screen family members for these aberrations and healthy family members diagnosed with a leukemia predisposition syndrome should be counseled regarding appropriate cancer surveillance (25).

The development of a globally adopted classification system crafted both by hematopathologists and clinicians has been of great benefit to the field of hematologic malignancies in general and of myeloid neoplasm in particular. Its flexible approach allows for the seamless integration of new data into a sound diagnostic scaffold. It remains an effective instrument for daily therapeutic decision-making and clinical trial design and will inform any future classification scheme.

Attilio Orazi is Professor and Chair in the Department of Pathology, Paul L. Foster School of Medicine, Texas Tech University Health Sciences Center, El Paso, Texas, USA.

CLICK HERE FOR REFERENCES



FEATURE

Clonal Cytopenia of Uncertain Significance or Myelodysplastic Syndrome?

The first case in our series on myeloid neoplasms

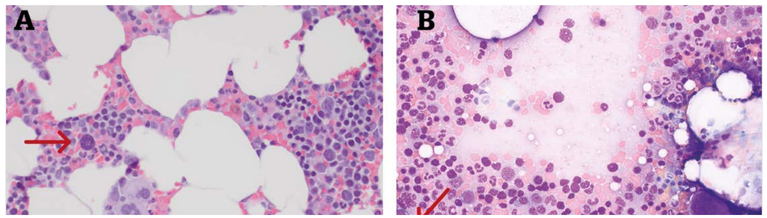
Clinical history

51-year-old man with progressive fatigue and exertional dyspnea.

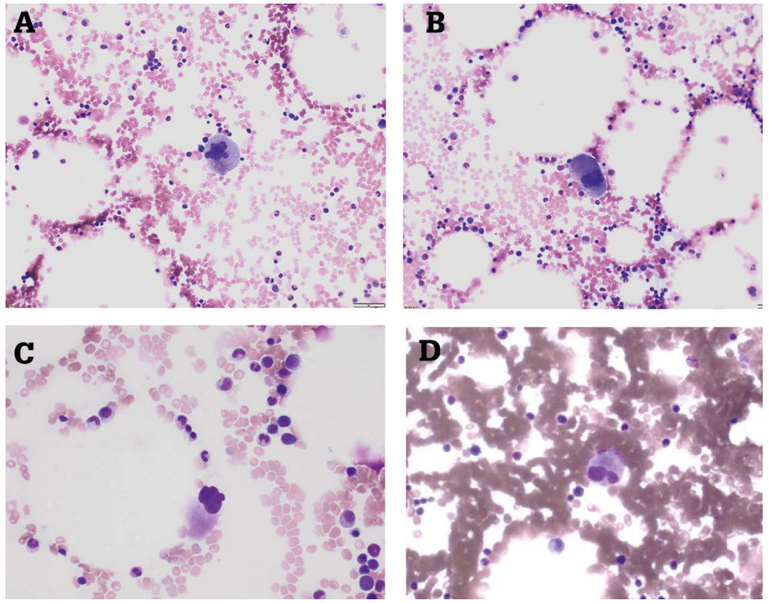
Complete blood count and differential (reference range)

WBC	5.8 x10^9/L (4.0–11.0)
Hgb	9.9 gm/dL (14.0–18.0)
MCV	101 fL (82–98)
Platelets	247 x10^9/L (140–440)
Neutrophils	66.5% (42.0–66.0)
Monocytes	11.9% (2.0–7.0)
Absolute neutrophils	3.85 x10^9/L (1.70–7.30)
Absolute monocytes	0.69 x10^9/L (0.08–0.70)

Bone marrow morphology



Bone marrow is hypercellular for age. Megakaryocytes are variably distributed and include occasional small, hyperchromatic forms (arrow) and other morphologically unremarkable forms (A; H&E 400x). Granulocytes are largely unremarkable in morphology; erythroid precursors show megaloblastoid maturation but no other features of dysplasia; megakaryocytes include occasional small, hyperchromatic forms (B; Giemsa 1000x).

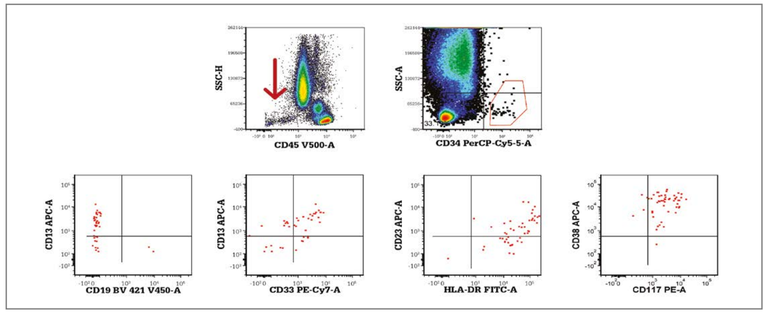


Bone marrow aspirate smears showed numerous morphologically unremarkable megakaryocytes (A–B; Giemsa 1000x), as well as occasional small and variably hyperchromatic forms (C–D; Giemsa 1000x).

Flow cytometry

Mildly decreased side scatter of granulocytes; myeloid progenitors with unremarkable phenotype.

Flow cytometric immunophenotyping of the bone marrow aspirate shows granulocytes with decreased side scatter and a small number of CD34+ myeloid progenitors with unremarkable immunophenotype.



Karyotype

46,XY[20]

Next-generation sequencing

Next-generation sequencing studies showed the following mutations:

Gene	HGVS	VAF (%)
SRSF2	NM_003016.4(SRSF2):c.281_283dupGCC p.R94dup	14
DNMT3A	NM_022552.4(DNMT3A):c.1376del p.K459fs*192	6
SETBP1	NM_015559.2(SETBP1):c.2608G>A p.G870S	2
SETBP1	NM_015559.2(SETBP1):c.2602G>A p.D868N	1
ASXL1	NM_015338.5(ASXL1):c.1283_1284del p.Q428fs*9	22

VAF: variant allele frequency.

Final diagnosis

Variably cellular (40–60 percent) bone marrow showing trilineage hematopoiesis with left-shifted erythropoiesis and granulopoiesis, mild dysmegakaryopoiesis, mild eosinophilia, and mild polytypic plasmacytosis.

ONLINE CASE DISCUSSION

The pathologists' view

Sanam Loghavi is Assistant Professor of Hematopathology, Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA.

Kamran Mirza is Associate Professor and Vice Chair of Education in the Department of Pathology and Laboratory Medicine, Loyola University Chicago Stritch School of Medicine, Maywood, USA.

CLICK HERE TO SEE DISCUSSION

ONLINE CASE DISCUSSION

The hematologist's view

David Steensma is Edward P. Evans Chair in MDS Research and Director, Center for Prevention of Progression (CPOP) of Hematological Malignancies, Division of Hematologic Malignancies, Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts, USA.

CLICK HERE TO SEE DISCUSSION

FEATURE

SF3B1-Mutant Chronic Myelomonocytic Leukemia

The second case in our series on myeloid neoplasms

Clinical history

82-year-old man with progressive exertional dyspnea for one year.

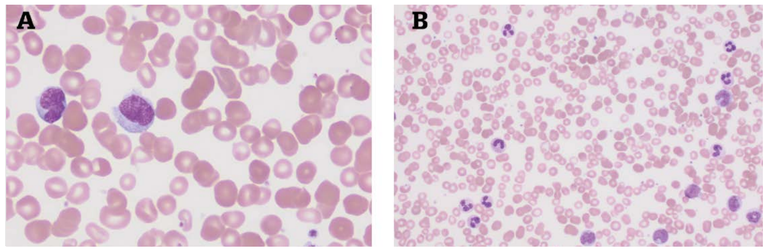
Pertinent physical exam

No hepatosplenomegaly.

