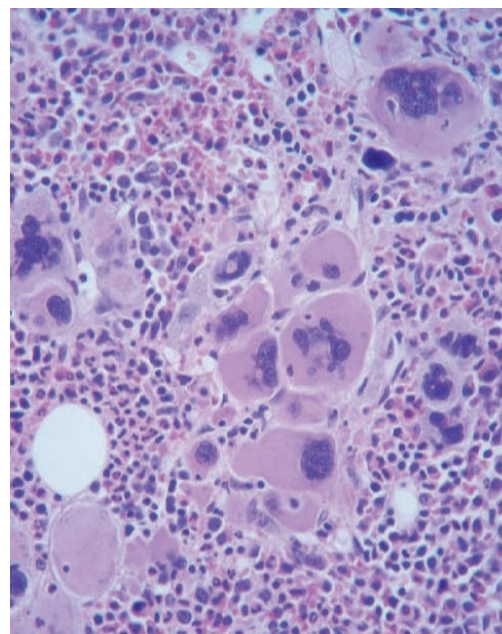
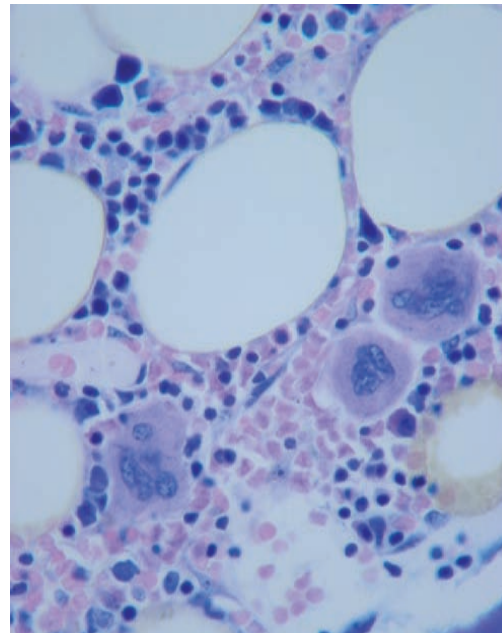
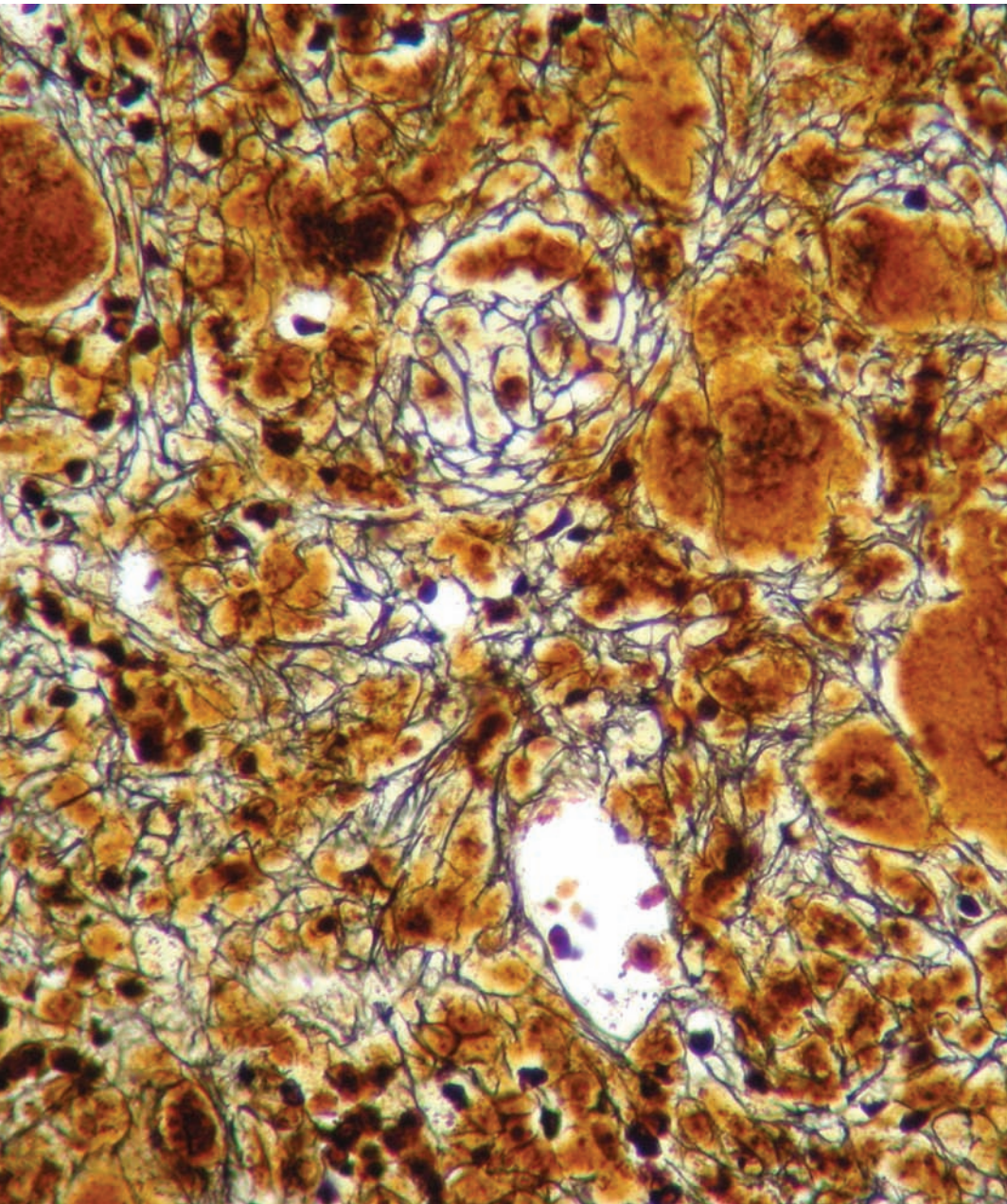


PATHOLOGY

Education Guide

The Illustrated Guide to Bone Marrow Diagnosis
Second Edition

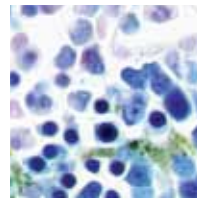


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La Plata School of Medicine, La Plata Argentina*

- *Large image: Osteomyelofibrosis stained with reticulin*
- *Top, right image: Myelodysplasia, with Giemsa stain*
- *Bottom, right image: Idiopathic Myelofibrosis with H&E*



The Illustrated Guide to Bone Marrow Diagnosis Second Edition

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Guide to Bone Marrow Diagnosis

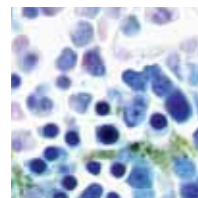
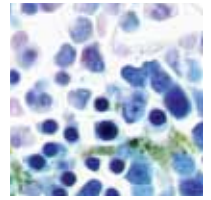


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Guide to Bone Marrow Diagnosis



Introduction

Bone marrow core biopsies introduced in the late 1950s have enabled pathologists to diagnose a wide range of hematopathological disorders. Most biopsies are taken from the posterior superior iliac spine and should be at least 1 cm in length. The advantages of a trephine over an aspirate are in showing the topography and cellularity of the marrow. Furthermore, in diseases which produce fibrosis, e.g. Hodgkin's disease, an aspirate often fails to produce an adequate diagnostic sample ('a dry tap').

Close liaison with hematologists is important since it makes the reporting of trephine biopsies easier and ensures that misdiagnoses are kept to a minimum.

Biopsies may be embedded in plastic or in paraffin wax with prior decalcification. The latter is most suitable for a routine laboratory and is used throughout this book. In this book both Giemsa and H&E counterstains are illustrated. When indicated, we include a reticulin stain in our bone marrow set.

Reasons for Performing Bone Marrow Biopsies

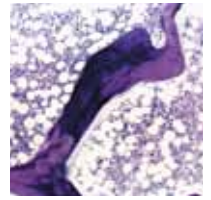
The majority of bone marrow biopsies are performed for the following reasons:

1. Dry tap, usually caused by fibrosis or extreme hypercellularity
2. Assessment of cellularity
3. Identification of focal disease
4. Lymphoma staging
5. Assessment of HIV infection

How to Examine a Trephine Section

It is important to have an organized approach to the examination of bone marrow sections and to assess sequentially cellularity, topography, morphology and accessory structures.

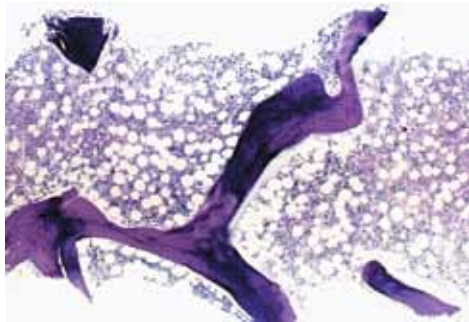
The bone marrow is a highly organized and specialized tissue. Confidence in examining a trephine biopsy is derived from a familiarity with the normal marrow appearances and an understanding of how these alter throughout life.



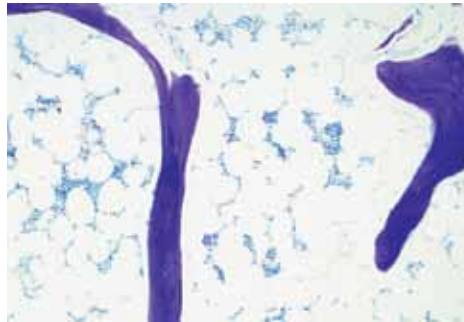
Chapter 1 ■ Normal Bone Marrow

Cellularity

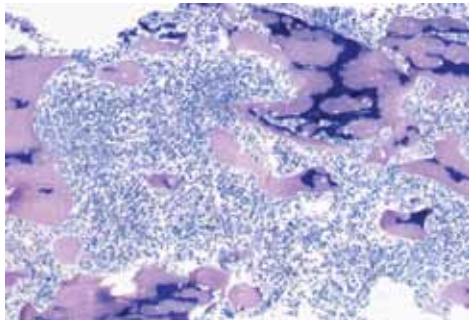
Bone marrow cellularity and site alter throughout life as indicated in the figures and the table below.



Normal adult



Bone marrow in old age

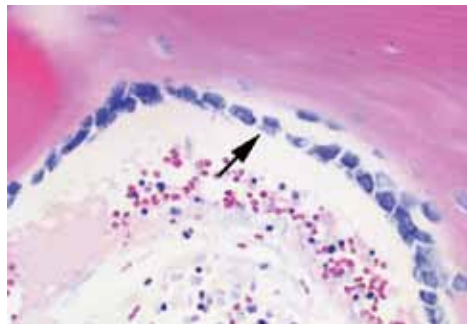


Neonatal bone marrow

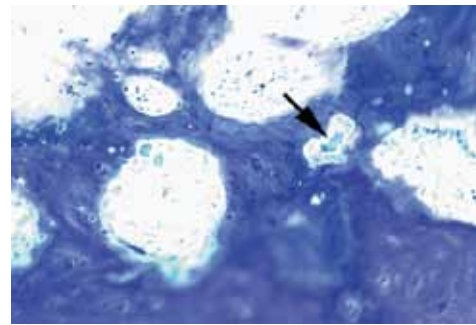
Bone Marrow Cellularity

Age	Site	Cellularity (hemopoietic cells/fat)
Neonate	All bones, liver, spleen	100/0
Child	Most bones	70/30
Adult	Axial skeleton	50/50
Old age	Axial skeleton	30/70

Cell Types Associated with the Bone

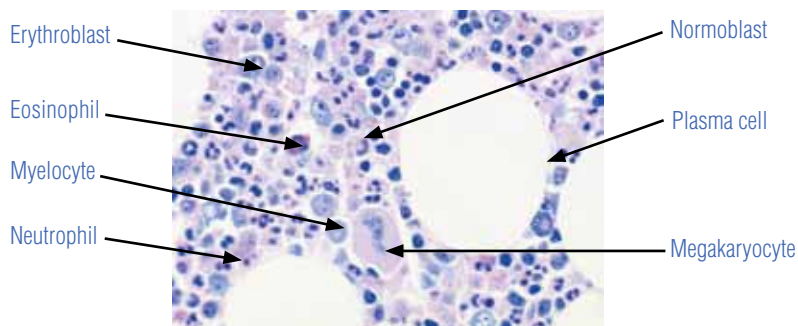


Osteoblasts



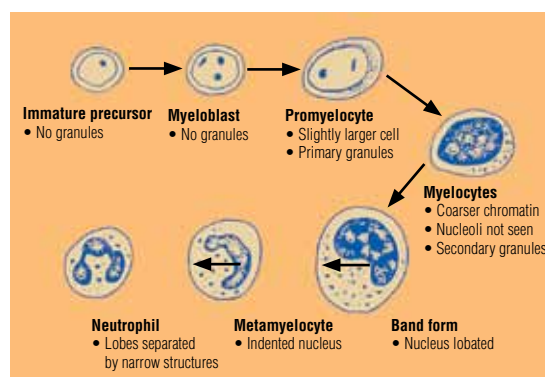
Osteoclasts

Cell Types Seen in Normal Bone Marrow



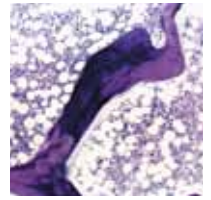
Hematopoietic Cells

In a good quality marrow section, especially, if stained by the Giemsa method, most of the normal differentiated cell types can be identified: Granulocytes, erythroid cells, megakaryocytes, monocytes, macrophages, mast cells, lymphocytes, plasma cells and bone forming cells.

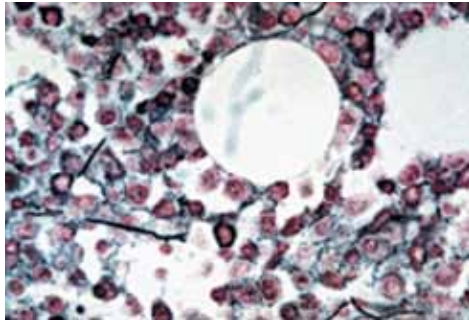


Schematic illustration of granulocytic cell differentiation.

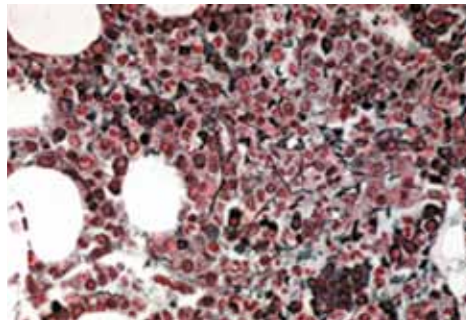
Normal Bone Marrow



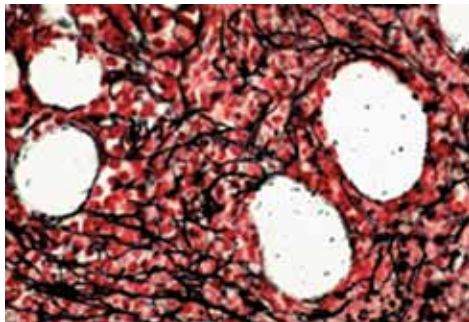
The reticulin in the bone marrow exists as a fine network. It is readily displayed by the Gomori's Silver Stain and can be graded semi-quantitatively when abnormal as shown in the figures below.



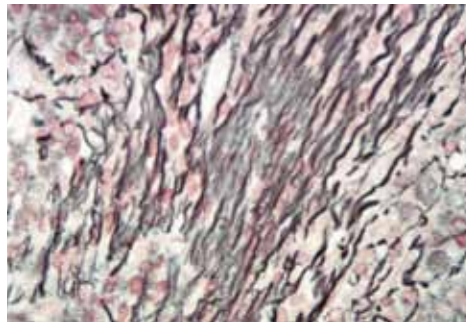
Grade 1. Focal fine reticulin. Normal. Gomori's Silver Stain.



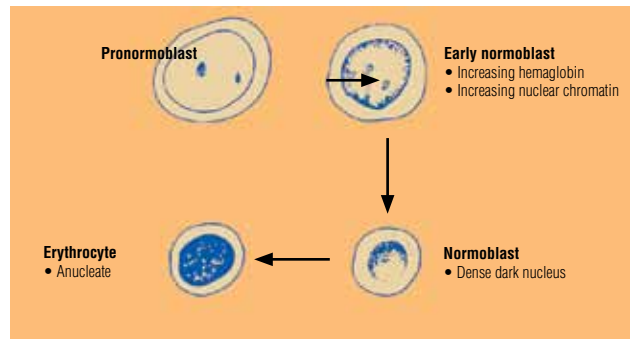
Grade 2. Diffuse fine reticulin. Normal. Gomori's Silver Stain.



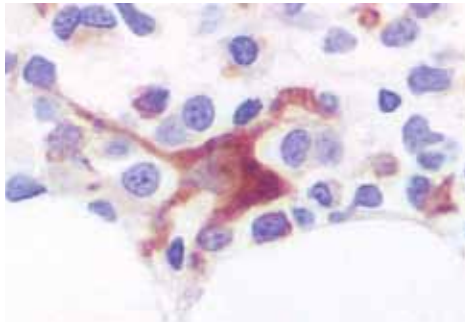
Grade 3. Diffuse fine reticulin, plus focal coarse reticulin. Abnormal. Gomori's Silver Stain.



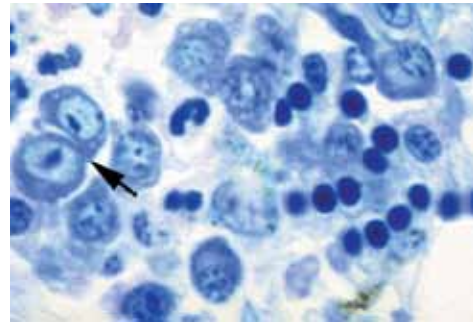
Grade 4. Diffuse coarse reticulin, including collagen, i.e. fibrosis. Abnormal. Gomori's Silver Stain.