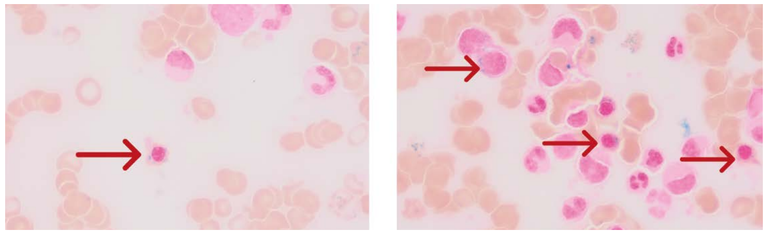
Complete blood count and differential (reference range)

WBC	12.5 x10^9/L (4.0–11.0)
Hgb	8.7 gm/dL (14.0–18.0)
MCV	104 fL (82–98)
Platelets	315 x10^9/L (140–440)
Neutrophils	65.0% (42.0–66.0)
Lymphocytes	14.0% (24.0–44.0)
Monocytes	18.0% (2.0–7.0)
Eosinophils	1.0% (1.0–4.0)
Basophils	1.0% (0.0–1.0)
Metamyelocytes	1.0% (<=0.0)
Absolute neutrophils	8.13 x10^9/L (1.70–7.30)
Absolute lymphocytes	1.75 x10^9/L (1.00–4.80)
Absolute monocytes	2.25 x10^9/L (0.08–0.70)

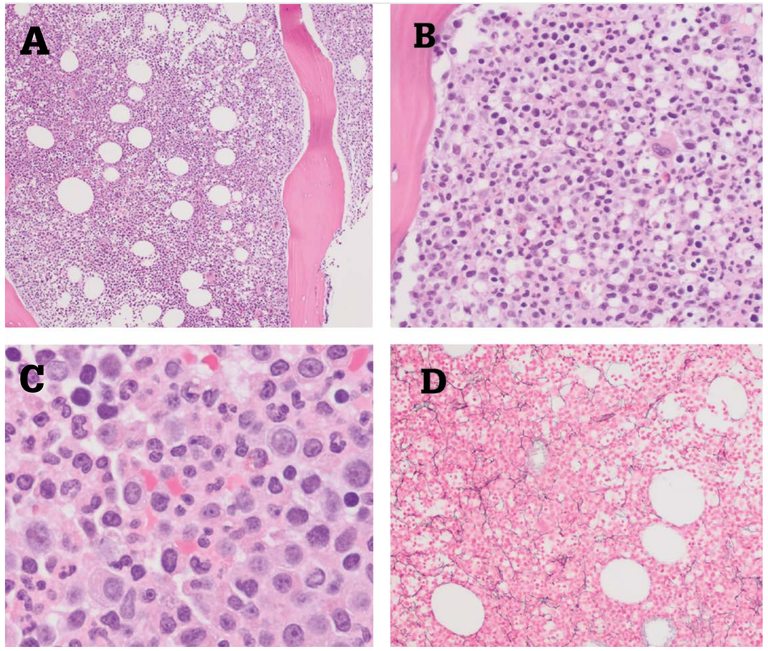
Bone marrow morphology



Peripheral blood smear shows macrocytic anemia with leukocytosis and absolute monocytosis. Monocytes are morphologically atypical and include increased immature forms (promonocytes) (Giemsa; A 200x, B 400x).

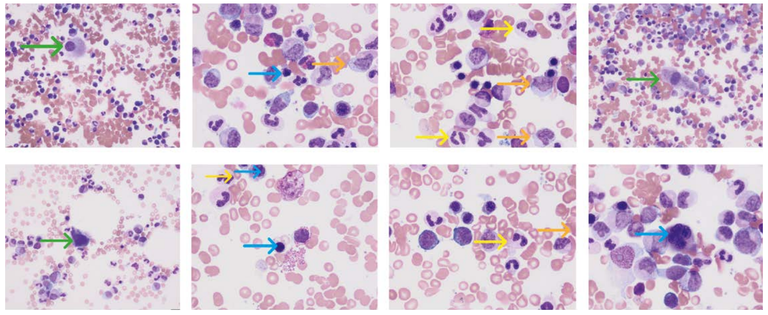


An iron stain performed on the bone marrow aspirate smear shows increased ring sideroblasts (red arrows) (Prussian blue 1000x).



The bone marrow core biopsy is hypercellular for age (A; H&E 100x). There is myelomonocytic hyperplasia; megakaryocytes are small and dysplastic (B; H&E 200x). Erythroid precursors include increased left-shifted forms (C; H&E 400x). A reticulin stain shows mild reticulin fibrosis (D; 100x).

The bone marrow aspirate smears show trilineage dysplasia. Erythroid dysplasia (blue arrows) is characterized by nuclear contour irregularities, basophilic stippling of cytoplasm, and asynchronous maturation of cytoplasm and nucleus. Dysmegakaryopoiesis (green arrows) is manifested by increased small, hypolobated forms. Granulocytes (yellow arrows) are also dysplastic with increased hypolobated and hypogranular forms.



Monocytes and monocytic precursors (orange arrows) are increased.

Karyotype

Routine cytogenetic studies show an abnormal male karyotype – 46,XY,del(12)(p12)[19]/46,XY[1]

Next-generation sequencing

Next-generation sequencing studies showed the following mutations: VAF: variant allele frequency.

Gene	HGVS	VAF (%)
CSF3R	NM_156039.3(CSF3R):c.1853C>T p.T618I	2
SETBP1	NM_015559.2(SETBP1):c.2602G>A p.D868N	4
RUNX1	NM_001754.4(RUNX1):c.964_965del p.S322fs*277	22
ASXL1	NM_015338.5(ASXL1):c.1934dupG p.G646fs*11	32
SRSF2	NM_003016.4(SRSF2):c.284C>T p.P95L	34
SF3B1	NM_012433.2(SF3B1):c.1998G>C p.K666N	40

Final diagnosis

SF3B1-mutant chronic myelomonocytic leukemia.

ONLINE CASE DISCUSSION

The pathologists' view

Sanam Loghavi is Assistant Professor of Hematopathology, Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA.

Kamran Mirza is Associate Professor and Vice Chair of Education in the Department of Pathology and Laboratory Medicine, Loyola University Chicago Stritch School of Medicine, Maywood, USA.

CLICK HERE TO SEE DISCUSSION

ONLINE CASE DISCUSSION

The hematologist's view

Mrinal M. Patnaik is Associate Professor of Internal Medicine, Chair of the Acute Leukemia and Myeloid Malignancies Group, and Co-Director of the Epigenetics Developmental Laboratory, Mayo Clinic, Rochester, Minnesota, USA.

CLICK HERE TO SEE DISCUSSION

FEATURE

Myelodysplastic Syndrome with Excess Blasts and Fibrosis

The third case in our series on myeloid neoplasms

Clinical history

68-year-old woman with progressive fatigue and exertional dyspnea.

Pertinent physical exam

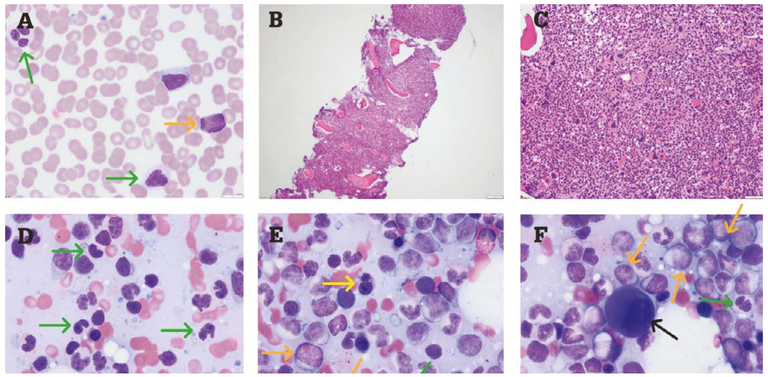
Mild splenomegaly.

WBC	7.4 x10^9/L (4.0–11.0)
Hgb	7.3 gm/dL (14.0–18.0)
Platelets	52 x10^9/L (140–440)
Absolute neutrophils	4.22 x10^9/L (1.70–7.30)
MCV	98 fL (82–98)
Absolute monocytes	2.25 x10^9/L (0.08–0.70)

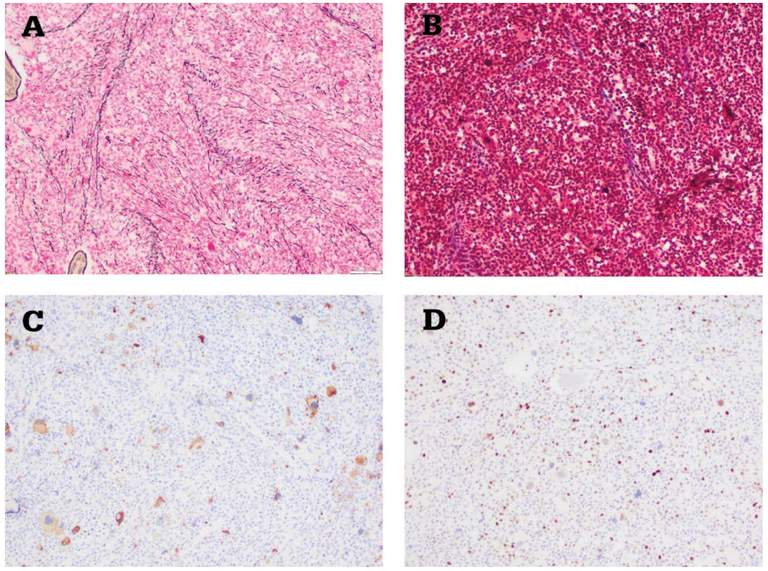
Complete blood count and differential (reference range)
Serum lactate dehydrogenase

497 U/L (135–214)

Peripheral blood and bone marrow morphology



Peripheral blood smear shows anemia, thrombocytopenia, dysplastic granulocytes (green), and occasional circulating blasts (orange) (A; Giemsa 1000x). Bone marrow core biopsy is markedly hypercellular (B; H&E 20x). Megakaryocytes are increased and variably distributed without significant clustering. They are frequently dysplastic, characterized by small size, nuclear hypolobation, and hyperchromasia (C; H&E 200x). Bone marrow aspirate smears show dysplastic (hypogranular, hypolobated) granulocytes; dysplastic erythroid precursors with nuclear budding (yellow); small, dysplastic megakaryocytes (black); and increased blasts (D–F; Giemsa 1000x).



The bone marrow core biopsy shows dense reticulin fibrosis forming intersections (A; reticulin stain 200x) and scattered fine bundles of collagen (B; Masson Trichrome 200x). A CD61 stain highlights increased dysplastic megakaryocytes and a p53 stain shows aberrant overexpression with moderate-to-bright nuclear staining in a subset of cells, including some megakaryocytes (C and D, respectively; immunohistochemistry with hematoxylin counterstain 200x).

Karyotype

46,XX,der(3)del(3)(p21p23)inv(3)(p21q27),del(5)(q13q33),del(11)(q22q24)[15]/46,XX[5]

Gene	HGVS	VAF (%)
TET2	NM_001127208.2(TET2):c.1270del p.S424fs*3	45
TP53	NM_000546.5(TP53):c.578A>T p.H193L	37
JAK2	NM_004972.3(JAK2):c.1849G>T p.V617F	6
ASXL1	NM_015338.5(ASXL1):c.3076G>A p.G1026R	4

Next-generation sequencing

Next-generation sequencing studies showed the following mutations:

VAF: variant allele frequency.

Final diagnosis

Myelodysplastic syndrome with excess blasts-2 and fibrosis.

ONLINE CASE DISCUSSION

The pathologists' view

Sanam Loghavi is Assistant Professor of Hematopathology, Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA.

Kamran Mirza is Associate Professor and Vice Chair of Education in the Department of Pathology and Laboratory Medicine, Loyola University Chicago Stritch School of Medicine, Maywood, USA.

CLICK HERE TO SEE DISCUSSION

ONLINE CASE DISCUSSION

The hematologist's view

Mikkael Sekeres is Chief of the Division of Hematology at Sylvester Comprehensive Cancer Center, University of Miami, Miami, Florida, USA.

CLICK HERE TO SEE DISCUSSION

FEATURE

Elevating the Treatment Standard in Older Patients with AML

The final case in our series on myeloid neoplasms

Clinical history

72-year-old woman with a history of chronic renal disease and coronary artery disease, referred to our institution with newly diagnosed acute myeloid leukemia.

Complete blood count and differential (reference range)

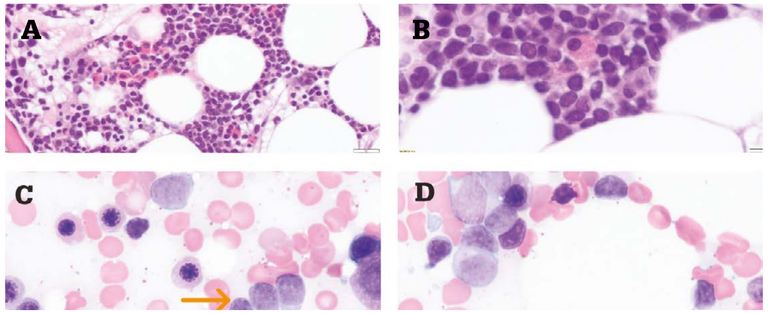
WBC	1.5 x10^9/L (4.0–11.0)
Hgb	12 gm/dL (14.0–18.0)
MCV	102 fL (82–98)
Platelets	155 x10^9/L (140–440)
Absolute neutrophils	0.69 x10^9/L (1.70–7.30)

Bone marrow morphology

The bone marrow core biopsy is hypercellular for age (A; H&E 200x) and shows clusters of immature-appearing mononuclear cells (B; H&E 1000x). The bone marrow touch imprint shows increased blasts. Blasts are small to intermediate in size with predominantly smooth nuclear contours, finely dispersed chromatin, and variably conspicuous nucleoli (orange arrows) (C,D; Giemsa 1000x).

Cytochemical stains

Blasts were negative for myeloperoxidase.



Flow cytometry immunophenotyping

Aberrant myeloid blasts (8%) positive for CD13, CD33, CD34, CD38, CD117, and CD123; negative for cytoCD3, CD7, CD19, HLA-DR, and MPO.

Karyotype

Routine cytogenetic studies show an abnormal female karyotype – 47,XX,+8[6]/46,XX[14]

Next-generation sequencing

Next-generation sequencing studies showed the following mutations:

Gene	HGVS	VAF (%)
RUNX1	NM_001754.4(RUNX1):c.1036dupC p.R346fs*254	2
IDH2	NM_002168.2(IDH2):c.515G>A p.R172K	3

VAF: variant allele frequency.

Final diagnosis

Acute myeloid leukemia with minimal differentiation.

ONLINE CASE DISCUSSION

The pathologists' view

Sanam Loghavi is Assistant Professor of Hematopathology, Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA.

Kamran Mirza is Associate Professor and Vice Chair of Education in the Department of Pathology and Laboratory Medicine, Loyola University Chicago Stritch School of Medicine, Maywood, USA.

CLICK HERE TO SEE DISCUSSION

ONLINE CASE DISCUSSION

The hematologists' view

Curtis Lachowiez is Hematology-oncology fellow in the Division of Cancer Medicine, MD Anderson Cancer Center, Houston, Texas, USA.

Courtney D. DiNardo is Associate Professor in the Department of Leukemia, Division of Cancer Medicine, MD Anderson Cancer Center, Houston, Texas, USA.

CLICK HERE TO SEE DISCUSSION

INTRODUCTION

Lymphoid Neoplasms

An introduction and brief historical perspective

In 1832, Thomas Hodgkin described seven patients with similar disease findings involving lymph nodes and spleen. Hodgkin made his observations on autopsy patient specimens and never used a microscope. Three decades later, Sir Samuel Wilks reported 15 similar cases, recognized the earlier work of Hodgkin, and coined the eponym "Hodgkin's disease" (now Hodgkin lymphoma). It subsequently became apparent that there are many types of lymphoma in addition to Hodgkin lymphoma, and these neoplasms became known as non-Hodgkin lymphomas.

Technological advances, in large part, facilitated insights into lymphomas. For almost 150 years after the study by Hodgkin, light microscopic evaluation was the principal technology employed. Pathologists compared lymphomas to normal lymph nodes and began to recognize entities. Patterns and cellular features were correlated with clinical features. We recognized that lymphomas composed of large cells were associated with aggressive clinical behavior; most composed of small cells were clinically indolent. Cases with a nodular pattern resembled germinal centers of lymphoid follicles. Various morphologic classification systems were proposed, most of which separated Hodgkin from non-Hodgkin lymphomas and then further subclassified these groups.

New technologies led to seminal discoveries that changed our understanding of lymphocytes and lymphoma classification:

- Lymphocyte lineages were discovered. This began with Glick and colleagues' recognition of B cells, followed by Miller and others' identification of T lymphocytes and Klein and others' discovery of NK cells.
- Lymphocytes – previously thought to be terminally differentiated cells – were shown to respond to antigens or mitogens by transforming into larger proliferating cells.
- Lymphocytes were discovered to have surface antigens we can exploit to identify normal and neoplastic cells – indicating their utility in diagnosing and classifying lymphomas. Simultaneous technological advances in immunophenotyping methods facilitated the characterization of lymphoid cells. Kohler and Milstein's discovery of hybridoma technology led to widespread availability of monoclonal antibodies.