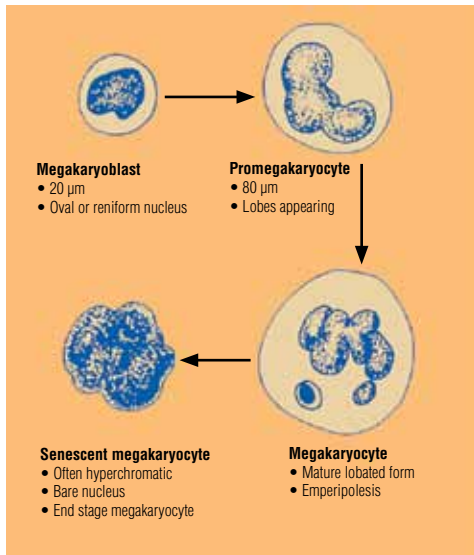
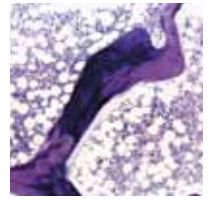
Schematic illustration of erythroid differentiation.



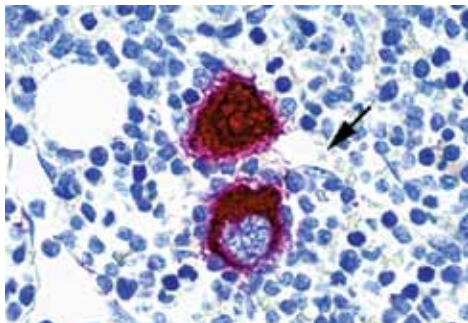
Erythroid colonies differentiate in the center of the marrow and frequently consist of small erythroid islands with central macrophage (immunostained for CD68 above).



Erythroid colonies are particularly well distinguished in Giemsa preparations by the distinct blueness of the erythroblasts (arrow).



Schematic illustration of megakaryocytic differentiation.



Two megakaryocytes immunostained for CD61 lying in close proximity to a venous sinusoid (arrow) are illustrated.

Mature megakaryocytes and their precursors are found uniformly distributed throughout the central region of the marrow, particularly in association with thin-walled venous sinuses.

Lymphocytes

Normal adult marrow contains an inconspicuous population of both B and T lymphocytes throughout the marrow. They may occur as small aggregates. These lymphoid aggregates increase in number with age, being frequently seen in the elderly population. If there are more than three lymphoid aggregates per trephine, it does suggest a neoplastic rather than a reactive process. Distinguishing between benign lymphoid aggregates and those which are neoplastic can be difficult and their significance remains unresolved (see table).

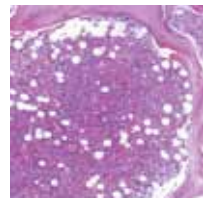
A comparison of the features which may help discriminate between benign lymphoid aggregates in bone marrow and neoplastic involvement:

Benign

- Rounded aggregates
- Well-circumscribed, regular, small lymphocytes
- Elderly population
- < 3 mm in diameter
- Never paratrabecular
- Germinal centers (5% of cases)
- May contain plasma cells and eosinophils
- Polyclonal light chain expression
- 1–3 aggregates per trephine

Neoplastic

- May be irregular
- Cellular atypia may be present
- Wide age range
- May be > 3 mm diameter
- May be paratrabecular
- No germinal centers
- Usually just lymphoid cells
- Monoclonal light chain pattern
- > 3 aggregates per trephine



Chapter 2 ■ HIV Infection

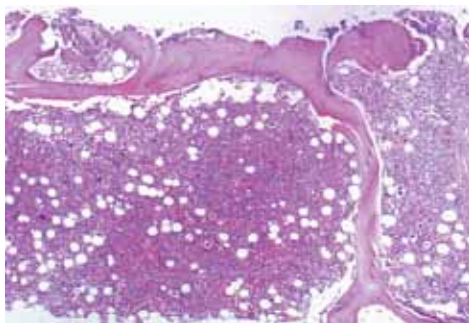
Introduction

HIV infection may result in many changes to the bone marrow. These include reactive processes, secondary infections and the development of malignancies.

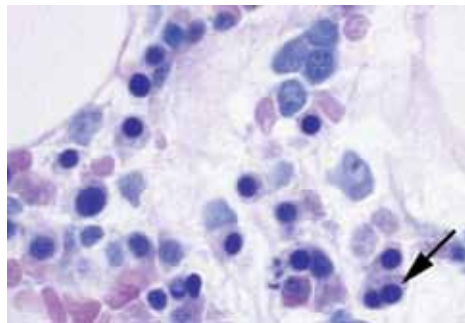
Histopathology of the Bone Marrow

Reactive Features

Up to three-quarters of HIV-infected marrow show dyshematopoiesis with myelodysplastic-like features affecting all three cell lines. Eosinophilia is common (seen in 15% of cases) as is a reactive plasmacytosis.

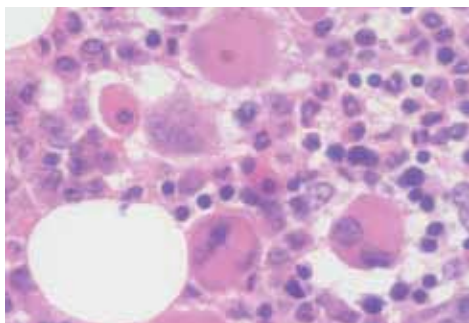


Typical hypercellular marrow seen in uncomplicated HIV infection. H&E.

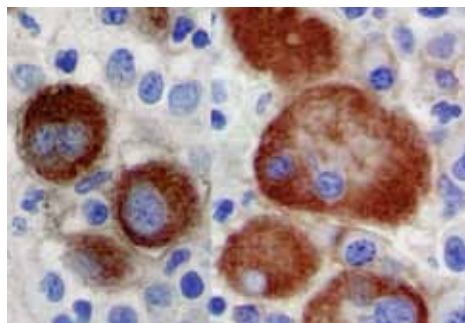


Dyserythropoiesis in HIV infection. Binucleate normoblast (arrow). Giemsa.

Micromegakaryocytes in HIV infection (a and b):



(a) Giemsa.



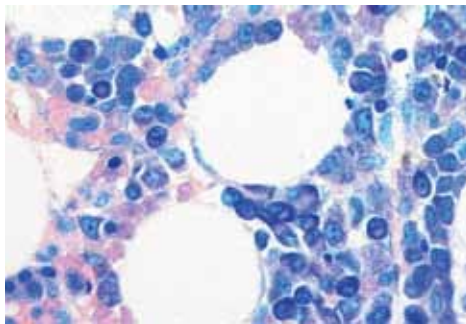
(b) CD31.

Secondary Infections

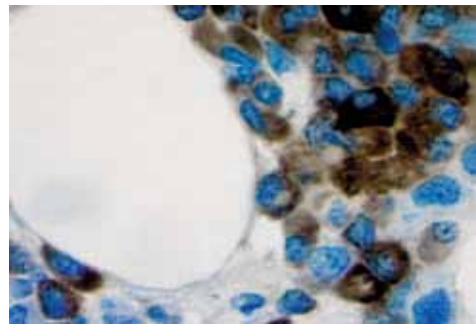
These are common and patients often have multiple infections. The most commonly encountered are those involving acid-fast bacilli, usually tubercle bacillus (TB) or Mycobacterium avium-intracellulare complex (MAIC).

Granulomas are usually present in marrows infected by these organisms (always stain all HIV+ tissues with a Ziehl-Neelsen (ZN) stain). Other common infections are Cryptococcus, Histoplasma, Leishmania, CMV and Pneumocystis carinii.

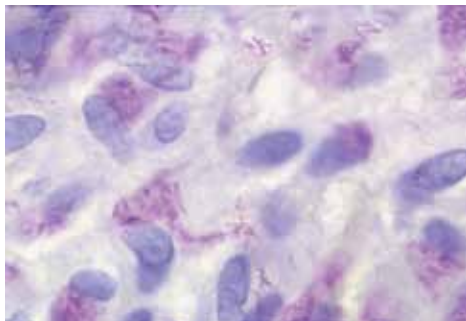
Increased numbers of plasma cells. (a) Giemsa. (b) Antibody VS38.



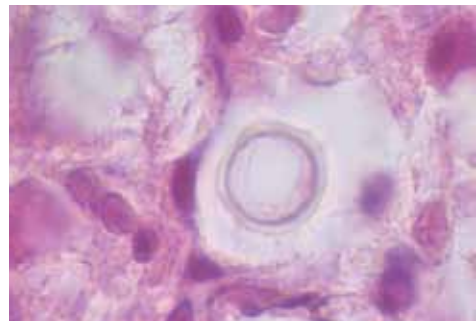
(a) Giemsa.



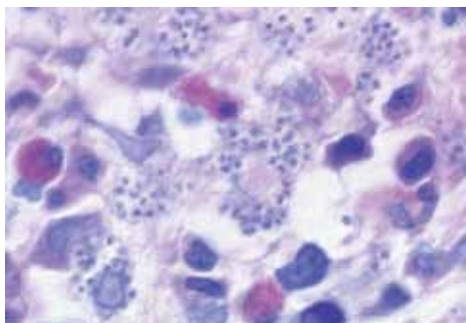
(b) Antibody VS38.



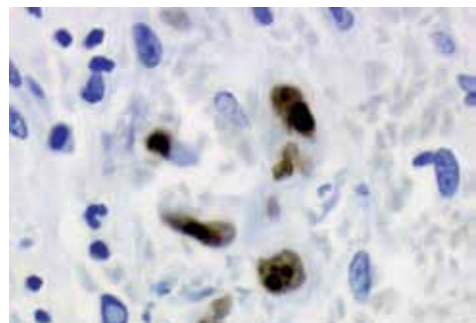
(a) Atypical tuberculosis in HIV marrow not associated with granuloma. ZN stain.



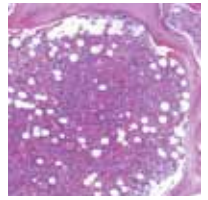
(b) HIV-associated cryptococcal infection. H&E.



(c) Leishmania donovani bodies in macrophage cytoplasm. Giemsa.



(d) CMV infection. Immunoperoxidase.



Malignancies

Patients with HIV infection have an increased risk of developing high-grade B-cell non-Hodgkin's lymphomas which often involve the marrow. Hodgkin's disease is also increased and Kaposi's sarcoma can involve most organs including, rarely, the bone marrow.

Summary of Features Seen in HIV-positive Bone Marrow Biopsies

Myelodysplastic-like features

- Seen in 75%
- All three cell lines affected
- 15% have eosinophilia
- Megaloblastoid features
- Nuclear abnormalities in normoblasts
- Nuclear abnormalities and decreased granularity in myeloid series
- Micromegakaryocytes
- Mononuclear megakaryocytes
- Naked megakaryocyte nuclei

Stromal features

- Increased plasma cells and macrophage
- Macrophages showing erythrophagocytosis
- Serous atrophy
- Lymphocytosis
- Lymphohistiocytic aggregates (AILD-like lesions)
- Vascular proliferation

Infections

- Leishmaniasis
- Pneumocystis carinii
- Cryptococcus neoformans
- Histoplasma capsulatum
- Mycobacterium avium-intracellulare
- CMV
- TB

Malignancies

- Kaposi's sarcoma
- Non-Hodgkin's lymphoma
- Hodgkin's disease

Diagnostic Problems

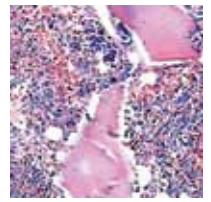
1. Misdiagnosis as MDS is unlikely since there are usually other features to suggest HIV infection. In addition, the clinical features including, the patient's age, would be unusual for a diagnosis of MDS.
2. A striking plasmacytosis may provide confusion with multiple myeloma. Immunohistochemistry will resolve this by demonstrating that the plasma cell population is polyclonal.
3. Megaloblastoid change seen in members of the erythroid series should not be misdiagnosed as leukemia or megaloblastic anemia, since the change is accompanied by other features of HIV infection.
4. Distinction between secondary infection by TB and by MAIC may be possible since MAIC tends to be PAS-positive in addition to being ZN-positive.

Summary of Key Points

Perform a ZN stain on all known HIV-infected marrow biopsies.

HIV infection may cause a number of histological changes, none of which are specific.

Consider HIV infection in any marrow which shows ill-defined abnormalities which are not diagnostic of other conditions.



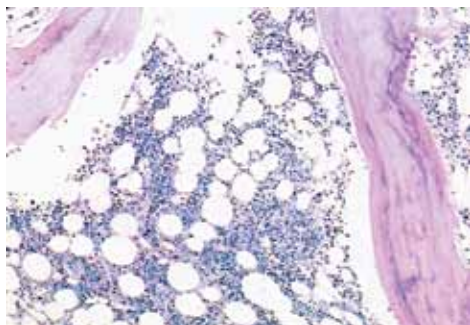
Chapter 3 ■ Anemias and Aplasias

Iron Deficiency Anemia

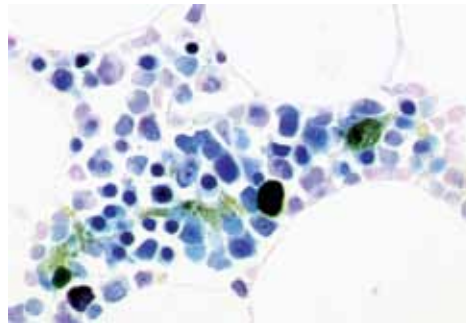
In uncomplicated iron deficiency anemia the marrow is usually slightly hypercellular due to increased erythropoiesis. Iron stores are reduced or absent.

Anemia of Chronic Disease

The cellularity is normal or slightly reduced with iron detectable in macrophages.



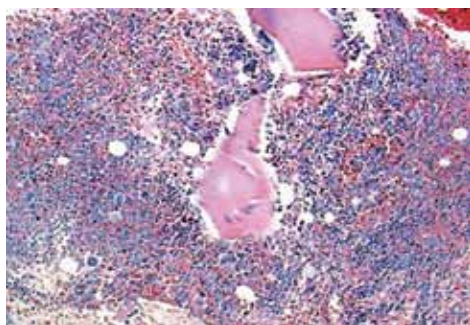
Iron deficiency anemia. Hypercellular marrow showing increased erythropoiesis. Giemsa.



Anemia of chronic disease. Iron in macrophages (green). Giemsa.

Hemolytic Anemia

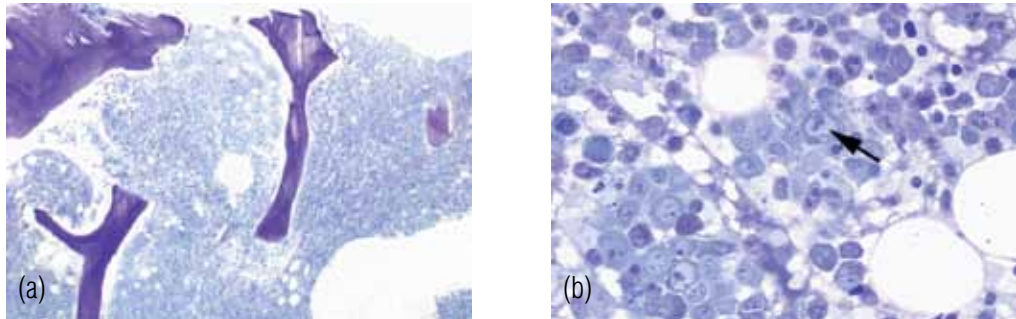
Uncomplicated hemolytic marrows are hypercellular with marked erythroid hyperplasia, but little else of note.



Hypercellular marrow. Giemsa.

Megaloblastic Anemia

The marrow shows erythroid hyperplasia with characteristic megaloblastic erythroid precursors which must not be misdiagnosed as leukemia. Other changes include giant metamyelocytes and small megakaryocytes with hyperlobated nuclei. Other macrocytic anemias with many causes, including drugs and alcohol, can give rise to megaloblastic changes.



Typical megaloblastic anemia. Note the characteristic elongated 'coin slot' nucleoli of the megaloblasts (arrow), both Giemsa. (a) Low power, (b) High power.

Aplastias

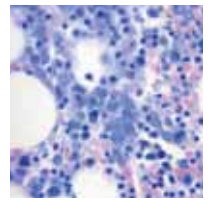
Bone marrow aplasia is a profound cellular hypoplasia combined with pancytopenia. Aplastic anemia may result from many drugs, including chemotherapy, wide field radiotherapy and a variety of viral infections.

Aplastic Anemias

The trephine features are similar regardless of the cause with the marrow being profoundly hypocellular. Hypocellular MDS/AML may be detected by the presence of clusters of blast cells.



Low power.



Chapter 4 ■ The Myelodysplastic Syndromes

Introduction

Myelodysplasia is commonly thought of as a precursor condition to AML. Five subtypes are recognized by the French-American-British (FAB) Co-operative Group. Apart from chronic myelomonocytic leukemia (CMML) these are difficult to differentiate on trephines.

Histopathology of the Bone Marrow

The appearances are mainly loss of cellular differentiation and disruption of normal architecture. It is a common misconception that marked abnormalities must be detected in each cell line in order to diagnose MDS on trephine sections. In fact, the identifiable abnormalities may be restricted to only one series, e.g. the erythroid in sideroblastic anemia.

Cellularity

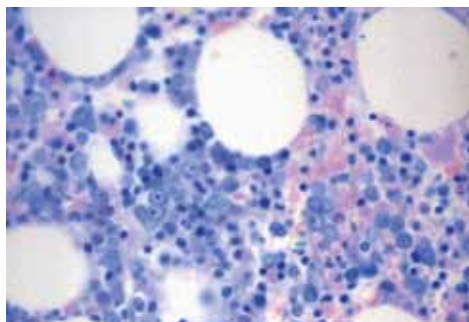
The marrow is hypercellular in the majority of cases (80%).