These immunologic insights led to the proposal of two lymphoma classifications in 1974: the Kiel classification and the Lukes-Collins classification. For the remainder of the decade, multiple immunology-based and morphology-based lymphoma classifications competed. The situation, confusing for clinicians and pathologists alike, led to a National Cancer Institute-sponsored study in 1982 that compared

all six classifications using morphology and outcomes. All systems rendered diagnostic categories that could broadly divide non-Hodgkin lymphomas into prognostic groups: low-grade indolent neoplasms (indolent), intermediate-grade neoplasms (aggressive), and high-grade neoplasms (very aggressive). A Working Formulation was proposed to serve as a common language for translating between systems. Although it became popular in the United States and functioned as a de facto classification, Europe and many other nations continued to use the Kiel system. A stalemate of sorts set in.

Alongside advances in immunology, our ability to study human chromosomes blossomed. Fluorescent dyes that facilitated chromosome banding allowed improved recognition of chromosomal abnormalities in lymphomas. Chromosome 8q24 translocations were linked to Burkitt lymphoma, t(14;18)(q32;q21) to follicular lymphoma, t(11;14)(q13;q32) to mantle cell lymphoma, and t(2;5)(q23;q35) to anaplastic large cell lymphoma.

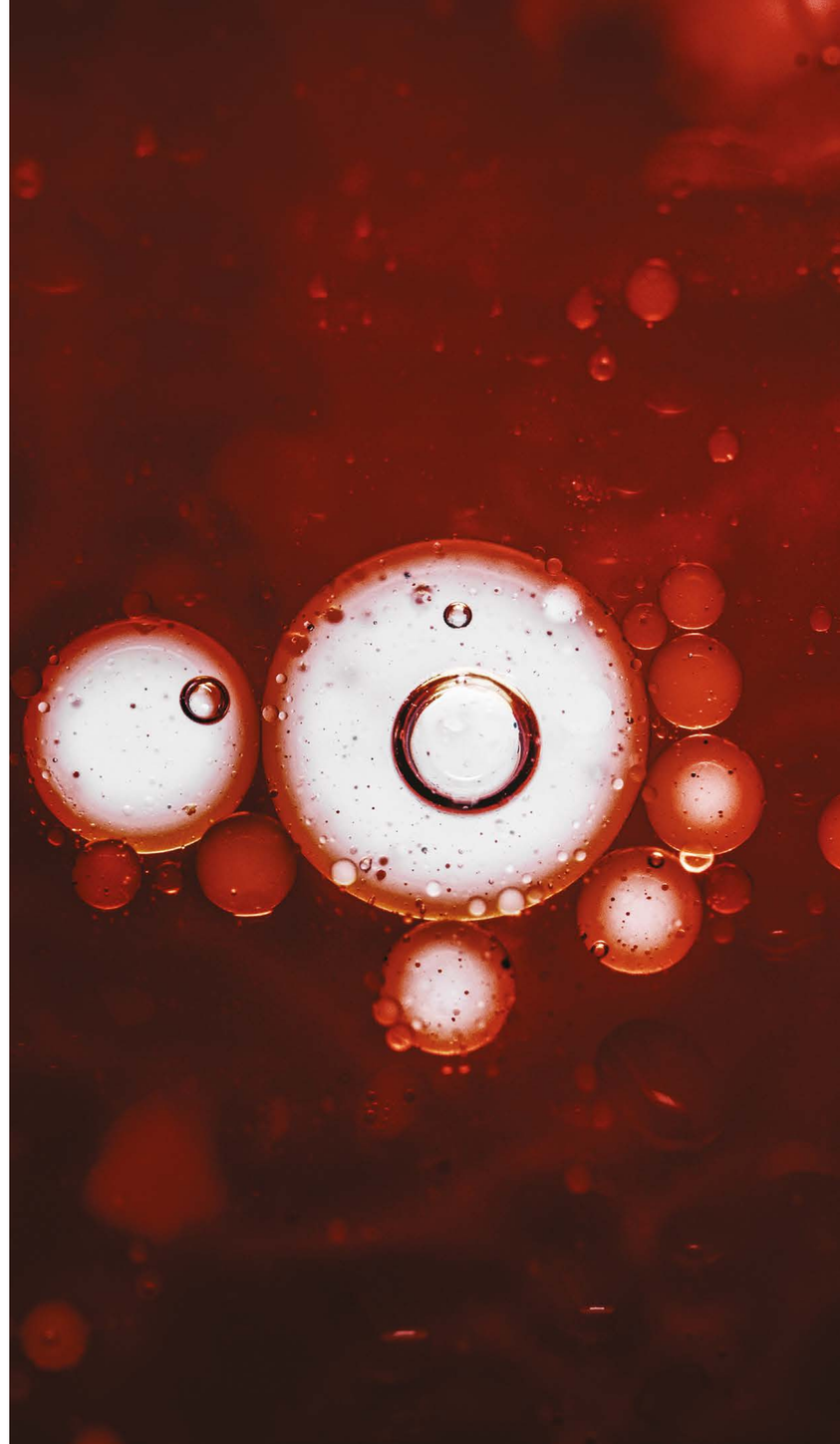
The field of genomics began to emerge with Nathans' 1971 discovery of restriction endonucleases, which allowed researchers to cut and manipulate DNA fragments. Subsequent application of molecular methods to the study of lymphomas yielded many insights. These methods enabled the cloning and characterization of oncogenes and tumor suppressor genes involved in various chromosome abnormalities. For example, *MYC* was identified at chromosome 8q24, *BCL2* at chromosome 18q21, *CCND1* at chromosome 11q13, and *NPM1-ALK* was shown to be a fusion gene created by t(2;5). This type of information greatly enhanced our understanding of lymphomas, and it didn't take long for the findings to be incorporated into lymphoma diagnosis, patient risk stratification, and prognostication. However, they were not incorporated in a consensus manner… and the stage was set for a new attempt at lymphoma classification.

In 1994, Nancy Harris and colleagues proposed the Revised European-American Lymphoma (REAL) classification, which used a multiparametric approach to lymphoma diagnosis by employing clinical data, morphology, immunophenotype, genetic information, and presumed cell of origin. The REAL classification was the foundation of the third edition of the WHO classification (2001), and the subsequent fourth edition (2008) and revision (2017). As part of the WHO classification effort, pathologists and clinicians worked collaboratively to develop a consensus classification of lymphomas, each more detailed and granular than the last. The current WHO classification is accepted internationally as a consensus classification for lymphoma diagnosis and as a tool to facilitate lymphoma discovery.

The completed human genome heralded the advent of high-throughput genomic testing to interrogate these genes in various cancers. In lymphomas, gene expression profiling was used for both discovery and classification. The results provided possible targets for therapy and showed the heterogeneity of a number of disease categories defined in the WHO classification. Then came next-generation sequencing of lymphomas. Targeted sequencing using gene panels has shown their molecular landscapes at the DNA and RNA level as never seen before. Molecular pathways critical for lymphomagenesis have been recognized and drugs that specifically target molecular abnormalities are being developed or are in clinical trials. Some novel agents are already approved, and many more will follow. Our understanding of lymphomas has never been greater, and the prospects for future targeted therapy that is more effective and less toxic have never been brighter.

L. Jeffrey Medeiros is Professor and Chair in the Department of Hematopathology, MD Anderson Cancer Center, Houston, Texas, USA.

CLICK HERE FOR REFERENCES



FEATURE

Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma – Accelerated Phase

The first case in our series on lymphoid neoplasms

Clinical history

67-year-old woman presented with bilateral neck swelling.

Imaging

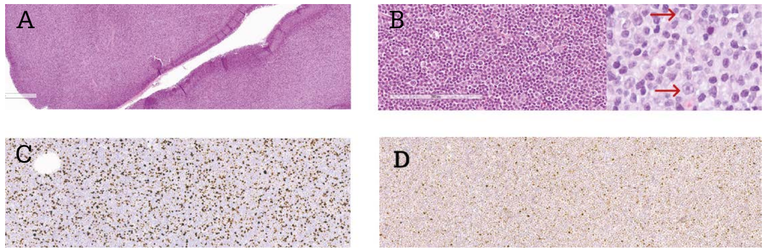
Multicompartmental lymphadenopathy above and below the diaphragm.