Abnormalities of Marrow Architecture

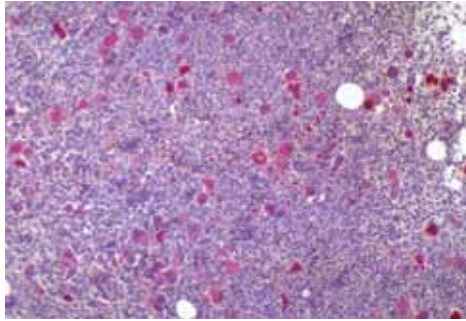
The most striking feature seen at low power is the disruption of the normal cellular distribution.

Dysmegakaryopoiesis

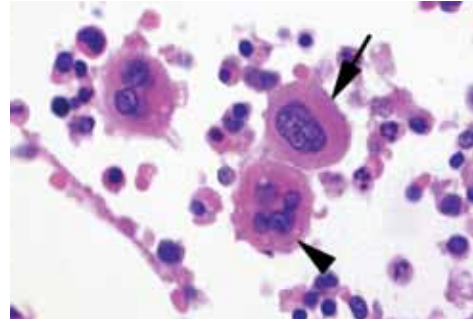
The number of megakaryocytes is increased. Their distribution is abnormal, with many having a paratrabecular location. Abnormalities in size, shape and nuclear lobe configuration include micromegakaryocytes, monolobated megakaryocytes (especially in the 5q-syndrome) and megakaryoblasts.



Typical jumbled appearance of cell types in myelodysplasia. Giemsa.



Increased numbers of megakaryocytes in MDS.
Highlighted by immunostaining for CD61.



Typical micromegakaryocyte (arrowhead) next to a
monolobated form (arrow) in MDS. H&E.

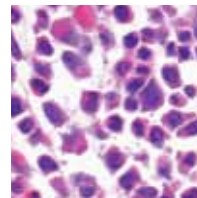
Dyserythropoiesis is characterized by enlarged irregular erythroid colonies containing increased numbers of immature cells with binucleate or irregularly shaped normoblasts and megaloblastic precursors.

Secondary MDS

MDS may follow the use of chemotherapy or radiotherapy. Secondary MDS, like primary MDS, may progress to AML. The marrow is often hypocellular and dyserythropoiesis is a prominent feature.

Overlap Between Myelodysplasia and Myeloproliferation

In a minority of cases it may not be possible on histological criteria alone to make a definite diagnosis of myelodysplasia or myeloproliferation



Chapter 5 ■ Myeloproliferative Disease (MPD)

Classification of MPD

There are four diseases, well-defined clinically, which constitute myeloproliferation:

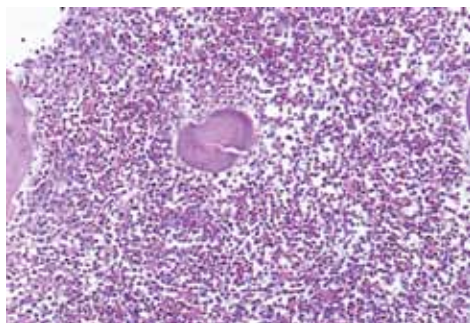
1. Chronic myeloid leukemia (CML)
2. Polycythemia rubra vera (PRV)
3. Essential thrombocythemia (ET)
4. Myelofibrosis (MF).

Chronic Myeloid Leukemia (CML) Introduction

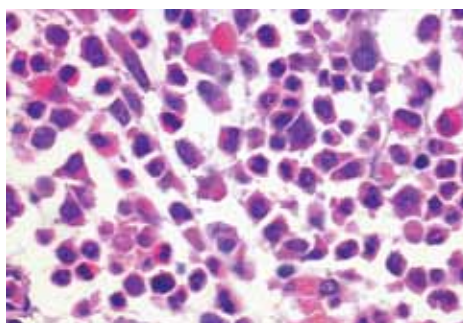
CML is characterized by the proliferation of differentiated granulocytes. The majority of cases (90%) will have the Philadelphia chromosome (Ph) which involves the translocation of the c-abl proto-oncogene from its site on chromosome 9 to the BCR region of chromosome 22.

Histopathology of the Bone Marrow

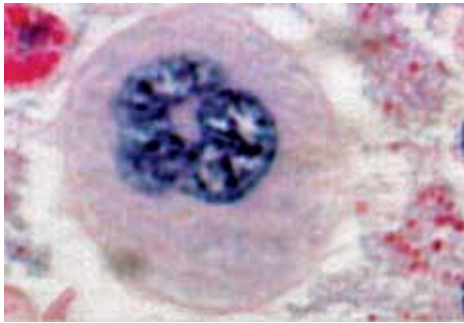
The bone marrow is typically hypercellular showing profound granulocytic differentiation. Erythroid changes are usually those of immaturity (left-shifting). Megakaryocytes are smaller than normal, increased in number, and usually mononuclear. With time, most cases will transform into an acute leukemia (blast crisis).



Typical low power appearances of CML. Note lack of fat spaces. H&E.



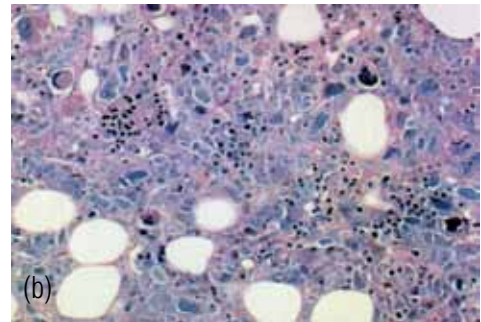
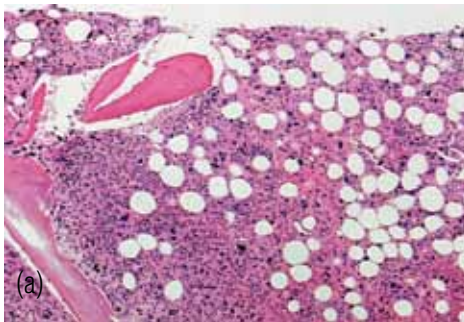
Low power view of CML, H&E



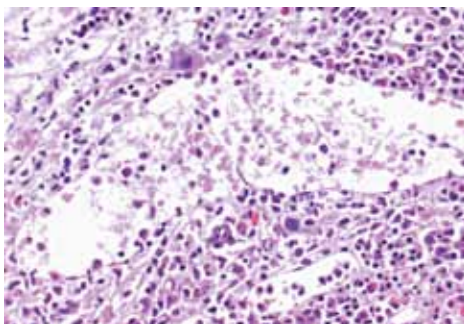
Atypical mononuclear megakaryocyte in CML. Giemsa.

Polycythemia Rubra Vera (PRV) Introduction

PRV is a neoplastic proliferation with erythroid differentiation resulting in an increased mass of red blood cells. Most cases of PRV will progress to myelofibrosis with subsequent blast transformation into AML.



Polycythemia rubra vera. (a) Low power. H&E. (b) Medium power, Giemsa..

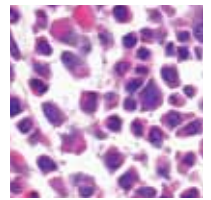


Large dilated sinus in PRV. H&E.

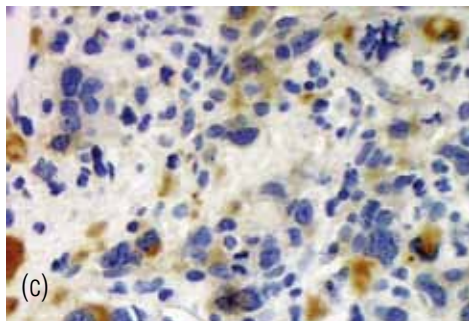
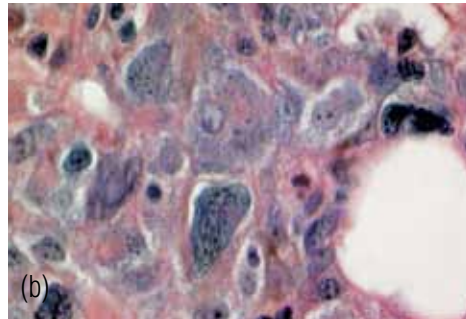
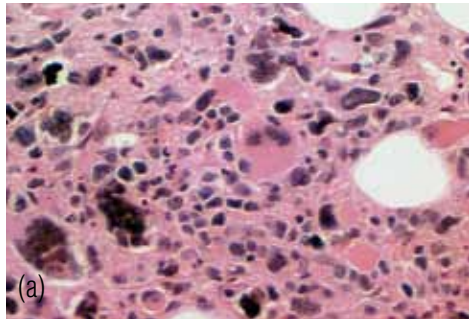
Histopathology of the Bone Marrow

The bone marrow is hypercellular with prominent erythroid differentiation. Florid megakaryocytic proliferation is usually present, many of which are large pleomorphic types often gathered together in prominent clusters. Large dilated sinuses filled with red

Myeloproliferative Disease (MPD)



blood cells are common and are usually associated with increased reticulin throughout the marrow.



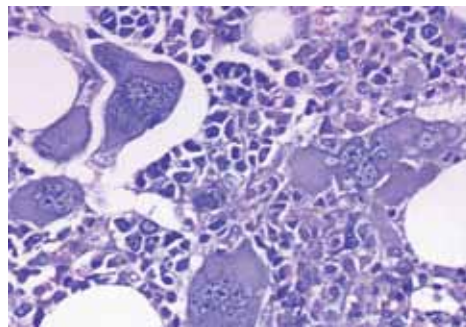
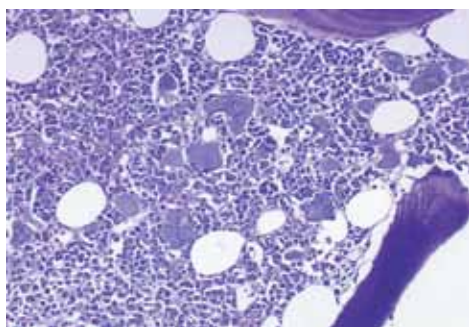
Abnormal megakaryocytes in PRV. (a) H&E. (b) Giemsa. (c) Immunostain for CD31.

Essential Thrombocythemia (ET)

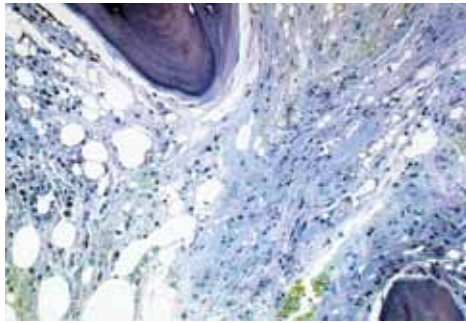
In this subgroup of MPD, megakaryocytes are the dominant cell line. They produce large numbers of platelets which are often functionally impaired. The disease tends to follow an indolent clinical course, but can transform into acute leukemia.

Histopathology of the Bone Marrow

The marrow is usually markedly hypercellular with increased numbers of megakaryocytes. Many are giant forms with marked nuclear atypia. They occur in clusters and often congregate near sinuses into which they discharge clouds of platelets.



Above and right: Typical low power appearance of ET. Giemsa.

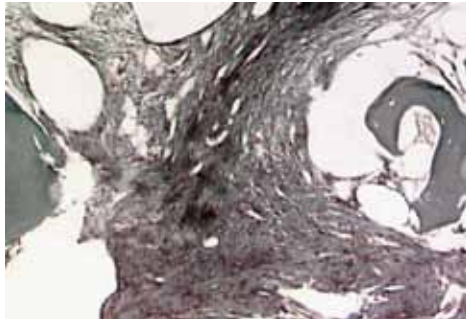


Fibrosis associated with abnormal megakaryocytes in ET. Giemsa.

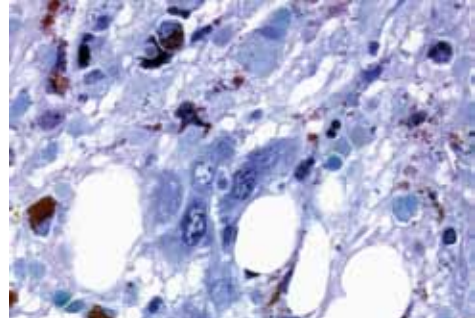
Primary Myelofibrosis

Chronic Myelofibrosis

This is probably not a separate entity, but represents a response by the marrow to the presence of the neoplastic cells in one of the other forms of MPD. The fibrosis results from fibroblast stimulation by platelet-derived growth factors released by dysfunctional megakaryocytes. When the fibrosis occurs as the dominant feature in the marrow at presentation it is referred to as chronic primary myelofibrosis.



Dense fibrosis in primary myelofibrosis.



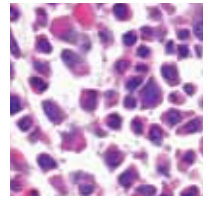
Abnormal megakaryocytes in primary myelofibrosis.

Histopathology of the Bone Marrows

The morphology is characterized by well-vascularized fibrosis. It is nearly always possible to identify abnormal megakaryocytes within this fibrosis.

Acute Myelofibrosis

This is usually a variant of acute megakaryoblastic (M7) leukemia, but is occasionally primary and of unknown etiology.



Diagnostic Difficulties

Distinguishing Between MDS and MPD

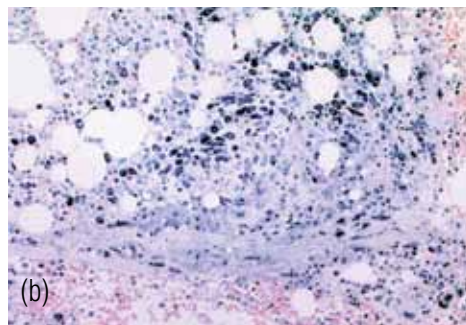
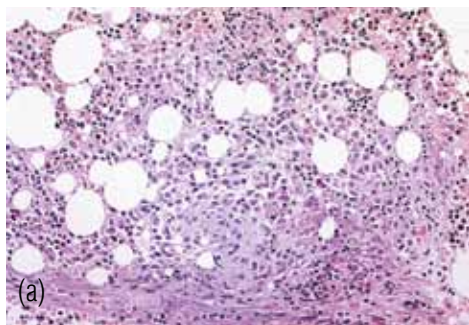
There is no doubt that a number of patients seem to show features, both clinically and histologically, of MDS and MPD. This may reflect imprecision in the current means of classifying these disorders although it is more likely that biologically there is considerable overlap between them. Karyotype analysis is becoming increasingly important in providing more objective criteria.

Mast Cell Disease

Neoplastic conditions of mast cells are rare and involve the bone marrow as systemic mastocytosis which has two forms:

1. Benign

This is associated with skin involvement (urticaria pigmentosa) and is rarely associated with myeloproliferative disorders.



So-called 'mast cell granulomas' in systemic mastocytosis. (a) H&E. (b) Giemsa.

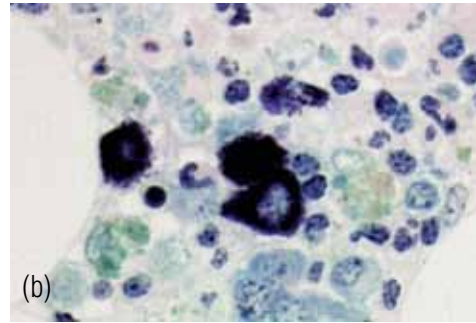
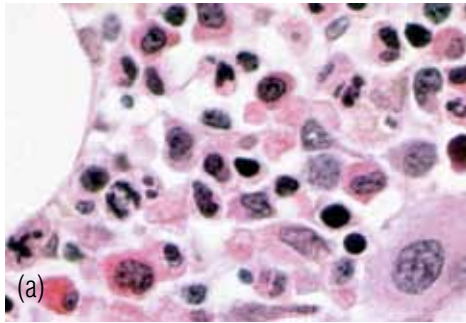
2. Malignant

This is not associated with urticaria pigmentosa, but is rather a myeloproliferative disorder and may progress to acute leukemia.

Histopathology of the Bone Marrows

The marrow is usually hypercellular and focally-infiltrated by aggregates of mast cells often known as mast cell granulomas. These may have a perivascular and paratrabecular distribution. The infiltrate consists of mast cells, lymphocytes, plasma cells, eosinophils and sea-green histiocytes surrounding the mast cell precursors in the granulomas.

The infiltrate is often associated with fibrosis, an increased number of eosinophils and bone changes which may include osteosclerosis, osteopenia and osteolysis.



Cytological appearances of mastocytosis. (a) H&E. (b) Giemsa.



Chapter 6 ■ Acute Leukemia

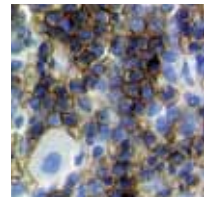
Introduction

There are two main types of acute leukemia, myeloid (AML) and lymphoblastic (ALL). Acute leukemias are blastic proliferations of white cells which usually, but not invariably, involve the peripheral blood.

Both acute leukemias affect all ages although their frequencies are quite different. AML is primarily an adult disease with the incidence rising with age whereas more than 85% of cases of ALL are in children aged under 15. The prognosis in acute leukemia lymphoblastic (ALL) in children is excellent whereas in AML it is generally poor, particularly, in the elderly.

Classification

The French-American-British (FAB) Co-operative Group classification has been widely accepted especially for both AML and ALL. This classification is based on cytological appearances that are not always best appreciated in trephine sections.



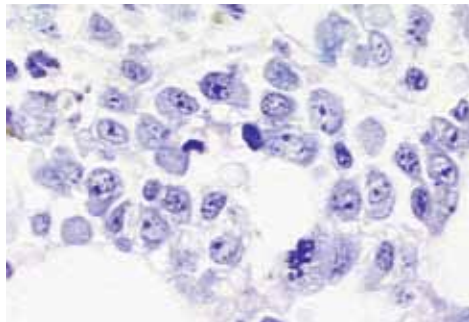
Chapter 7 ■ Acute Myeloid Leukemia (AML)

Histopathology and Immunophenotyping

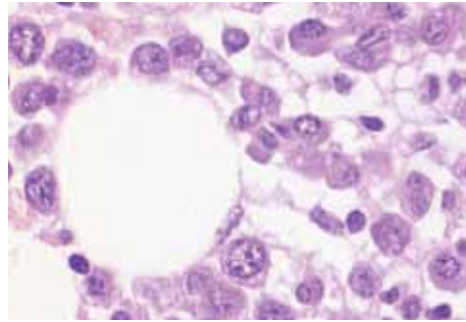
Acute Myeloid Leukemia M0-M5

With the exception of acute promyelocytic leukemia M3 (bi-lobed nuclei, cytoplasmic granules) the first five categories of the FAB classification look remarkably similar on histology. In general, there is increased positivity for myeloperoxidase and elastase in M1/M2 and for CD68 in M4 and M5 though this is not specific.