Complete blood count and differential (reference range)

WBC	9.3 x10^9/L (4.0–11.0)
Hgb	13.3 gm/dL (14.0–18.0)
MCV	101 fL (82–98)
Platelets	247 x10^9/L (140–440)
Absolute lymphocytes	6.32 x10^9/L (1.00–4.80)
Absolute neutrophils	2.23 x10^9/L (1.70–7.30)

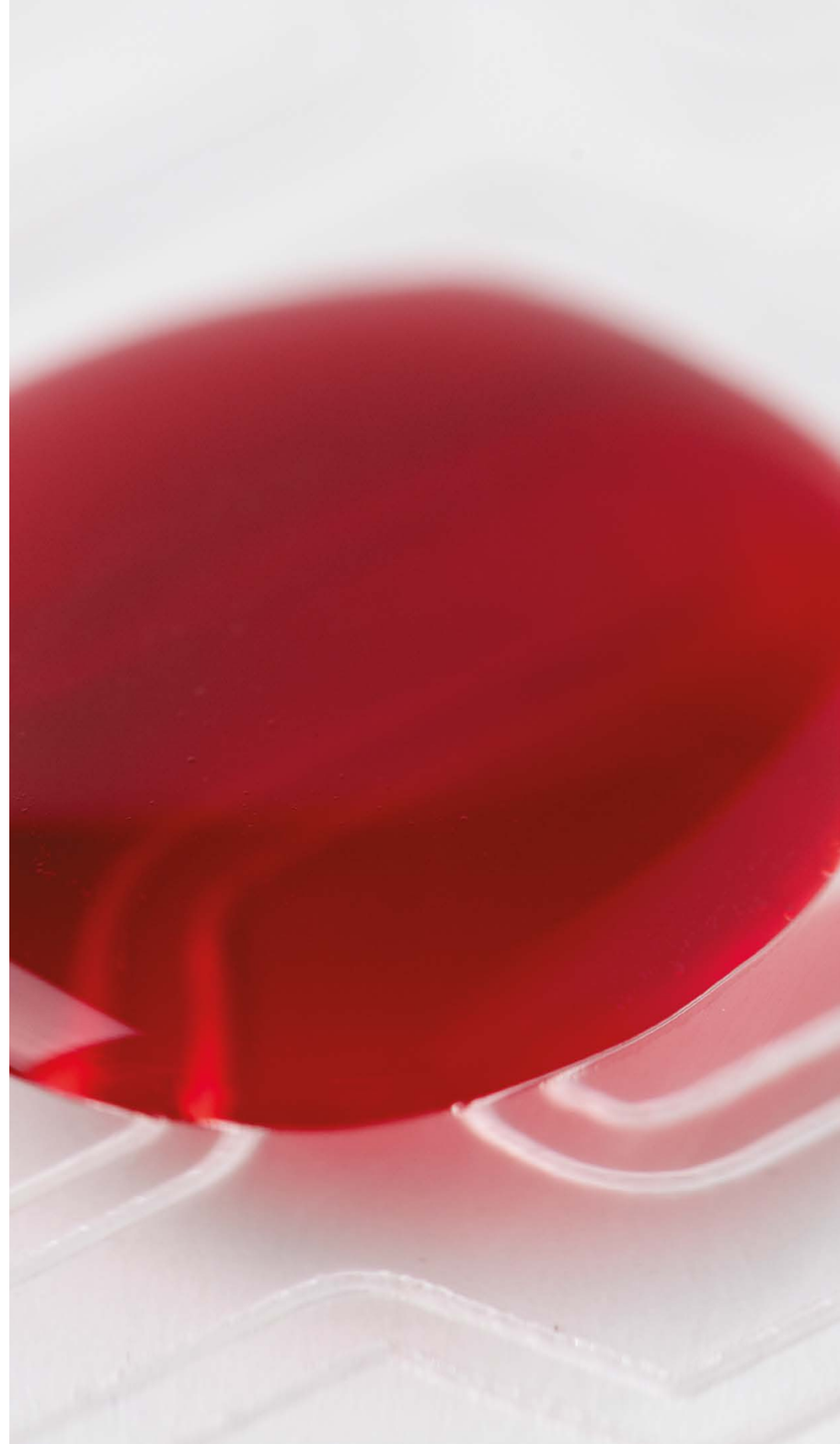
Additional laboratory results

Serum lactate dehydrogenase	358 U/L (135–214)
Serum beta 2 microglobulin	2.6 U/L (0.8–2.3)



Lymph node morphology

Histologic examination of a left cervical lymph node shows near-total replacement of the normal architecture by lymphoma. At low power (A; H&E 10x), large, vague, pale nodules are identified, consistent with proliferation centers. In some areas, these proliferation centers appear to be beginning to fuse with one another. On higher-power examination (B; H&E 200x, inset 1000x), there are increased paraimmunoblasts (red arrows) and prolymphocytes; however, there are no sheets of large cells to indicate histologic transformation to diffuse large B cell lymphoma. The Ki-67 proliferation index is increased; there is weak expression of p53 protein in the small lymphoid cells and stronger (aberrant) nuclear staining in the larger neoplastic cells (C and D, respectively, immunohistochemistry with hematoxylin counterstain).



Immunohistochemical characterization of neoplastic lymphoid cells

Gene	HGVS	VAF (%)
NOTCH1	NM_017617.3(NOTCH1):c.7541_7542del p.P2514fs*4	20
BIRC3	NM_001165.4(BIRC3):c.1654del p.Q552fs*16	4

VAF: variant allele frequency.

Examination of bone marrow showed extensive involvement by a small B cell neoplasm. Aberrant B cells represented 63 percent of all cells by flow cytometry and were positive for CD5, CD23, and CD200.

Fluorescence in situ hybridization performed on bone marrow

Positive for +12 and del(13q) in 41 percent and 2.2 percent of analyzed interphases, respectively. The *TP53* locus was intact.

DNA sequencing

DNA sequencing studies performed on a bone marrow sample showed unmutated *IGHV*.

Next-generation sequencing

Next-generation sequencing performed on a bone marrow sample showed mutation involving *NOTCH1* and *BIRC3*. *TP53* was wild-type.

CD20	Positive
CD23	Positive
CD5	Positive
Ki-67	Positive in ~25% of cells
p53	Weakly positive in ~50% of cells
Cyclin D1	Negative

Final diagnosis

Chronic lymphocytic leukemia/small lymphocytic lymphoma with increased large cells and proliferation rate (25 percent) suggestive of accelerated phase.

ONLINE CASE DISCUSSION

The pathologists' view

Sanam Loghavi is Assistant Professor of Hematopathology, Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA.

Kamran Mirza is Associate Professor and Vice Chair of Education in the Department of Pathology and Laboratory Medicine, Loyola University Chicago Stritch School of Medicine, Maywood, USA.

CLICK HERE TO SEE DISCUSSION

ONLINE CASE DISCUSSION

The hematologists' view

Amy Wang is Hematology-oncology fellow at the University of Chicago, Chicago, Illinois, USA.

Sonali M. Smith is Elwood V. Jensen Professor in Medicine, Chief of the Section of Hematology/Oncology, and Director of the Lymphoma Program at the University of Chicago, Chicago, Illinois, USA.

CLICK HERE TO SEE DISCUSSION

FEATURE

Lymphoma-Driven Hemophagocytic Lymphohistiocytosis

The second case in our series on lymphoid neoplasms

Clinical history

21-year-old man with a two-month history of worsening fatigue and fever. He was transferred to our hospital from an outside institution for acute-onset renal failure and suspected tumor lysis syndrome.

Pertinent physical exam

Temperature: 103.2°F; hepatosplenomegaly.