Careful liaison with the Hematology Department is the key to distinguish these subtypes.



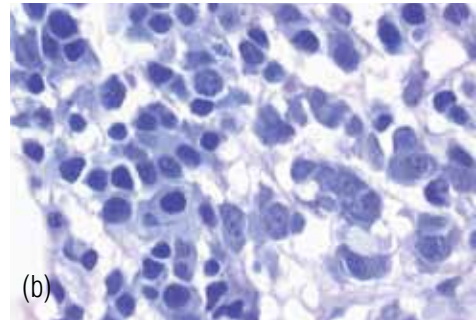
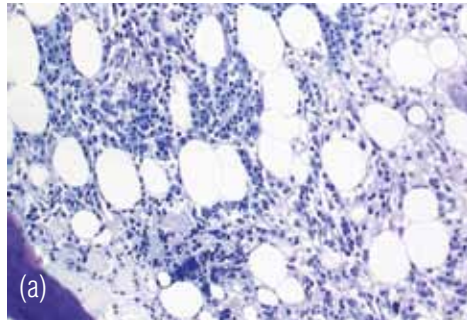
AML M2. Giemsa.



AML M2. H&E.

Erythroleukemia M6

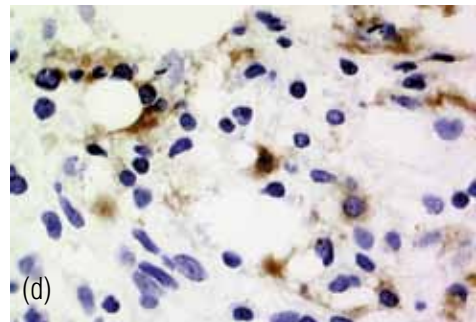
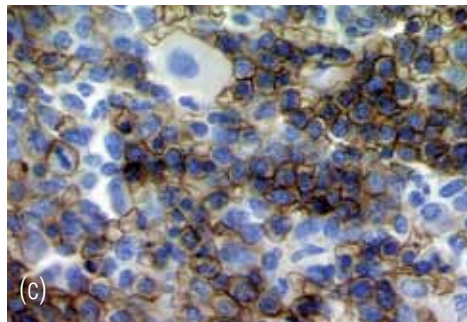
This is a rare category which is easy to overlook as myelodysplasia with excess blasts. The erythroid element is predominantly associated with dysplastic erythroid colonies whereas the lineage of most of the true leukemic blasts is uncertain.



M6 erythroleukemia. The histology is shown in (a) and (b) Giemsa.

Megakaryoblastic and Megakaryocytic Leukemia M7

This acute leukemia has a variable morphology from the recognizably megakaryocytic to the frankly bizarre where it can be identified only on the basis of immunocytochemistry.

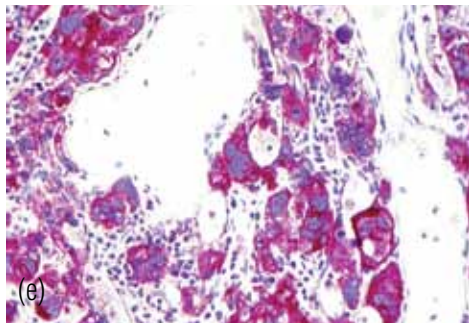
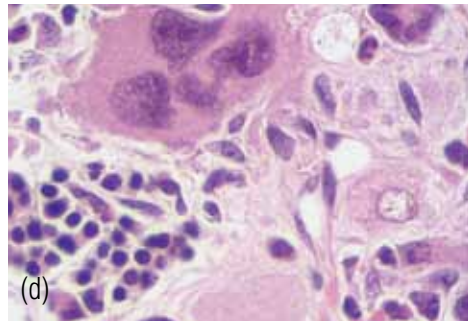
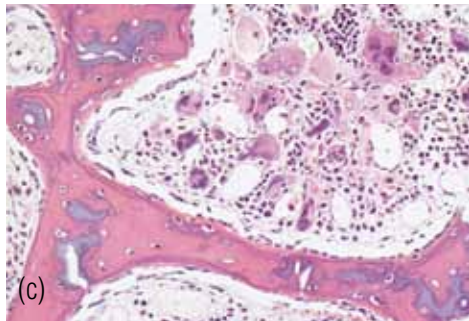
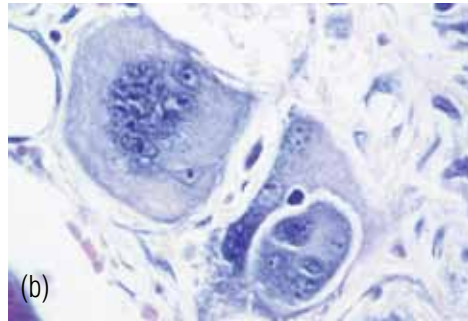
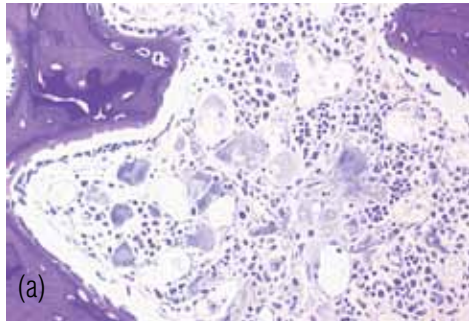
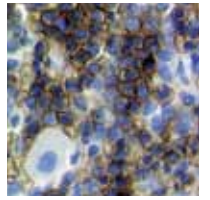


The majority of the cells are erythroid as shown by glycophorin C staining (c), but many abnormal cells express other lineage markers such as the megakaryocytic antigen CD61 (d).

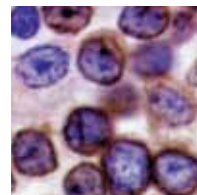
Hypoplastic AML

These patients present with pancytopenia and a 'dry tap' at aspiration. Aplastic anemia is usually the main differential, though leukemia is often suspected clinically due to a few suspicious blast-like cells being seen in the peripheral blood. The trephine usually makes the diagnosis by showing discrete collections of primitive blast cells in an otherwise empty marrow.

Acute Myeloid Leukemia (AML)



Example of an obviously megakaryocytic leukemia. (a and b) Giemsa. (c and d) H&E. (e) CD31 immunostain.



Chapter 8 ■ Acute Lymphoblastic Leukemia and Lymphoma

Diagnostic Problems

AML or ALL

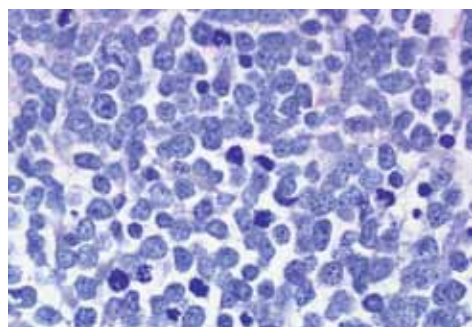
It is usually possible to distinguish these using a small panel of antibodies including myeloperoxidase, elastase, CD68, B- and T-cell markers and CD10.

General Features

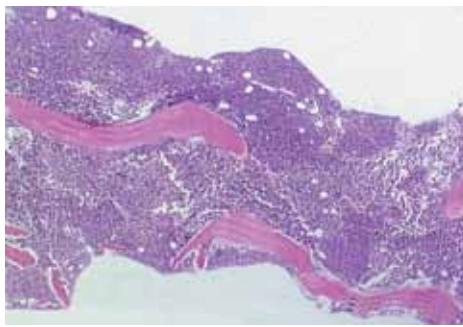
These neoplasms may be of B- (80%) or T- (20%) cell type. The T-cell lymphoblastics give rise to the well-described clinical syndrome of mediastinal tumors without an obvious leukemic phase.

Histopathology of the Bone Marrow

B- and T-cell cases have identical morphological features. The neoplastic lymphoblasts are slightly larger than lymphocytes and have round or convoluted nuclei, fine chromatin, often with a smudged appearance, inconspicuous nucleoli and scant, faintly basophilic cytoplasm. Mitotic figures are numerous. The marrow is packed with little of the hematopoietic tissue or fat remaining though, occasionally, a more patchy infiltration is evident. Areas of necrosis (often a sinister sign in a marrow) may be present. The distinction between B- and T-cell cases can only be made immunocytochemically. An important practical note is that B-lymphoblasts have a precursor phenotype being CD79a-positive, but usually lacking mature B markers such as CD20.



Cytology of lymphoblastic lymphoma. Giemsa.



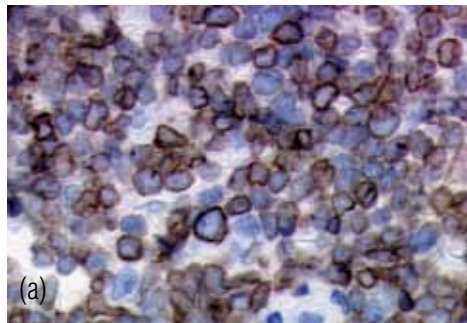
Hypercellular marrow in lymphoblastic lymphoma. H&E.

Immunophenotype B-cell:

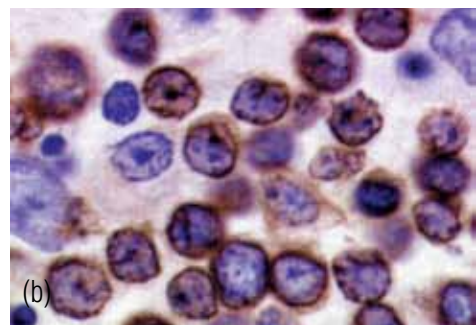
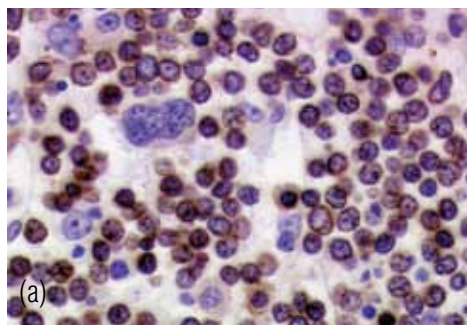
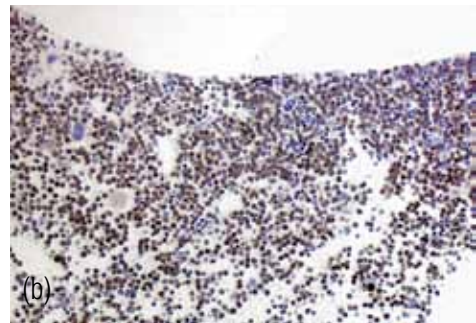
TdT+, PAX5 +, CD10+/-, CD19+, CD20-, CD22-, CD79a+, SIg-

Immunophenotype T-cell:

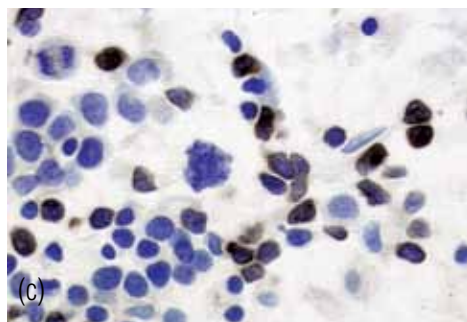
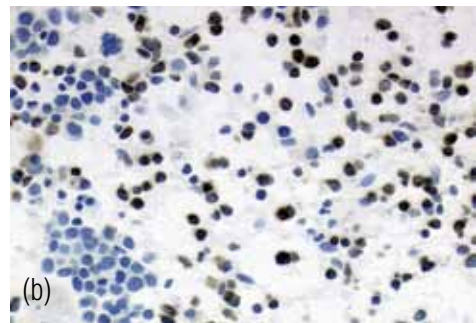
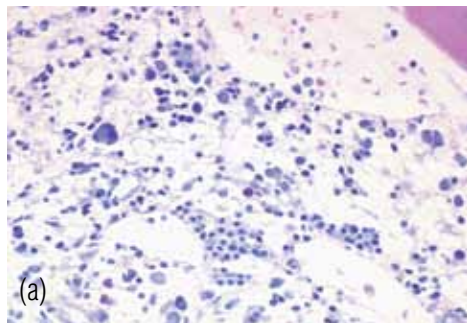
TdT+, CD3+, CD7+, CD2 and 5 variable, CD4+/-, CD8+/-



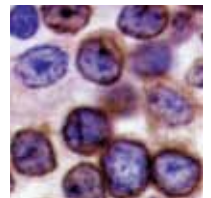
(a) B lymphoblastic immunostained for CD79a. (b) T lymphoblastic immunostained for CD3.



(a and b) T lymphoblastic leukemia immunostained for CD3.



Scanty infiltration by B lymphoblastic lymphoma/leukemia is easily overlooked morphologically (a: Giemsa), but is revealed (b and c) by immunostaining for CD79a.



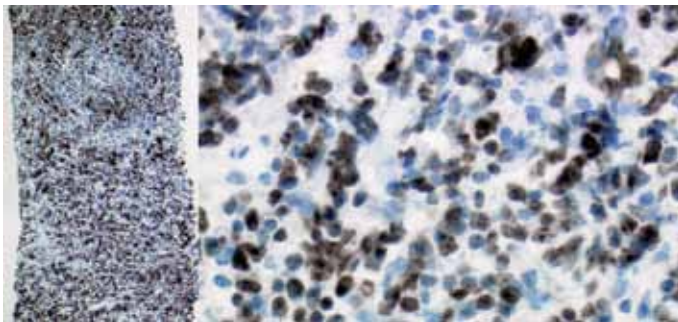
Diagnostic Problems

1. Sparse Infiltrate

Occasionally, the infiltrate is sparse with blast cells outnumbered by normal marrow cells so that it is easy for the histologist to overlook them. This may occur at diagnosis as well as at early relapse and is relatively easily recognized by immunostaining for B- or T-cell antigens.

2. Distinction from CLL

The small size of the lymphoblasts and the packed nature of the marrow can produce a picture in an adult which is surprisingly easy to confuse with CLL. It can be avoided by checking for a high mitotic rate in ALL which will be confirmed by abundant positively stained nuclei for proliferation markers such as Ki67.



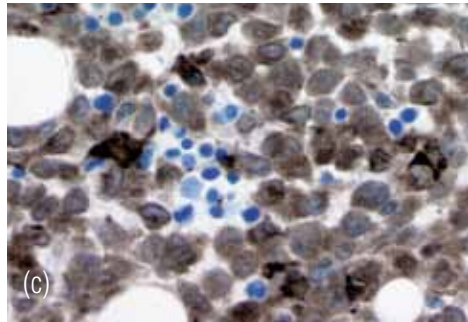
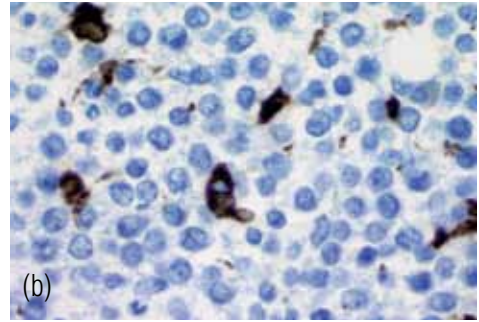
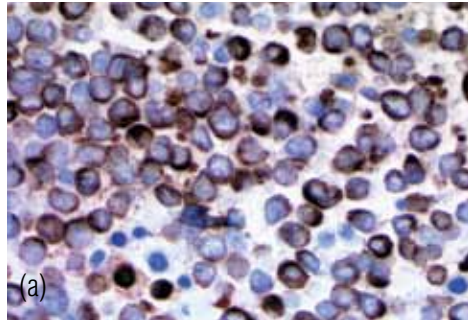
Immunostain for Ki67, low power (left) and high power (right), showing high proliferative activity in lymphoblastic lymphoma.

3. Distinction from AML

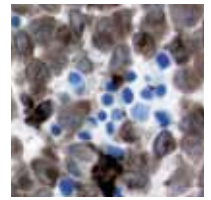
Histologically AML (M 0–5) looks like ALL. The only currently available distinction is the recognition of ALL by B- and T-cell or AML by myeloid-associated immunostaining.

Summary of Key Points

- Aggressive, but potentially curable disease.
- Small blast cells with high mitotic rate.
- Packed marrow (often virtually a total replacement).
- May resemble CLL at low power.



Typical immunostains for AML show (a) M1 myeloperoxidase-positive, but (b) CD68-negative whereas (c) an M5 case is strongly CD68-positive.



Chapter 9 ■ Lymphomas

Introduction

The classification of lymphoma is now generally agreed following the publication of the new WHO classification in 2008, which includes most of the previous “Revised European-American Classification of Lymphoid Lymphomas” = REAL (2004) and WHO (2001) classifications as well as new entities.

The following chapter discusses those lymphomas most likely to be encountered in the bone marrow.

The 2008 World Health Organisation Lymphoma Classification (main types)

Precursor B-cell Neoplasms

- Precursor B-lymphoblastic leukemia/lymphoma

Peripheral B-cell Neoplasms

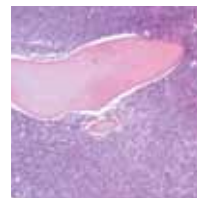
- B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma
- Lymphoplasmacytic lymphoma
- Mantle cell lymphoma
- Follicle center cell lymphoma.
- Marginal zone B-cell lymphoma
- Hairy cell leukemia
- Plasmacytoma/plasma cell myeloma
- Diffuse large B-cell lymphoma
- Burkitt’s lymphoma

Precursor T-cell Neoplasms

- Precursor T lymphoblastic leukemia/lymphoma

Peripheral T-cell and NK-cell Neoplasms

- T-cell chronic lymphocytic leukemia/prolymphocytic leukemia
- Large granular lymphocyte leukemia (LGL)
- Mycosis fungoides/Sézary syndrome
- Peripheral T-cell lymphomas, unspecified
- Angioimmunoblastic T-cell lymphoma (AILD)
- Extranodal NK/T-cell lymphoma, nasal type
- Intestinal T-cell lymphoma
- Adult T-cell lymphoma/leukemia (ATL/L)
- Anaplastic large cell lymphoma (ALCL)



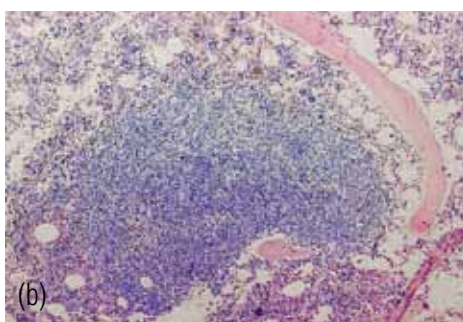
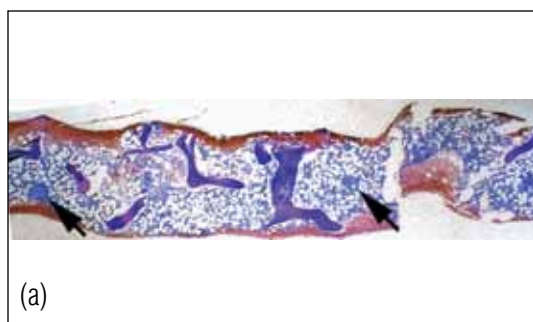
Chapter 10 ■ Chronic Lymphocytic Leukemia (CLL)/ Small Lymphocytic Lymphomas

General Features

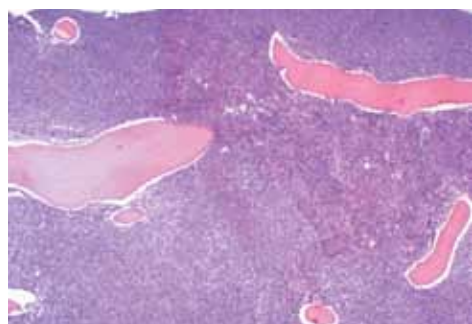
The separation of small lymphocytic lymphoma and chronic lymphocytic leukemia (CLL) is arbitrary and is based on the tissue involved, i.e. blood, bone marrow or lymphoid organs. The neoplastic cell is usually (99%) a B-cell. The rare T-cell variant is difficult to recognize on cell morphology alone.

Histopathology of the Bone Marrow

The most frequent pattern at presentation contains central nodules of neoplastic cells with a diffuse background infiltrate intimately admixed with the hematopoietic tissue. As disease progresses the marrow is progressively filled.



Early presentation of CLL with small centrally located nodules (arrows). (a) Low power. (b) Medium power.

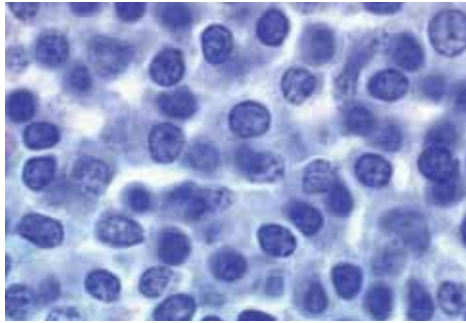


Hypercellular packed marrow of advanced CLL. H&E.

The neoplastic cell is a small lymphocyte with a round nucleus, clumped chromatin and inconspicuous nucleolus.

Richter's syndrome, the transformation of CLL to a large-cell lymphoma, occurs in a small proportion of cases.

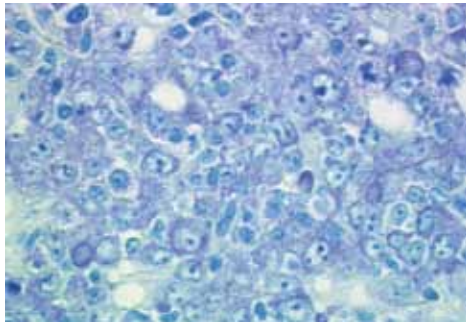
Variations such as prolymphocytic leukemia are more reliably identified on smears. These patients have a more aggressive disease and respond less well to the treatment regimes used for CLL. Prolymphocytes, which must form more than half the neoplastic cell population, have a prominent central nucleolus and more cytoplasm than CLL cells.



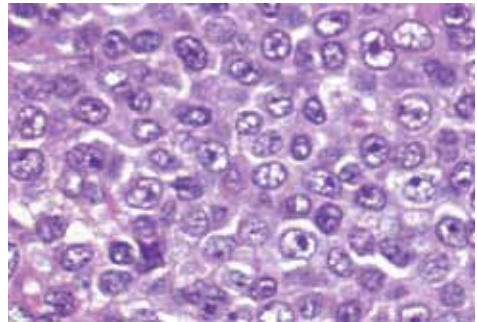
Cytological appearances of CLL in bone marrow sections Giemsa.

Immunophenotype:

CD19+, CD20+, CD79a+, CD5+,
CD23+, CD43+



Richter's syndrome: A large cell lymphoma has arisen in the marrow of a patient previously showing typical CLL.



The appearances of prolymphocytic leukemia in bone marrow.

Many cases of CLL show a degree of plasmacytic differentiation but differ from immunocytoma (Waldenström's macroglobulinemia) both morphologically and immunophenotypically.



Diagnostic Problems

Distinction Between Reactive Lymphoid Nodules and CLL

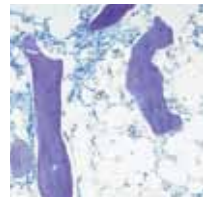
In CLL, the number of nodules is usually more than three in a trephine of >1 cm in length, although confirmation of clonality may be needed to confirm the diagnosis.

Is it possible to distinguish between CLL and small-cell lymphocytic lymphoma on marrow histology?

There are no definite features which allow a reliable distinction to be made.

Summary of Key Points

- This is a low-grade, indolent lymphoma although it is currently incurable.
- The majority are B-cells.
- The marrow is hypercellular and usually has a nodular pattern.
- The nodules have a central, intertrabecular distribution.
- CLL variants are difficult to identify on marrow histology.



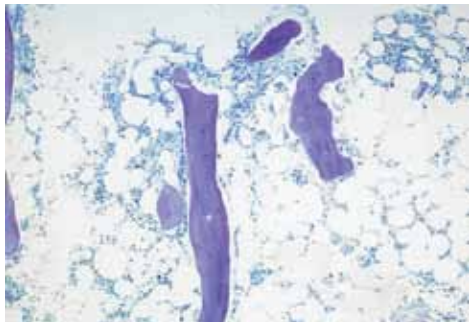
Chapter 11 ■ Lymphoplasmacytic Lymphoma (Immunocytoma) Including Waldenström's Macroglobulinemia

General Features

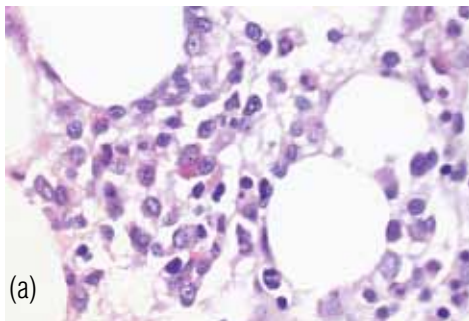
This low-grade B-cell malignancy shows a particular tendency towards plasma cell differentiation and is often associated with the clinical syndrome of Waldenström's macroglobulinemia.

Histopathology of the Bone Marrow

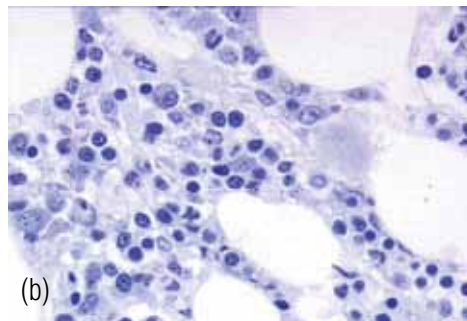
At presentation, the marrow is often hypocellular, but becomes increasingly infiltrated as the disease progresses. The normal hematopoietic tissue is replaced by neoplastic B-cells showing a range of differentiation between lymphocytes and plasma cells.



Immunocytoma: hypocellular marrow at presentation. Giemsa.

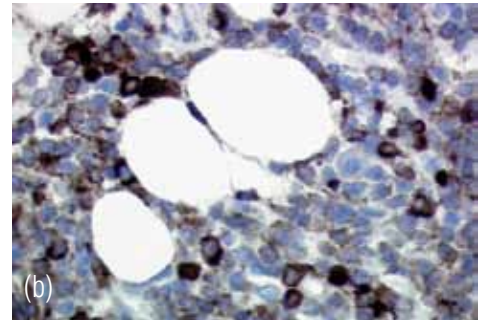
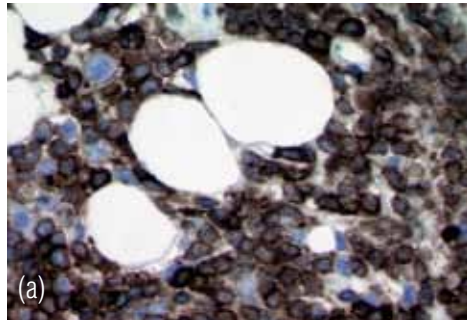


(a)



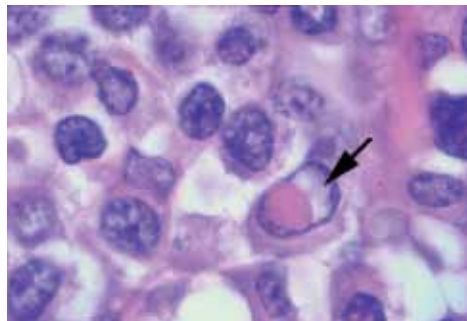
(b)

Immunocytoma is composed of a range of cells from lymphocytes to plasma cells. (a) H&E. (b) Giemsa.



Immunocytoma is a B-cell proliferation (a: CD79) which shows a characteristic expression of the antigen (VS38), ranging from weak on lymphoid to strong on plasma cells. (b) Immunostain (VS38).

IgM and light chain restriction can be demonstrated immunohistochemically in the neoplastic cells. Some of the plasmacytoid cells and plasma cells contain perinuclear accumulations of IgM which indent the nucleus and appear to be intranuclear (Dutcher bodies).

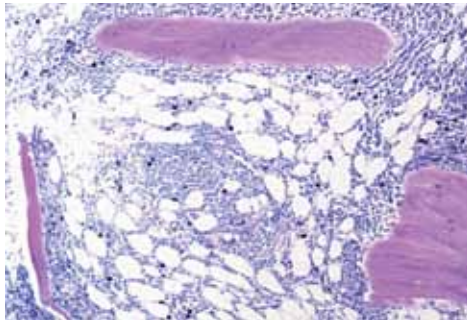
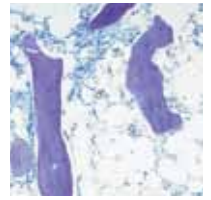


Dutcher body (arrow) in an immunocytoma.

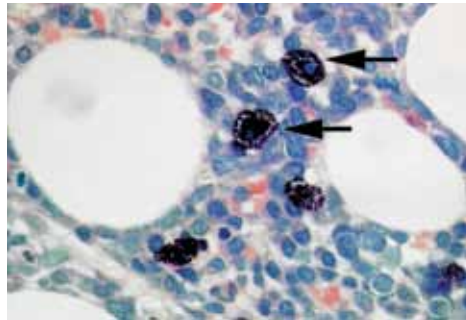
Early involvement is frequently patchy, sometimes resembling CLL with centrally located nodules and at other times follicular lymphoma with paratrabeular aggregations. There is also a sparse diffuse infiltrate spreading through the rest of the marrow easily seen with immunocytochemical assistance. As the disease progresses this enlarges to fill the entire marrow.

Additional features include numerous mast cells and the deposition of an amorphous, eosinophilic, PAS-positive substance, probably IgM.

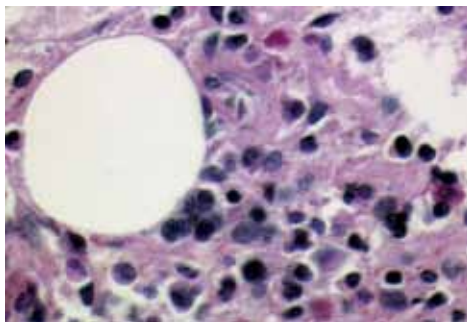
Lymphoplasmacytic Lymphoma (Immunocytoma)



Infiltration of the bone marrow by immunocytoma showing both paratrabecular and central involvement. Giemsa.



Mast cells (arrows) in a case of immunocytoma giving rise to Waldenström's macroglobulinemia. Giemsa.

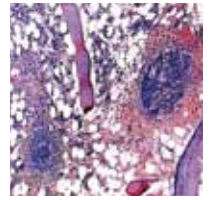


Interstitial deposition of presumed IgM in a case of Waldenström's macroglobulinemia. PAS.

Immunophenotype: positive for surface and cytoplasmic Ig (usually IgM), CD19, CD20, CD22, CD79a, MUM1 and VS38c (plasmacytic component). They lack CD5 and CD10. The strong cytoplasmic Ig staining and lack of CD5 help distinguish it from B-CLL.

Summary of Key Points

- The neoplastic cells have features of both small lymphocytes and plasma cells.
- The neoplastic cells produce cytoplasmic Ig (usually IgM) and lack CD5 and CD23.
- Mast cells are often very prominent.



Chapter 12 ■ Mantle Cell Lymphoma (MCL)

General Features

Originally known as centrocytic lymphoma, the neoplastic cells are now believed to originate from the mantle zone and not from the germinal center. It has a more aggressive course than other low-grade lymphomas and is currently incurable.

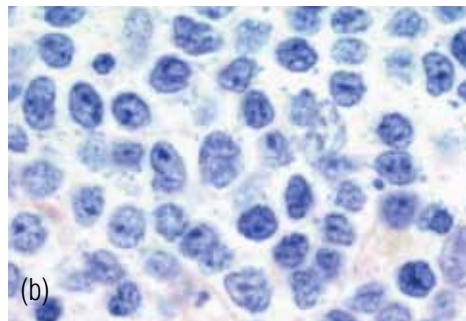
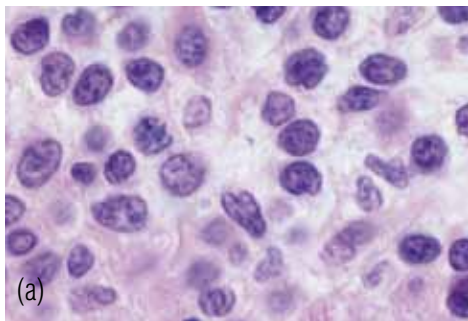
Transformation to a blast cell variant carries an even worse prognosis.

Histopathology of the Bone Marrow

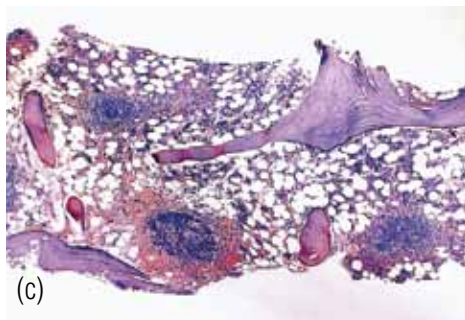
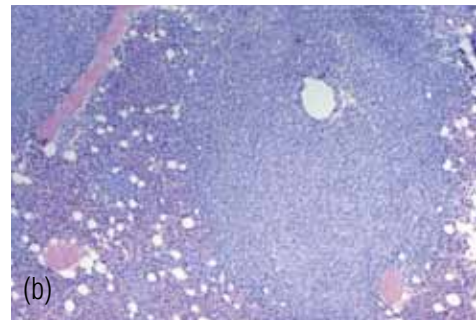
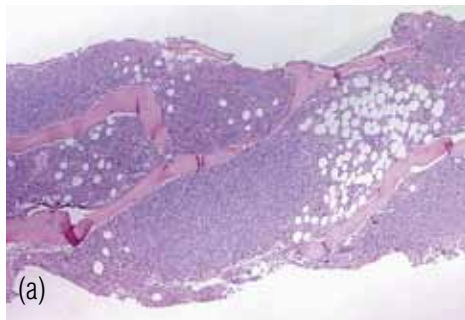
The marrow infiltrate consists of a uniform population of small to medium lymphoid cells with cleaved or angular nuclei, dispersed chromatin, inconspicuous nucleoli and scanty cytoplasm.

Unlike follicular lymphoma virtually no larger cells, i.e. centroblasts, are present. Marrow involvement may be both paratrabecular and diffuse.

Immunophenotype: Positive for surface IgM, CD19, 20, 22, 79a, CD5, CD43 and cyclin D1, and negative for CD23.



Cytological appearances of mantle cell lymphoma. (a) H&E. (b) Giemsa.



Marrow involvement by mantle cell lymphoma shows a variety of patterns as illustrated by three different cases. (a) H&E. (b and c) Giemsa.

Differential Diagnosis of Small B-cell Lymphoma

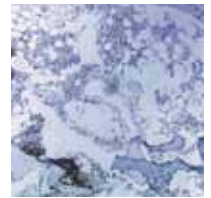
	CLL	Follicular Lymphoma	MCL
CD5	+	-	+
CD23	+	-/+	-
Cyclin D1	-	-	+
BCL6	-	+	-
CD10	-	+	-

Diagnostic Problems

Immunohistochemistry is particularly useful in distinguishing CLL, follicular lymphoma and MCL.