Complete blood count and differential (reference range)

WBC	1.9 x10^9/L (4.0–11.0)
Hgb	7.5 gm/dL (14.0–18.0)
Hct	22.4% (40.0–54.0)
MCV	79 fL (82–98)
MCH	26.4 pg (27.0–31.0)
Platelets	71 x10^9/L (140–440)
Absolute lymphocytes	0.1 x10^9/L (1.00–4.80)
Absolute neutrophils	1.71 x10^9/L (1.70–7.30)

Additional laboratory results

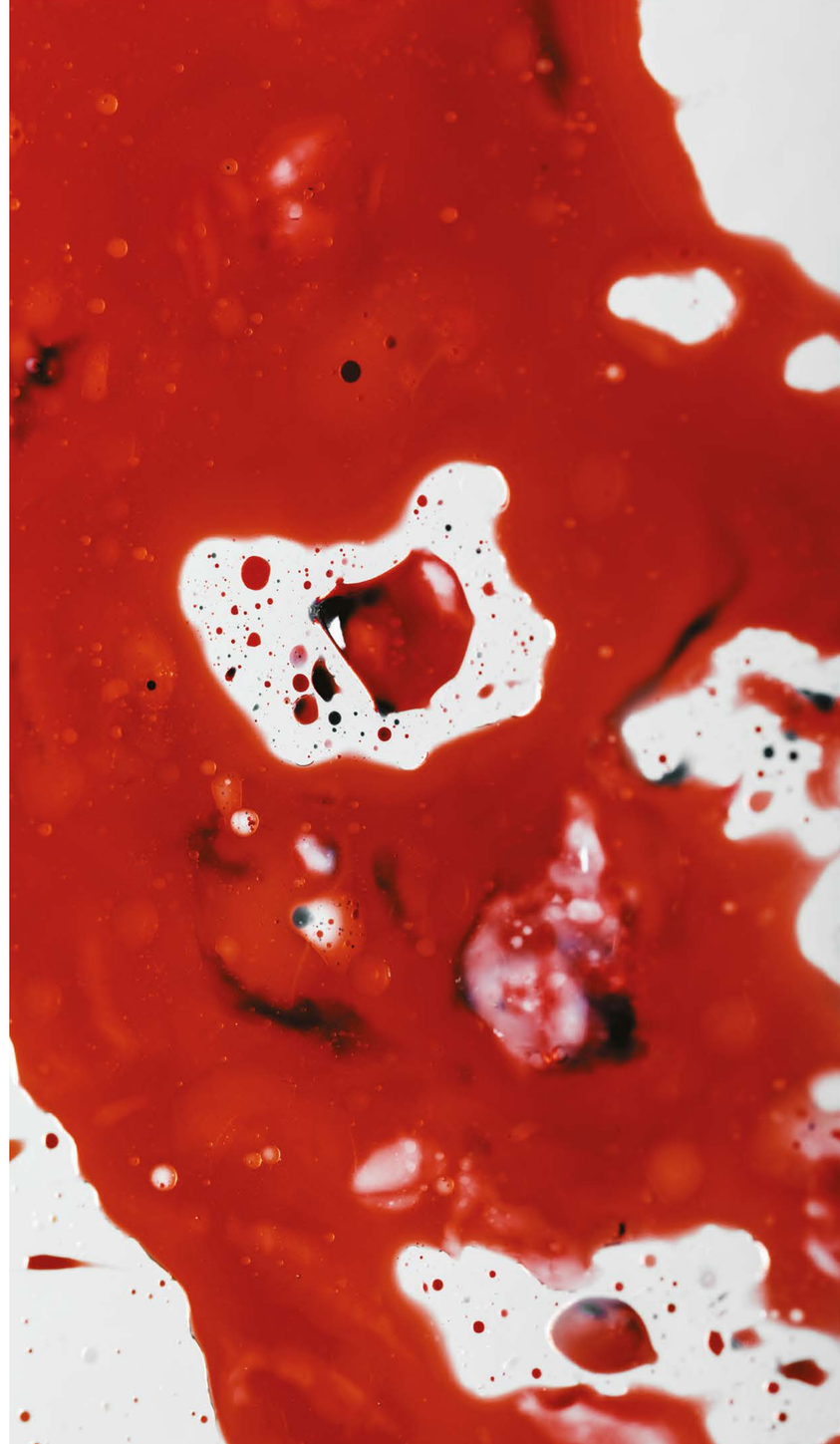
ALT	108 U/L (≤41)
AST	93 U/L (≤40)
Ferritin	11,484 ng/mL (30–400)
LDH	292 U/L (135–225)
Creatinine	4.88 mg/dL (0.67–1.17)
Triglycerides	205 mg/dL (≤149)

PET-CT

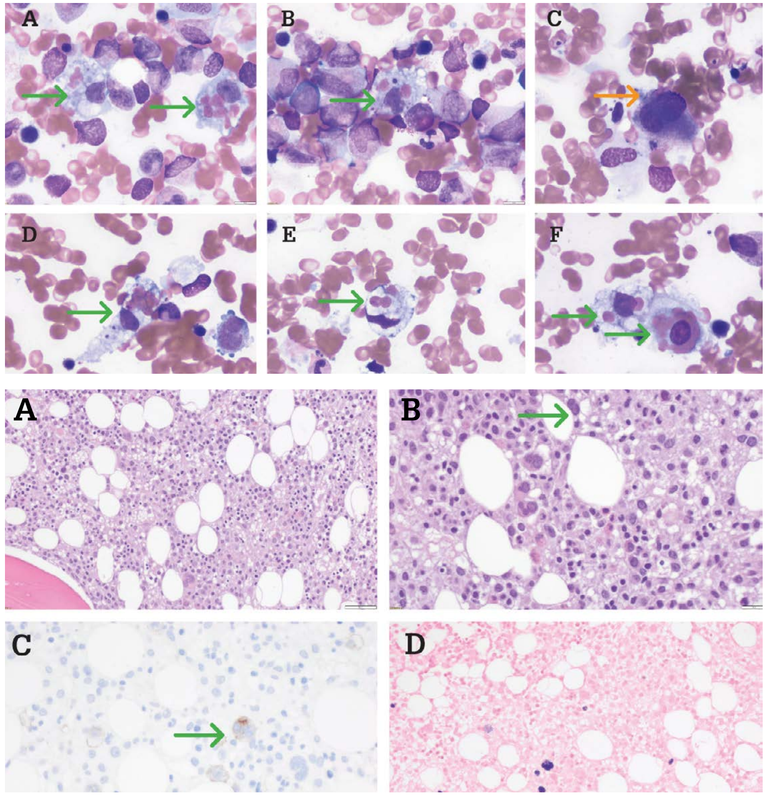
FDG-avid multicompartmental lymphadenopathy above and below the diaphragm. Heterogeneously increased activity within the spleen and several foci of activity within the skeleton.

Bone marrow morphology

Bone marrow aspirate smears show numerous histiocytes exhibiting hemophagocytosis (green arrows). Megakaryocytes include increased small, hypolobated forms; an example is highlighted by the orange arrow.

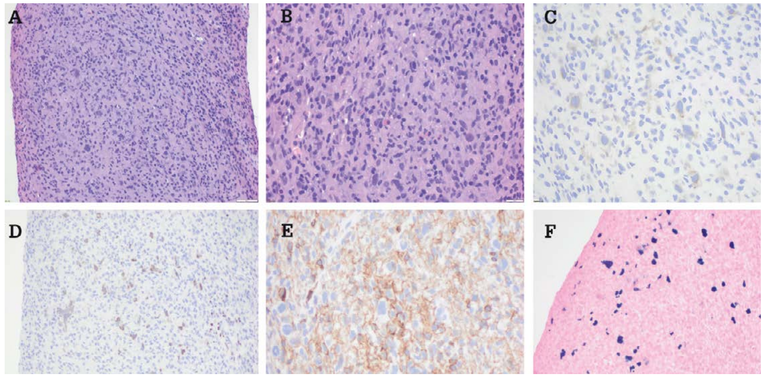


Granulocytes show left-shifted maturation; erythroid precursors were unremarkable in morphology (A–F; Giemsa 1000x).



The bone marrow core biopsy shows an extensive infiltrate of histiocytes and a few scattered larger cells (green arrows). The differential morphologic consideration for the larger cells includes dysplastic megakaryocytes and possibly Hodgkin cells. Of note, the typical stromal reaction and inflammatory infiltrate usually associated with Hodgkin lymphoma is lacking. (A–B; H&E 100x and 200x). A CD30 immunohistochemical stain is weakly positive in a few plasma cells as well as a large, atypical cell (green arrow) (C; immunohistochemistry with hematoxylin counterstain). In situ hybridization for EBV-encoded RNA highlights a few EBER-positive cells (D).

Lymph node biopsy



Core biopsy of the pelvic lymph node shows complete replacement of the nodal architecture by a proliferation of large atypical cells (A; H&E 100x) on a background of histiocytes and rare eosinophils (B; H&E 400x). The large cells are weakly positive for CD30 and PAX5 and negative for CD45/LCA (C–E, respectively; immunohistochemistry with hematoxylin counterstain) and positive for EBV-encoded RNA (EBER) by in situ hybridization (F).