Summary of Key Points

- Morphologically the neoplastic cell may resemble a centrocyte or lymphocyte.
- MCL has a more aggressive clinical course than most other lymphomas.
- Rare blastoid variants have an even worse prognosis.
- Immunohistochemistry may be required to differentiate between CLL and follicular lymphoma.



Chapter 13 ■ Follicular Lymphoma

General Features

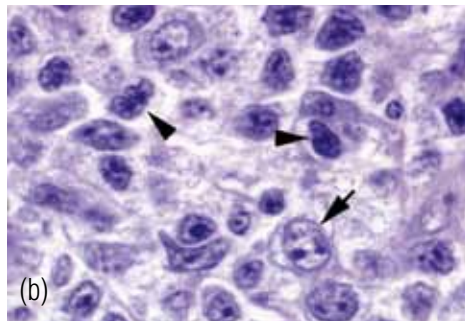
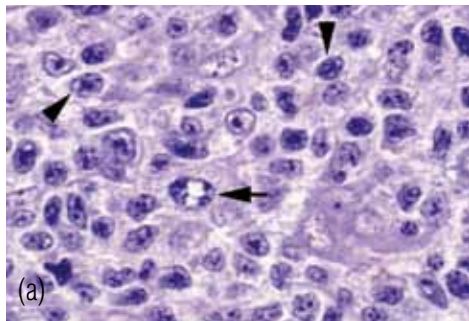
Follicular lymphoma (also known as centroblastic/centrocytic lymphoma) is a common type of non-Hodgkin's lymphoma in the Western hemisphere.

Histopathology of the Bone Marrow

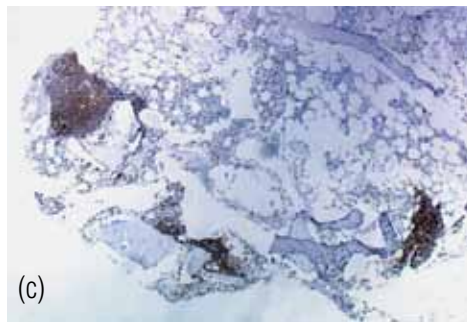
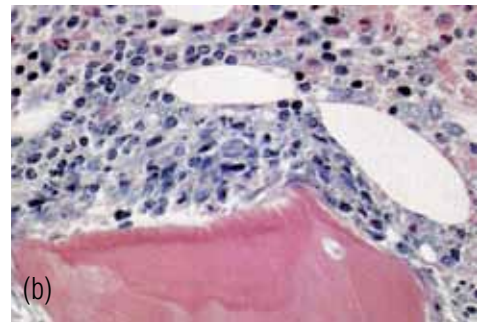
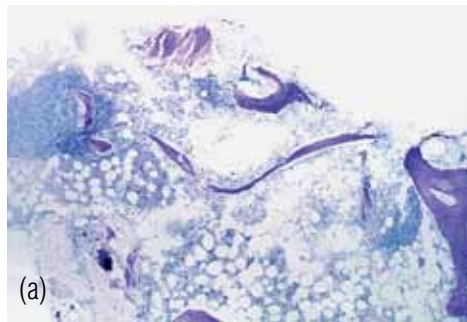
These lymphomas are composed of two cell types: centrocytes (cleaved follicle center cells) which predominate and centroblasts (large non-cleaved follicle center cells).

Early involvement is paratrabecular but with progression this increases in size and coalesces to fill the marrow space.

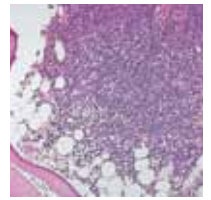
Follicular lymphoma may undergo transformation into a large-cell NHL.



(a and b) Centroblasts (arrow) and centrocytes (arrowheads) in a follicular lymphoma in the bone marrow. Giemsa.



The paratrabecular location of follicular lymphoma in bone marrow (a and b: Giemsa) is highlighted by B-cell immunostaining (c).



Chapter 14 ■ Marginal Zone Lymphoma (Including MALT)

General Features

The WHO 2008 classification uses the term 'marginal zone lymphoma' to include extra-nodal (MALT), splenic and nodal (former monocytoid B-cell lymphoma) lymphomas.

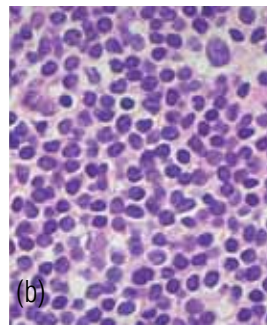
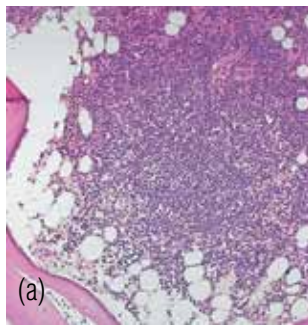
Histopathology of the Bone Marrow

Marginal Zone Lymphomas

Involvement of the bone marrow is relatively uncommon, but occurs either as central nodules or a patchy diffuse infiltrate.

Splenic Marginal Zone Lymphomas

Most cases first involve the bone marrow as central nodules before becoming diffuse.



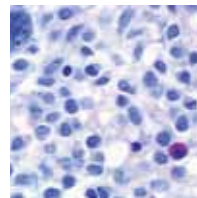
Splenic lymphoma involving the bone marrow. (a) H&E. (b) CD20 (B cell immunostain).

Immunophenotype:

IgM+, IgD+, CD19+, CD20+, CD22+, CD79a+, CD5-, CD10-, CD23-, BCL6-.

Summary of Key Points

- Marginal zone lymphomas are mainly comprised of MALT lesions and their extensions into lymph nodes and other tissues.
- MALT lesions rarely involve bone marrow unlike splenic lymphomas where marrow infiltration is common.
- Splenic lymphoma is probably an entity distinct from marginal zone lymphoma.



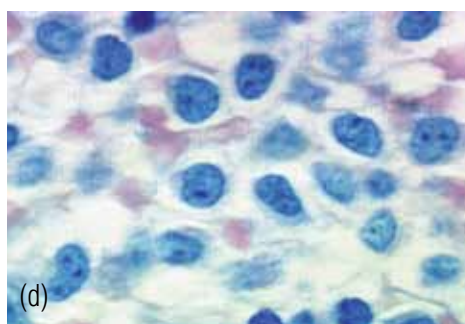
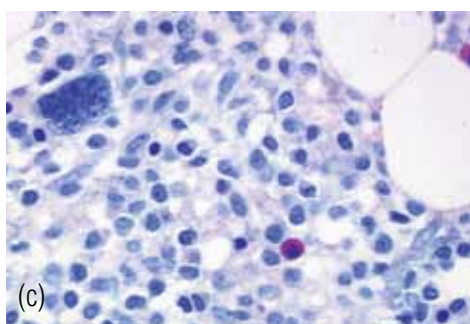
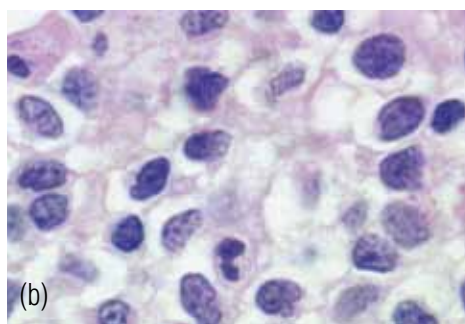
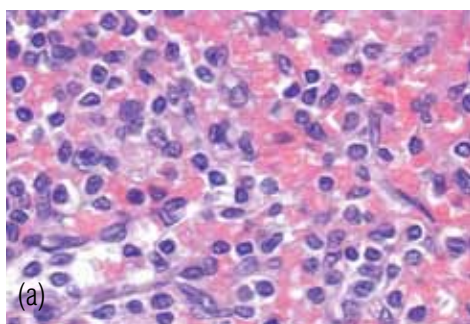
Chapter 15 ■ Hairy Cell Leukemia

Introduction

Hairy cell leukemia is a low-grade lymphoproliferative disorder of B lymphocytes now recognized as a distinct entity.

Histopathology of the Bone Marrow

The cells are small (their nuclei being slightly larger than a resting lymphocyte) and lie spaced apart. The nuclei are oval or 'bean'-shaped, have one or two small inconspicuous nucleoli and are surrounded by a clear zone of cytoplasm. The so-called hairs seen in smears and by electron microscopy are not apparent in conventional tissue sections. Mitotic figures are rare.



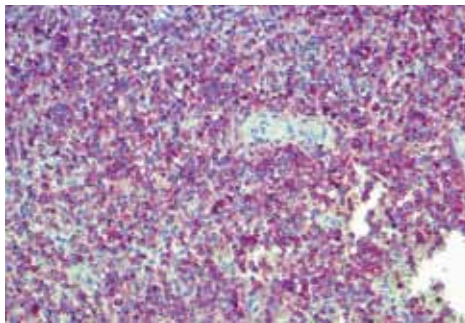
Typical case of hairy cell leukemia in a bone marrow trephine. (a and b) H&E, medium and high power. (c and d) Giemsa, medium and high power.

Patterns of Involvement

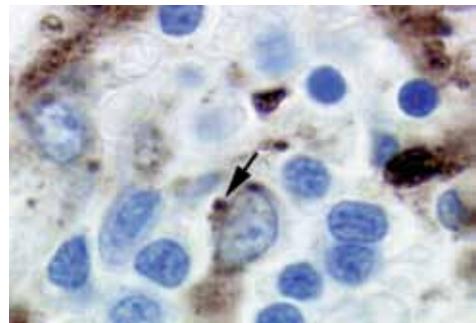
The marrow is usually hypercellular with three patterns of involvement:

1. Focal involvement. The most common pattern.
2. Diffuse involvement.
3. Interstitial involvement. The hairy cells lie amongst fat spaces in a hypoplastic marrow.

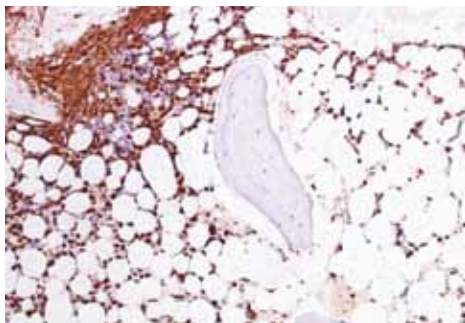
Hairy cells have a characteristic immunophenotype on paraffin sections being positive for CD79a and CD20, combined with Annexin1, CD123, CD103, CD68 and DBA.44.



CD79a



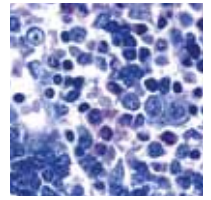
CD68



DBA.44

Summary of Key Points

- Marrow involvement by hairy cells may be subtle.
- Characteristic histology and cytology.
- Immunostains show mixed B and histiocytic phenotype.



Chapter 16 ■ Multiple Myeloma

Introduction

Multiple myeloma is characterized by:

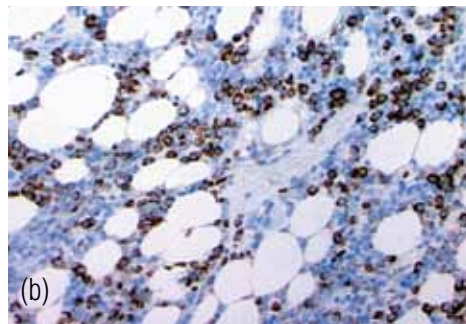
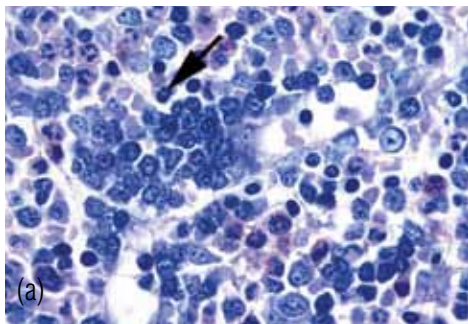
- increased numbers of clonal plasma cells within the bone marrow (> 10% of the nucleated cell population),
- lytic bone lesions, and
- the presence of an M (or paraprotein) band identified electrophoretically in serum or urine.

Histopathology of the Bone Marrow

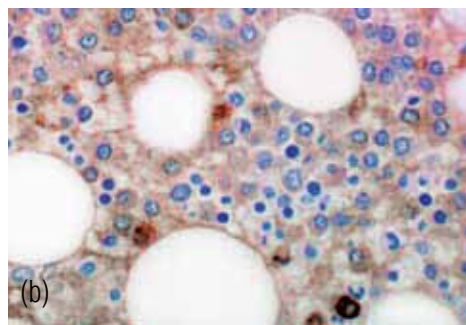
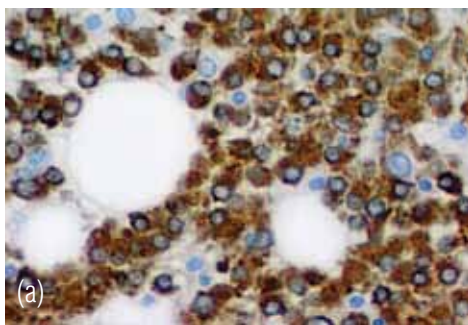
The marrow is usually hypercellular and overrun by neoplastic plasma cells. Abnormal forms (bi- or multi-nucleate and blast cells) are typically present, but the only certain means of separating these is by demonstrating monoclonality for light chains.

Immunophenotype:

Cytoplasmic Ig+, light chain restriction+, CD79a+, MUM1+, Vs38c+.



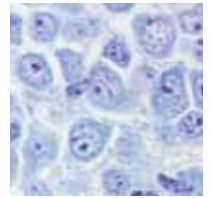
Neoplastic plasma cells (arrow) in (a) are often better appreciated by immunostaining with an antibody such as VS38 which identifies plasma cells (b).



Light chain restriction in myeloma. Lambda-positive myeloma cells (a) greatly outnumber a few remaining benign kappa-positive cells (b).

Summary of Key Points

- Immunohistochemistry is invaluable in separating reactive plasmacytosis from multiple myeloma and in identifying cases of 'early' myeloma where the number of plasma cells is low.
- The degree of plasma cell differentiation and pattern of marrow involvement may relate to prognosis.
- Whilst most cases of myeloma are diagnostically straightforward, a few cases will involve the marrow more subtly. Careful inspection of the marrow section and the use of immunocytochemistry will reveal them.



Chapter 17 ■ Diffuse Large B-Cell Lymphoma

General Features

Within the WHO 2008 classification, this entity encompasses centroblastic, immunoblastic, T-cell-rich B-cell lymphoma and other subtypes since even experienced pathologists have great difficulty in recognizing those subdivisions.

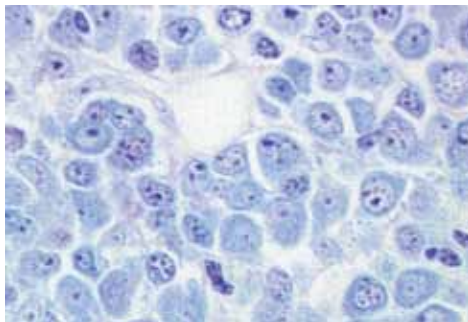
Histopathology of the Bone Marrow

The neoplastic cells have large vesicular nuclei, prominent nucleoli, basophilic cytoplasm and easily found mitotic figures. There is a variability in the morphology of these large cells which gave rise to their description as separate categories in other classifications. In practice these lymphomas usually have a heterogeneous cell population which has made for high intra- and inter-observer error in classification.

Immunophenotype:

Surface Ig+/-, CD19+, CD20+, CD22+, CD79a+, CD5-/+ , CD45+/- . Subtypes: GC-LIKE CD10+, BCL6+, MUM1-, AC-LIKE CD10-, BCL6-, MUM1+

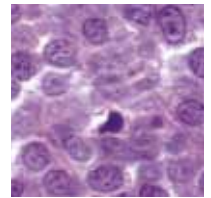
GC= Germinal Center-Like; AC= Activated Cell-Like



Typical example of large-cell lymphoma in the bone marrow. Note the high degree of cellular heterogeneity. Giemsa.

Summary of Key Points

- Diffuse large B-cell lymphomas consist of several morphological types.
- They are clinically aggressive, but potentially curable.
- Distinction must be made from anaplastic carcinoma.



Chapter 18 ■ Burkitt's Lymphoma

General Features

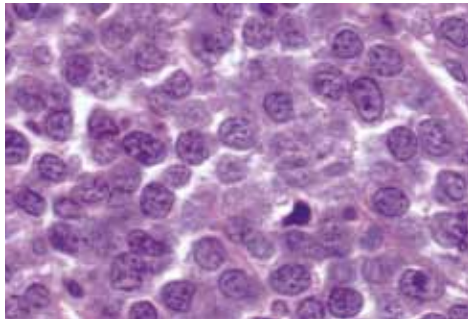
This lymphoma is endemic in children (4–7 years) in equatorial Africa and New Guinea. It is associated with Epstein–Barr Virus (EBV) and involves a chromosomal translocation commonly t(8;14). The non-African (non-endemic) form is rare, occurs over a wider age range and is associated with HIV infection.

Histopathology of the Bone Marrow

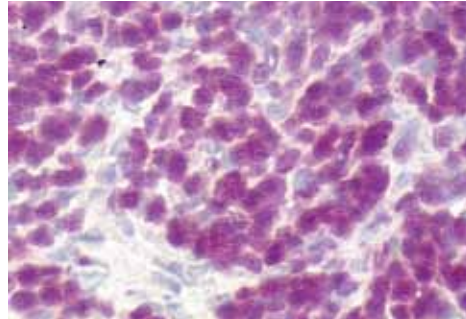
The marrow is not usually involved at presentation except with HIV. The neoplastic cell is a medium-sized blast cell with a moderate amount of basophilic cytoplasm and a regular oval or round nucleus containing 2–5 small nucleoli. Mitotic figures are numerous, although the 'starry sky' appearance seen in other organs is not a feature. The marrow is usually hypercellular.