Final diagnosis

Acute myeloid leukemia with minimal differentiation.

ONLINE CASE DISCUSSION

The pathologists' view

Sanam Loghavi is Assistant Professor of Hematopathology, Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA.

Kamran Mirza is Associate Professor and Vice Chair of Education in the Department of Pathology and Laboratory Medicine, Loyola University Chicago Stritch School of Medicine, Maywood, USA.

CLICK HERE TO SEE DISCUSSION

ONLINE CASE DISCUSSION

The hematologist's view

Paolo Strati is Assistant Professor in the Department of Lymphoma and Myeloma, Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA.

CLICK HERE TO SEE DISCUSSION

FEATURE

High-Grade B Cell Lymphoma with Leukemic Presentation

The third case in our series on lymphoid neoplasms

Clinical history

42-year-old man with no prior medical history had emergent neurosurgical decompression of a cervical spinal tumor after acute onset of paraplegia. At presentation, he was noted to have marked leukocytosis and was transferred to our institution for treatment of suspected acute leukemia.

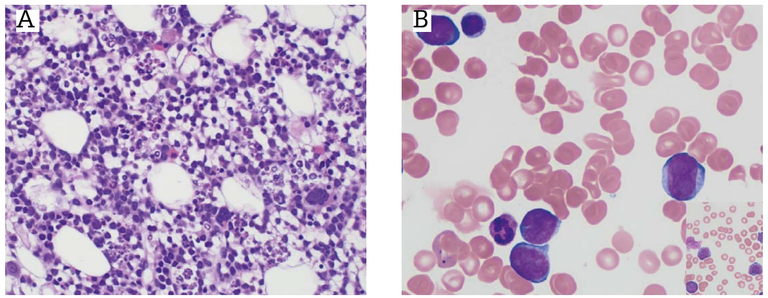
Imaging

No lymphadenopathy or focal PET+ lesions other than the cervical spine lesion.

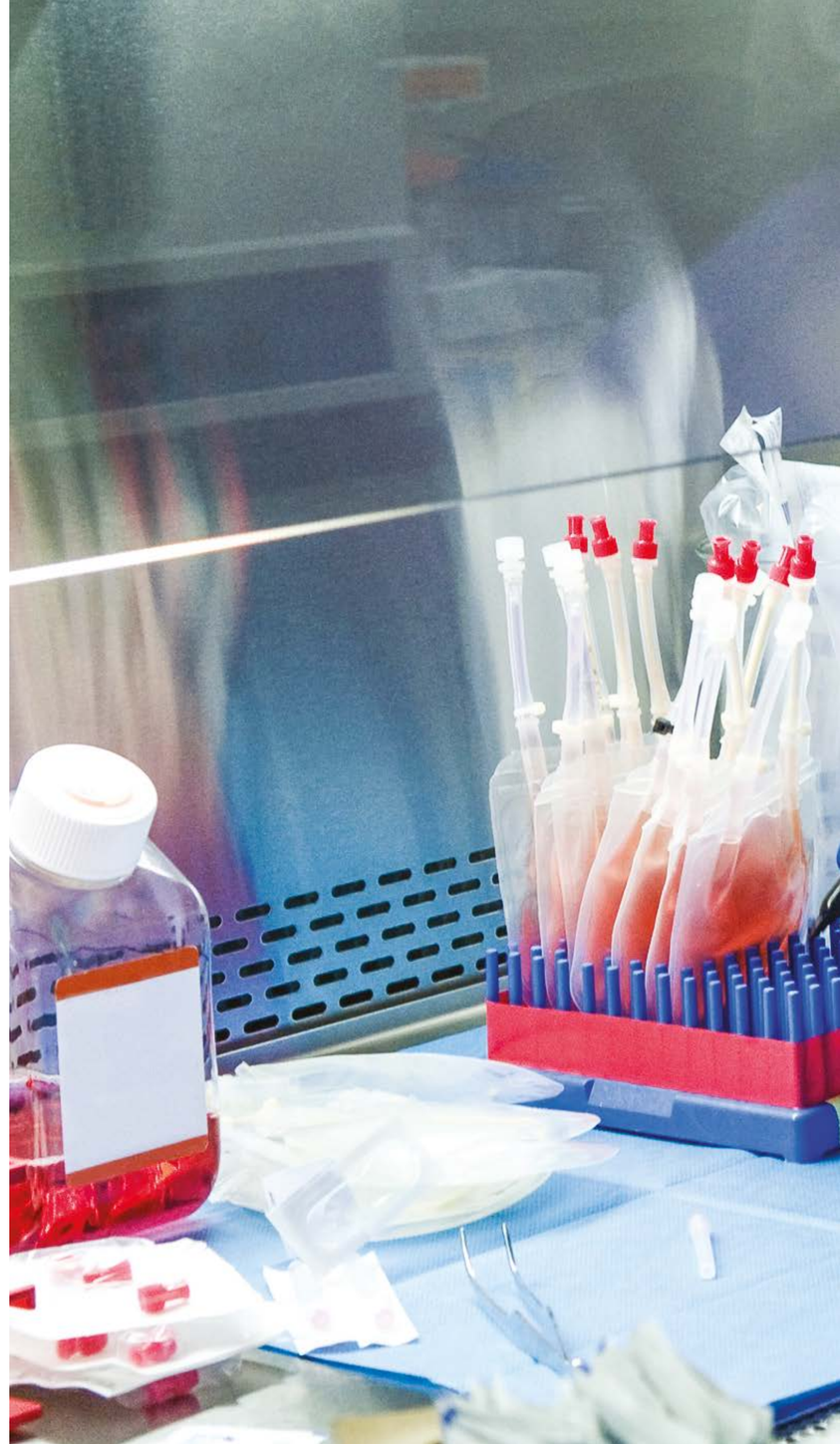
Complete blood count and differential (reference range)

WBC	287.4 x10^9/L (4.0–11.0)
Hgb	7.5 gm/dL (14.0–18.0)
MCV	90 fL (82–98)
Platelets	67 x10^9/L (140–440)
"Blasts"	94.0% (<=0.0)
Lymphocytes	2% (24.0–44.0)
Neutrophils	3.0% (42.0–66.0)
Metamyelocytes	1.0% (<=0.0)

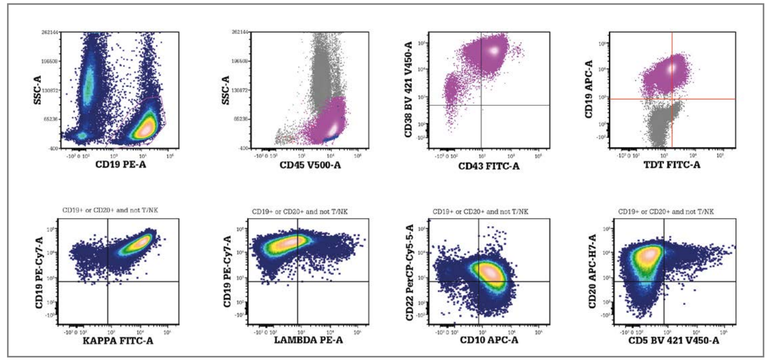
Peripheral blood and bone marrow morphology



Bone marrow trephine biopsy shows extensive replacement of normal hematopoietic elements by an infiltrate of immature-appearing mononuclear cells with an interstitial pattern of involvement. Apoptotic cells are readily identifiable (A; H&E 400x). The bone marrow aspirate smear shows numerous large, atypical mononuclear cells with scant basophilic cytoplasm, finely dispersed nuclear chromatin, and prominent nucleoli (B; Giemsa 1000x). Examination of the peripheral blood smear shows numerous circulating cells similar to those observed in the bone marrow aspirate smear (B, inset; Giemsa 1000x). Images courtesy of Chi Young Ok, MD Anderson Cancer Center.