Immunophenotype:

SIgM+, CD19+, CD20+, CD22+, CD79a+, CD10+, BCL6+, CD5-, CD23-, TdT-, BCL2-



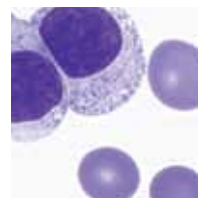
H&E



Ki67

Summary of Key Points

- Two forms exist: An endemic and a non-endemic. Although histologically identical, they differ clinically.
- Very high proliferation rate as demonstrated by antibodies, e.g. by the Ki-67 marker clone MIB-1.



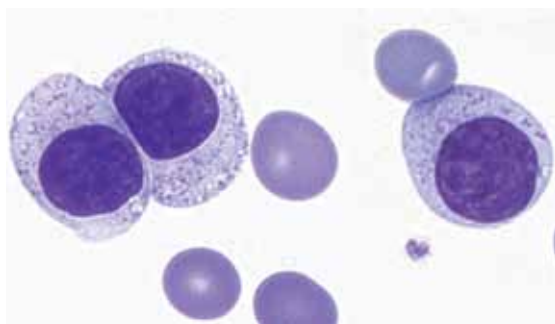
Chapter 19 ■ Large Granular Lymphocyte (LGL) Leukemia

General Features

This is an uncommon condition also described as CD8 lymphocytosis with neutropenia or T-lymphoproliferative disease. The peripheral blood lymphocytosis is composed of cells with round or oval nuclei with moderately condensed chromatin and rare nucleoli, eccentrically placed in abundant pale blue cytoplasm with azurophilic granules. There are two major lineages, one being T-cell (indolent) and the other having a natural killer (NK) cell phenotype (more aggressive).

Histopathology of the Bone Marrow

Bone marrow infiltration is usually sparse with the diagnosis being made on the peripheral blood smear or splenic histology.



The cytology of large granular lymphocytes in peripheral blood.

Immunophenotype T-cell:

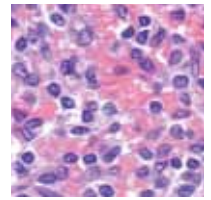
CD2+, CD3+, CD5-, CD7-, TCR $\alpha\beta$ +, CD4-, CD8+, CD16+, Cytotoxic granules (TIA-1, GRANZYME B)+, CD56-, CD57+/-, CD25-

Immunophenotype NK-cell:

CD2+, CD3-, TCR $\alpha\beta$ -, CD4-, CD8+/-, CD16+, CD56+/-, CD57+/-, Cytotoxic granules (TIA-1, GRANZYME B)+

Summary of Key Points

- LGL leukemia has two types, T-cell and NK-cell.
- The clinical course is usually indolent apart from a subset of aggressive Asian NK cases.
- Marrow infiltration is subtle and may easily be missed.



Chapter 20 ■ Cutaneous T-Cell Lymphoma

General Features

Most primary lymphomas of the skin are of two T-cell types, mycosis fungoides or Sézary syndrome.

Histopathology of the Bone Marrow

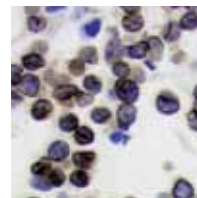
The marrow is rarely involved, even in patients with advanced disease or Sézary syndrome. The neoplastic cell is usually of the T-cell helper (CD4) type and morphologically is small with a convoluted (cerebriform) nucleus. Occasional larger cells may be present and these too have convoluted nuclei. There is no characteristic pattern of marrow involvement.

Immunophenotype:

CD2+, CD3+, CD5+, CD7+ (30%), CD4+, CD25-

Summary of Key Points

- Primary cutaneous T-cell lymphoma has two clinical forms.
- It usually is of T helper immunophenotype.
- The marrow is rarely involved.



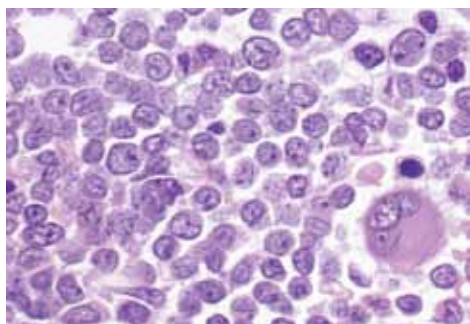
Chapter 21 ■ Peripheral T-Cell Lymphomas, Unspecified

General Features

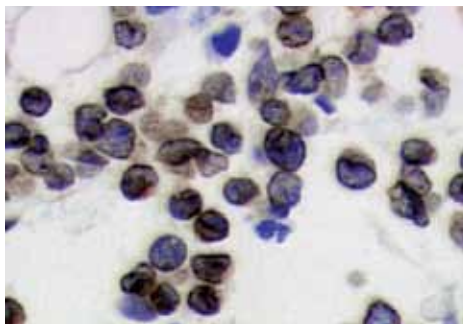
The WHO 2008 classification recognizes the difficulty faced by histopathologists in subclassifying T-cell lymphomas and has created a category called 'peripheral T-cell lymphomas, unspecified'. This includes many of the morphological subtypes which do not correspond to any of the specifically defined entities in that classification.

Histopathology of the Bone Marrow

The pattern of infiltration is variable and can be interstitial, paratrabeular or both. The infiltrate is similar to that in the tissues being polymorphous with variable numbers of small, medium and large neoplastic atypical lymphoid cells admixed with eosinophils and histiocytes, some of which may be epithelioid. The neoplastic cells have irregular nuclei and some larger cells may resemble Reed–Sternberg cells.



H&E



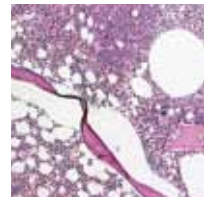
T-cell CD3

Immunophenotype:

CD3+/-, CD2+/-, CD5+/-, CD7-/+, CD4>CD8 may be CD4-, CD8-, may be CD45RA+ and CD45RO-

Summary of Key Points

- Histological classification of T-cell lymphomas is difficult.
- The WHO 2008 classification groups together a number of lymphomas previously recognized as separate entities in other classifications.
- Histological grading of T-cell lymphomas is less predictive of clinical behavior than histological grading of B-cell lymphomas.



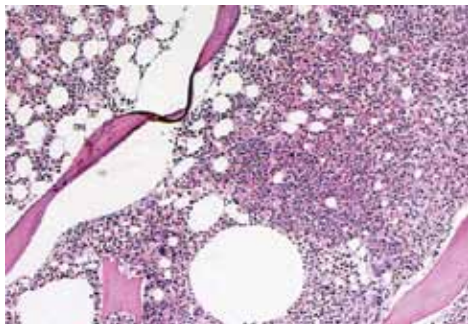
Chapter 22 ■ Angioimmunoblastic Lymphoma (AIL)

General Features

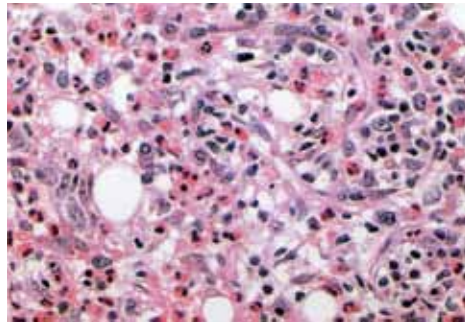
This is an uncommon condition with a variable clinical course. Patients usually have fever, generalized lymphadenopathy, skin rash and raised polyclonal gammaglobulins.

Histopathology of the Bone Marrow

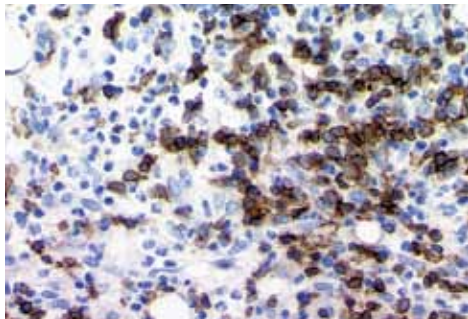
The marrow is involved in a significant number of cases. Focal areas of fibrosis and increased vascularization are seen though the complex arborizing vasculature with PAS-positive walls seen in affected lymph nodes is much less obvious and may be absent. Within these areas a highly polymorphic T-cell population is found.



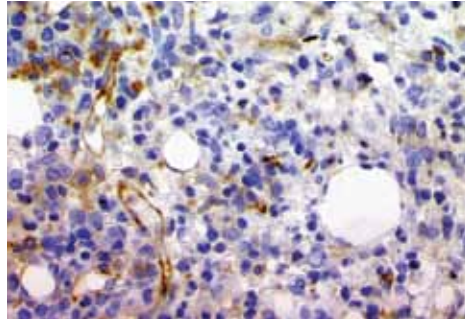
H&E



H&E



CD3 T-cell



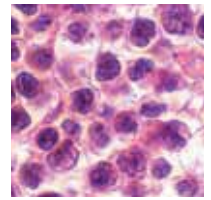
CD31

Immunophenotype:

CD2+, CD3+, CD4+/-, CD5+, CD7+, CD10+, BCL6+, CD8-

Summary of Key Points

- Angioimmunoblastic lymphoma has a variable clinical course.
- Marrow lesions lack the characteristic architectural features seen in lymph nodes.



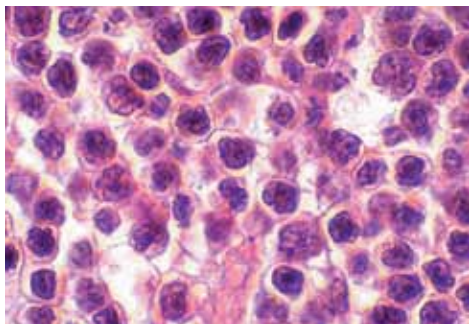
Chapter 23 ■ Adult T-Cell Lymphoma/Leukemia

General Features

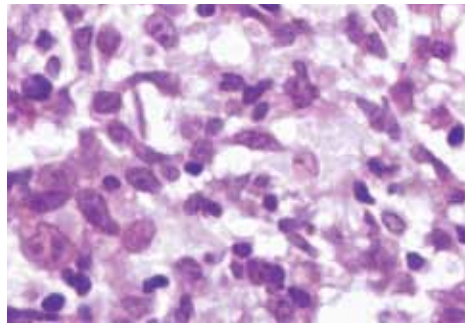
This occurs mainly in Japan and the Caribbean. It is caused by the human T-cell lymphotropic virus (HTLV-1), a type C retrovirus. It usually involves adults and has an aggressive course with leukemia, hypercalcemia, hepatosplenomegaly and lytic bone lesions.

Histopathology of the Bone Marrow

The marrow is involved in the majority of cases and tends to have a diffuse pattern which ranges from sparse to packed. There is marked cellular pleomorphism and the variation in cell size from case to case is great.



Packed



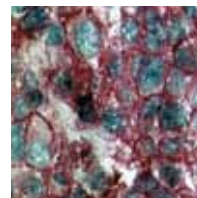
Sparse

Immunophenotype:

CD2+, CD3+, CD4+, CD5+, CD25+, CD7-

Summary of Key Points

- Adult T-cell lymphoma is caused by a retrovirus, HTLV-1.
- The disease is usually clinically aggressive.
- The marrow is involved to varying degrees in the majority of patients with highly pleomorphic neoplastic cells.
- Patients will have antibodies to HTLV-1.



Chapter 24 ■ Anaplastic Large-Cell Lymphoma

General Features

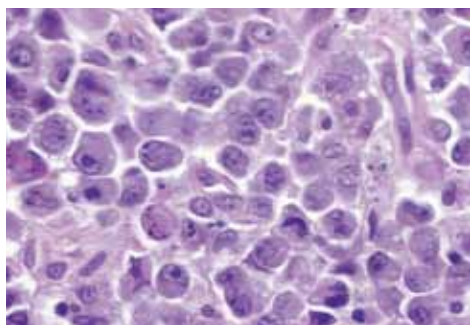
This is a recently recognized entity which expresses CD30 and in many cases (up to 75% in some series) T-cell markers. Many cases have a translocation between chromosome 2 and 5 juxtaposing two genes, nucleophosmin (NPM) and anaplastic lymphoma kinase (ALK), creating a new fusion protein p80. This may be recognized by nuclear staining for alk protein.

There appears to be two forms of the disease:

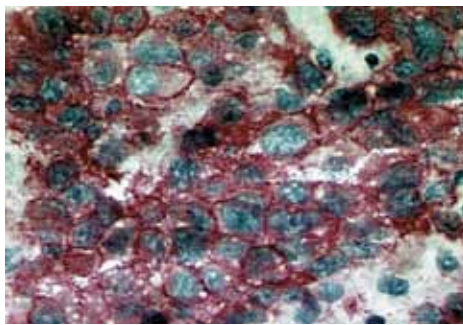
1. The systemic form involves lymph nodes or extranodal sites. It has a bimodal age distribution, i.e. older children (mostly alk+) and the elderly (many alk-). Alk+ lymphomas appear to have a better prognosis than other high-grade large-cell lymphomas.
2. The primary cutaneous form (alk-) tends to occur in adults and has a rather indolent and incurable course although it may spontaneously regress. It may be related to lymphomatoid papulosis type A.

Histopathology of the Bone Marrow

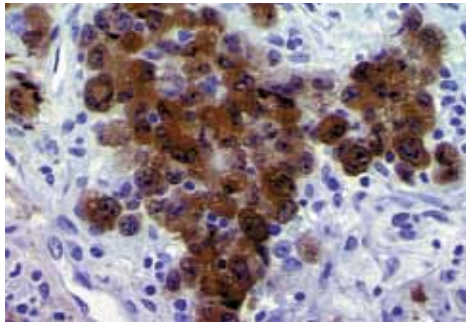
The neoplastic cells are large, have abundant cytoplasm and large pleomorphic nuclei with prominent multiple eosinophilic nucleoli. Large sheets of cells often occur and impart a cohesive appearance. Marrow involvement may be focal or a diffuse pattern with the diagnosis resting on the immunohistochemistry.



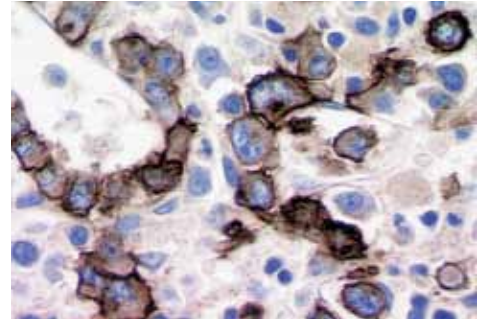
H&E



CD30



ALK



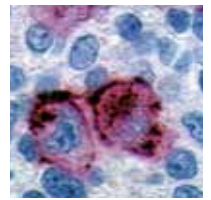
EMA

Immunophenotype:

CD30+, EMA+/-, CD43+/-, CD2+/-, CD5+/-, CD4+/-, TIA-1+, GRANZYME B+,
PERFORIN+, CD45-/+ , CD25+/-, CD15-/+ , CD3+/-, p80+/-

Summary of Key Points

- ALCL has a characteristic morphology and immunophenotype.
- ALCL has two clinical forms: Systemic and primary cutaneous.
- Its cohesive growth pattern may mimic metastatic carcinoma.



Chapter 25 ■ Hodgkin's Lymphoma

Introduction

The WHO 2008 classification divides Hodgkin's lymphoma into nodular lymphocyte predominant and classical varieties (mixed cellularity, nodular sclerosis, lymphocyte-rich and lymphocyte depletion). It is now clear that lymphocyte predominance is a separate entity and is a form of B-cell follicular proliferation.

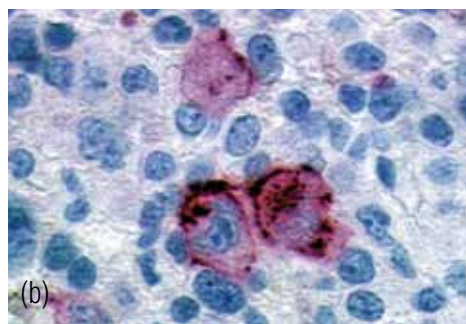
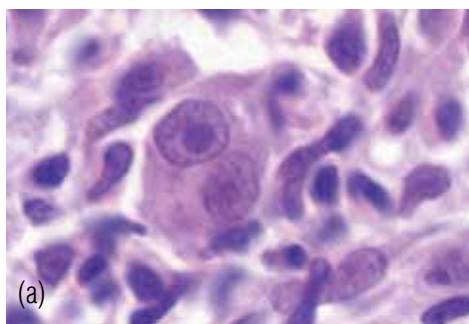
Histopathology of the Bone Marrow

Classical Hodgkin's Disease:

The bone marrow appearances of mixed cellularity or nodular sclerosis are indistinguishable so that subtyping must be performed on a lymph node biopsy. Usually the marrow shows focal involvement. In most cases of nodal disease with spread to the marrow, diagnostic cells are rare but a combination of focal fibrosis and abnormal mononuclear cells (RS cells are not essential if node involvement has been confirmed histologically) with appropriate immunostaining will allow a confident diagnosis. It is very unusual for Hodgkin's disease of any subtype to present solely as a bone marrow infiltration. In such cases it will be essential to identify a definite Reed-Sternberg cell.

Lymphocyte Predominance Hodgkin's Disease:

It is doubtful whether bone marrow involvement occurs other than when there has been a transformation to a large B-cell lymphoma.