Flow cytometry

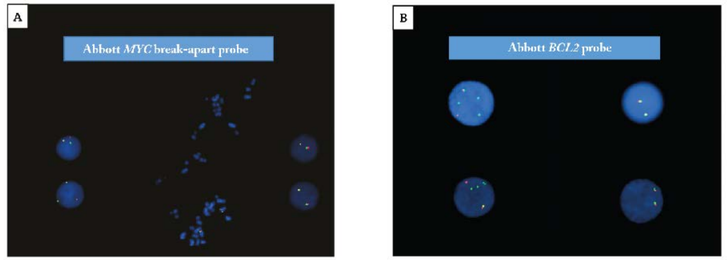


Flow cytometric immunophenotyping of peripheral blood samples shows an expanded population of CD19+ cells. Backgating the CD19+ population (pink gate) shows the CD19+ events with bright CD45 expression. The CD19+ cells show bright CD38 and dim TdT expression (top panel). The population of interest shows monotypic expression of surface kappa light chain, bright expression of CD20, dim expression of CD22, and is positive for CD10 and negative for CD5, supporting germinal center B cell derivation (bottom panel).

Immunohistochemical characterization of neoplastic lymphoid cells

CD20	Positive
BCL2	Positive
MYC	Positive for strong overexpression (3+ staining in >90% of cells)
p53	Aberrantly overexpressed
TdT	Negative

Fluorescence in situ hybridization performed on bone marrow



Fluorescence in situ hybridization performed on the bone marrow aspirate sample showed rearrangement of *MYC* and *BCL2* in 83 and 90 percent of analyzed interphases, respectively. The *MYC* probe (A) hybridizes to band 8q24.2 (5′ *MYC* is centromeric and labeled in spectrum orange; 3′ *MYC* is telomeric and labeled in spectrum green). The *BCL2* probe (B) hybridizes to band 18q21 (3′ *BCL2* is centromeric and labeled in spectrum green; 5′ *BCL2* is telomeric and labeled in spectrum orange).

Cytologic and flow cytometric examination of cerebrospinal fluid showed involvement by B cell neoplasm.

Final diagnosis

High-grade B cell lymphoma with *MYC* and *BCL2* rearrangements with leukemic presentation and central nervous system involvement.

ONLINE CASE DISCUSSION

The pathologists' view

Sanam Loghavi is Assistant Professor of Hematopathology, Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA.

Kamran Mirza is Associate Professor and Vice Chair of Education in the Department of Pathology and Laboratory Medicine, Loyola University Chicago Stritch School of Medicine, Maywood, USA.

CLICK HERE TO SEE DISCUSSION

ONLINE CASE DISCUSSION

The hematologists' view

Hua-Jay Cherng is Hematology-oncology fellow in the Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA.

Jason Westin is Director of Lymphoma Clinical Research, Section Chief of Aggressive Lymphoma, and Associate Professor in the Department of Lymphoma and Myeloma, Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA.

CLICK HERE TO SEE DISCUSSION

FEATURE

Follicular Lymphoma

The final case in our series on lymphoid neoplasms

Clinical history

73-year-old with a history of follicular lymphoma, presenting with palpable inguinal lymphadenopathy.

Complete blood count and differential (reference range)

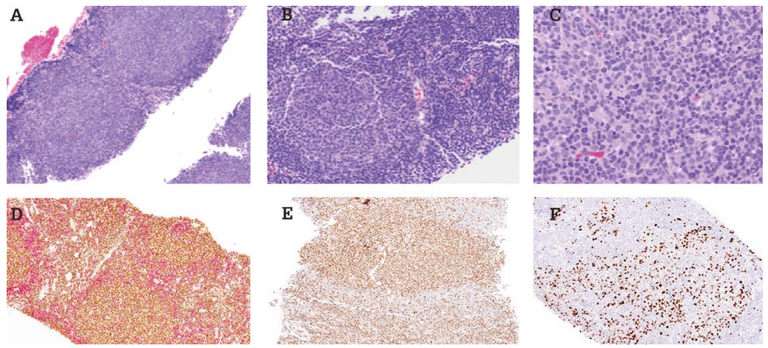
CD10	Positive
PAX5	Positive
BCL6	Positive
Ki-67	Positive in ~40% of cells

Serum lactate dehydrogenase

272 (135–214 U/L)

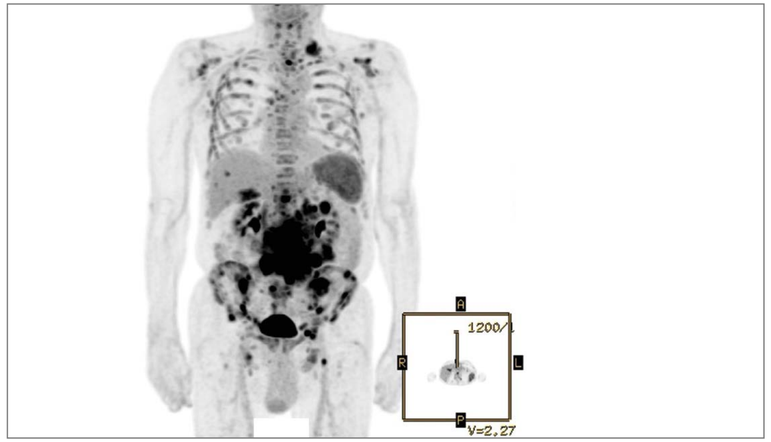
Lymph node morphology

Core biopsy of an abdominal mass shows fragments of lymphoid tissue involved by lymphoma. The neoplasm has a follicular/nodular pattern of growth (A and B; H&E 40x and 200x, respectively). Cytologically, the neoplastic follicles are composed predominantly of centrocytes with few admixed centroblasts in the range of histologic grade 2 (C; H&E 400x). Dual-stain immunohistochemistry shows that the lymphoma cells are positive for PAX5 (brown); residual small, admixed CD5+ T cells are seen in the interfollicular regions (red) (C). The neoplastic cells are positive for LMO2, supporting germinal center derivation (D). The Ki-67 proliferation index is ~40 percent (E). Photomicrographs courtesy of Francisco Vega-Vasquez, MD Anderson Cancer Center.



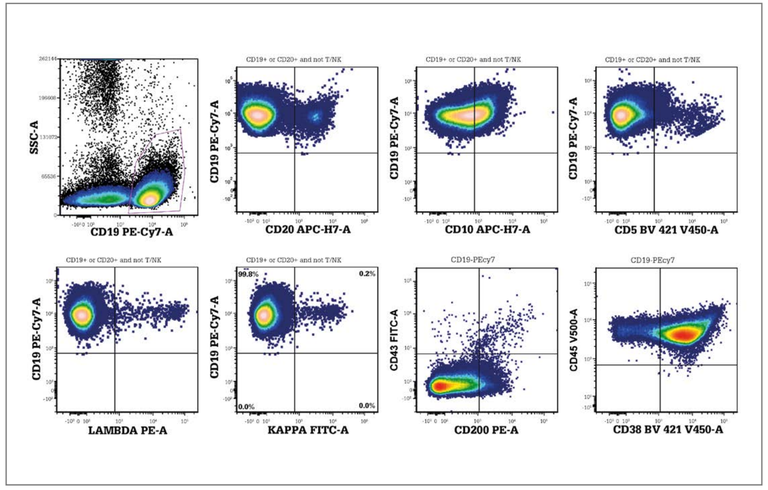
PET/CT

A PET/CT study showed hypermetabolic adenopathy above and below the diaphragm and widespread osseous lesions.



Flow cytometry

Flow cytometric immunophenotyping of a concurrent fine needle aspirate sample showed an expanded population of CD19+ B cells that were positive for CD10 (dim), predominantly negative for CD20, negative for CD5, and lacked surface light chain expression. These cells lacked expression of CD43 and CD200 and showed moderate/dim expression of CD38 and bright expression of CD45.



Lymph node morphology

WBC	3.9 x10^9/L (4.0–11.0)
Hgb	12.1 gm/dL (14.0–18.0)
MCV	96 fL (82–98)
Platelets	114 x10^9/L (140–440)

Final diagnosis

Follicular lymphoma, grade 2, with high Ki-67 proliferation index.

ONLINE CASE DISCUSSION

The pathologists' view

Sanam Loghavi is Assistant Professor of Hematopathology, Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA.

Kamran Mirza is Associate Professor and Vice Chair of Education in the Department of Pathology and Laboratory Medicine, Loyola University Chicago Stritch School of Medicine, Maywood, USA.

CLICK HERE TO SEE DISCUSSION

ONLINE CASE DISCUSSION

The hematologist's view

Loretta J. Nastoupil is Associate Professor, Director of the Lymphoma Outcomes Database, and Section Chief of New Drug Development in the Department of Lymphoma and Myeloma, Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA.

CLICK HERE TO SEE DISCUSSION