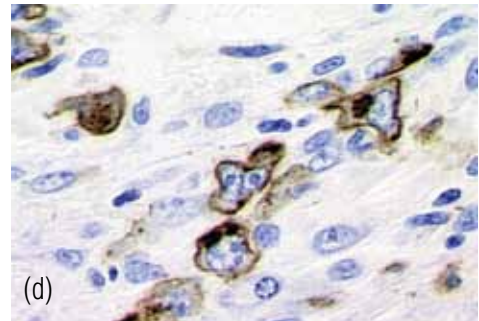
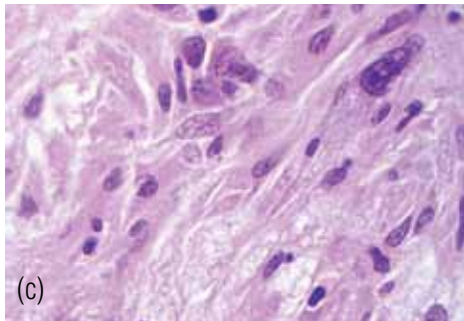
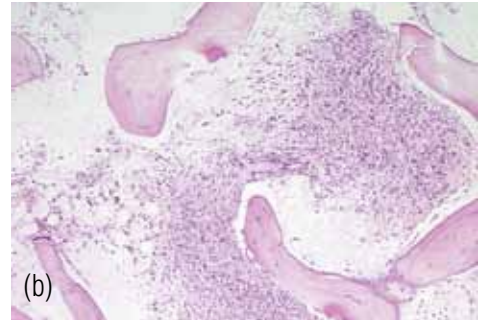
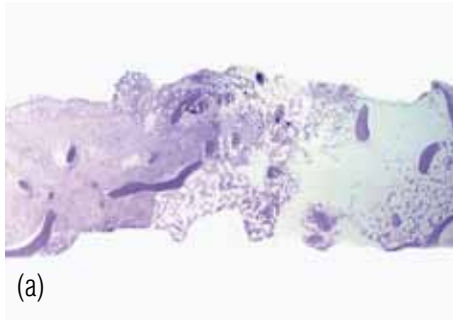
Diagnostic Reed-Sternberg cell in the bone marrow. (a) H&E. (b) Immunostain for CD30.

Immunophenotype Classical Reed-Sternberg cells:

CD15+, CD30+, PAX5+/-, CD45-, EMA-, OCT2-, BOB1-, PU.1-, J chain- B-cell antigens rare (except CD20).

Immunophenotype Lymphocyte Predominance (L&H) Cells:

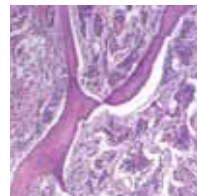
CD15-, CD30-, PAX5+, BCL6+. CD45+, EMA+/-, J chain+ B-cell antigens +, OCT2+, BOB1+, PU.1+.



Typical involvement of the bone marrow by Hodgkin's disease. (a-c) H&E. (d) Immunostain for CD30.

Summary of Key Points

- Classical Hodgkin's disease causes a fibrotic response, typically, patchy in the bone marrow.
- Involvement of the marrow is rare at presentation, but relatively common at relapse.
- Immunocytochemical examination is helpful in distinguishing it from other fibrosing conditions of the bone marrow.



Chapter 26 ■ Metastatic Disease

Introduction

Metastatic spread of carcinoma and sarcoma to the bone marrow is relatively common as a terminal event when there is usually little or no clinical justification for a biopsy.

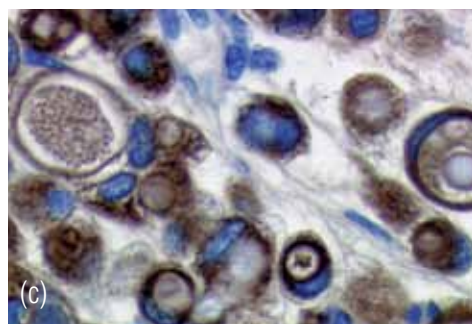
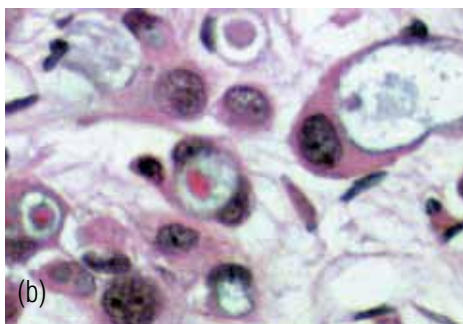
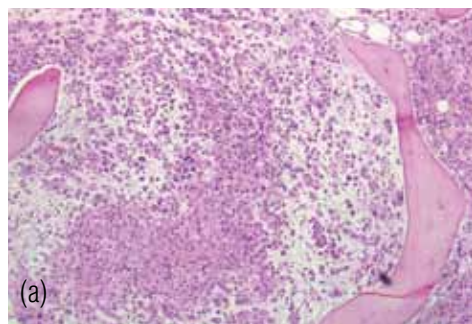
Histopathology of the Bone Marrow

Carcinoma

The bone marrow is virtually always grossly abnormal even at low power. Either the marrow will be heavily infiltrated by recognizable tumor or extensively fibrosed. In both cases immunocytochemistry is useful to ensure that lymphoma is not overlooked. Occasionally, the site of the primary tumor is unclear clinically so that immunostaining for endocrine and prostatic antigens can be helpful.

Lymphocyte Predominance Hodgkin's Disease:

It is doubtful whether bone marrow involvement occurs other than when there has been a transformation to a large B-cell lymphoma.



Secondary breast cancer in the bone marrow. (a and b) H&E. (c) Epithelial membrane antigen (EMA) immunostain.

Pediatric Tumors

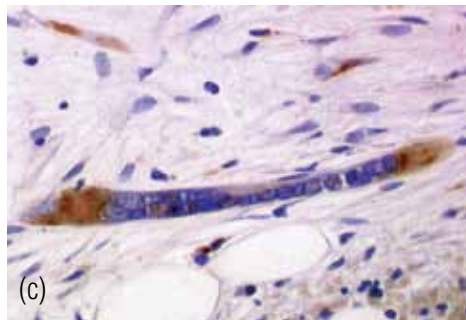
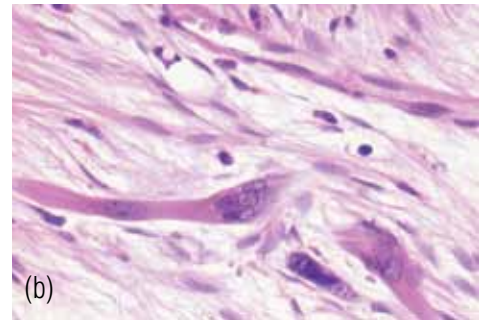
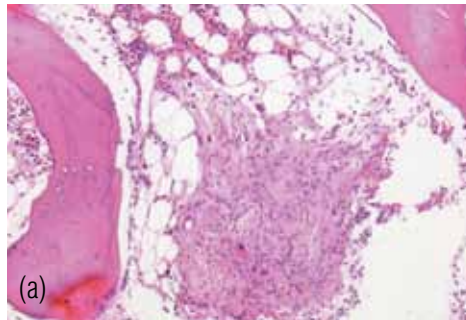
Bone marrow trephines are taken as part of most oncology protocols for the assessment of solid tumors in childhood.

Carcinoma:

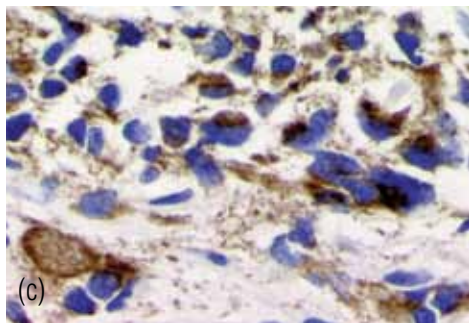
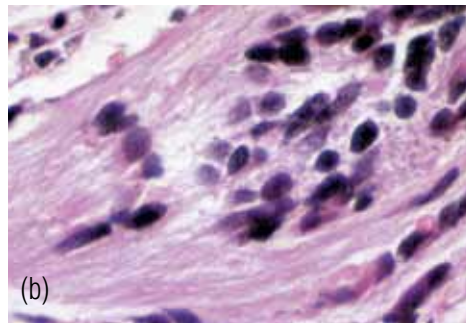
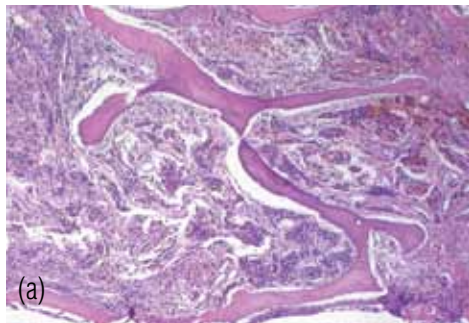
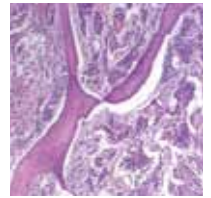
Thyroid:	Cytokeratin+, Epithelial membrane antigen (EMA)+, CD45-Thyroglobulin+, Calcitonin+ (medullary), TTF-1+
Prostate:	Prostatic acid phosphatase or prostate-specific antigen+, PSMA+ and AMACR+

Pediatric tumors:

Neuroblastoma:	NB84+, Neurofilaments+, NCam+
Rhabdomyosarcoma:	Desmin+, Myoglobin+, Myogenin+, MyoD1+.
Ewing's tumor:	NB84+, Neurofilaments-, NCam-



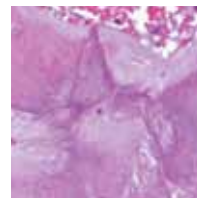
Involvement of the bone marrow by rhabdomyosarcoma. (a and b) H&E. (c) Immunostain for desmin.



Neuroblastoma in the bone marrow. (a and b) H&E. (c) Immunostain for neural marker NB84.

Summary of Key Points

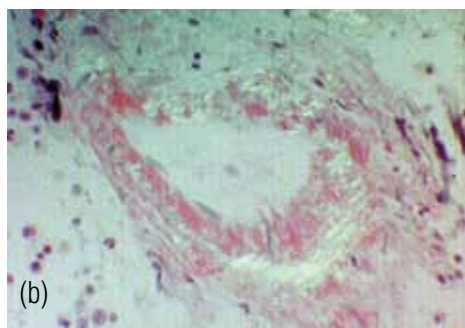
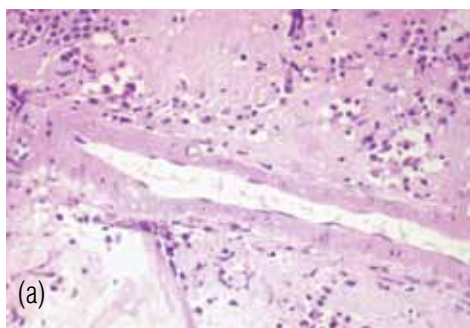
- Metastatic disease is a relatively uncommon indication for a bone marrow trephine.
- Involvement of the marrow is usually obvious.
- Immunocytochemical examination is helpful in distinguishing it from other fibrosing conditions of the bone marrow and in identifying different types of tumor.



Chapter 27 ■ Miscellaneous

Amyloidosis

This is a rare finding in a marrow biopsy. The amount of amyloid present is variable and the histological changes may be subtle. It is present in the walls of vessels and also within the stroma surrounding the hematopoietic tissue. As in other tissues it is extracellular and can be demonstrated histochemically using the Congo-Red stain. In marrows containing amyloid derived from immunoglobulin light chain, a neoplastic clone of plasma cells may also be detected.



Amyloidosis in bone marrow. (a) H&E. (b) Congo-Red under polarized light.

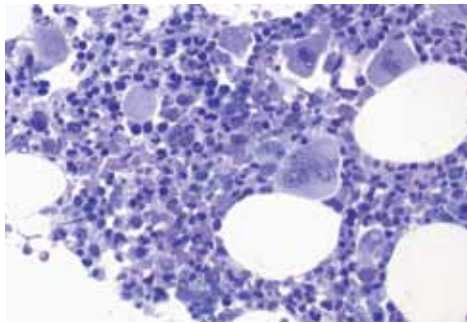
Immune Thrombocytopenic Purpura (ITP)

ITP is due to the removal of antibody-coated platelets from the circulation.

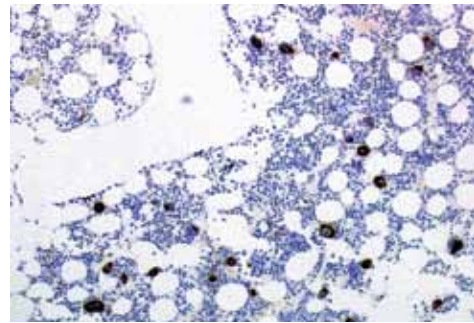
The auto-antibodies are produced by the body in a number of different circumstances, the most common being:

- autoimmune disorders, e.g. SLE (systemic lupus erythematosus);
- viral infections, e.g. HIV, EBV, CMV;
- drug-induced, e.g. carbamazepine, chlorothiazide;
- lymphoproliferative disorders, e.g. Hodgkin's disease, CLL;
- idiopathic, i.e. of unknown origin.

The marrow is relatively normal apart from an increased number of small megakaryocytes.



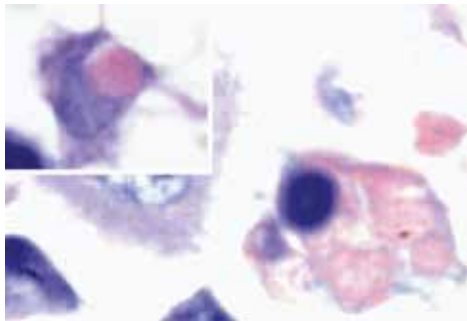
Increased numbers of small megakaryocytes in chronic ITP. Giemsa.



Increased numbers of megakaryocytes in ITP are easier to detect by immunostaining (CD31 in this case).

Erythrophagocytosis

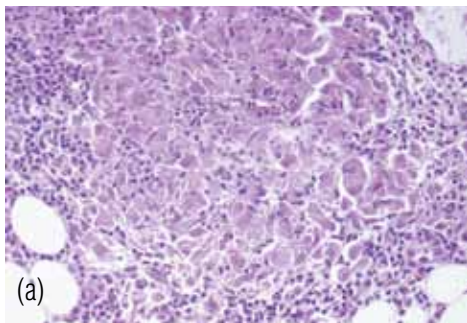
Erythrophagocytosis refers to those conditions where many, if not the majority of the macrophages contain phagocytosed red cells. This is usually of viral origin or associated with a T-cell lymphoma.



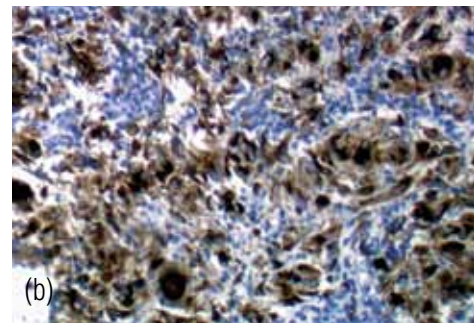
Erythrophagocytosis: Macrophage stuffed with erythrocytes compared with an example containing a single red cell (insert). Giemsa.

Granulomas

There are numerous causes for granuloma formation and the pathologist can often only provide a differential diagnosis relevant to the clinical details. In a percentage of cases no cause will ever be found.

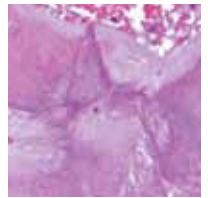


(a)



(b)

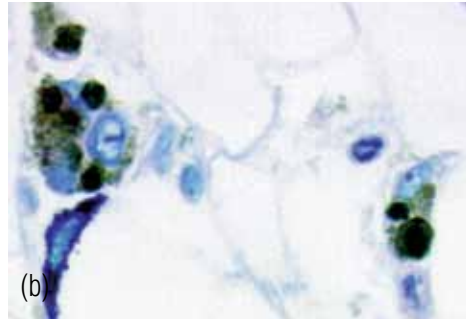
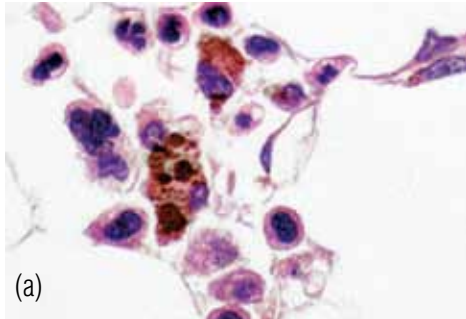
Granuloma in bone marrow. (a) H&E. (b) Immunostain for CD68 (macrophage marker).



Hemosiderin

Hemosiderin can be detected in both H&E and Giemsa stained sections. It takes the form of fine and coarse granules which appear brown on H&E and olive green on Giemsa stained sections.

The granules are present within macrophages and may be associated with erythroid colonies.



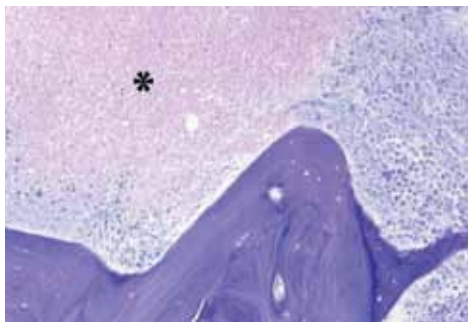
Iron in macrophages following blood transfusion. (a) H&E. (b) Giemsa.

Necrosis

When examining a marrow trephine, it is important to make the distinction between:

- necrosis involving the bone and marrow, such as is seen in sickle cell disease, embolism, caisson disease (the 'bends'), sepsis, and
- necrosis predominantly affecting the marrow tissue, which is almost invariably associated with malignancy, such as acute leukemia, non-Hodgkin's lymphoma, Hodgkin's disease and metastatic carcinoma.

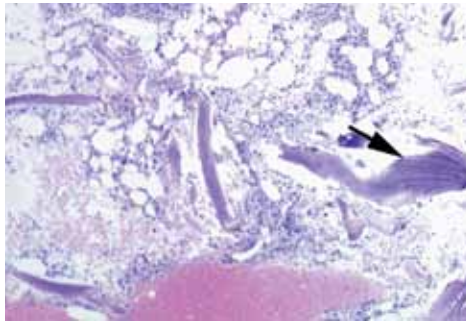
Immunohistochemistry may be useful in identifying the nature of the dead cells, e.g. anti-cytokeratin antibodies have been shown to be useful in this respect.



Necrosis: Transition from viable leukemia cells to total necrosis (*). Giemsa.

Osteoporosis

Osteoporosis is a decreased amount of bone per unit volume seen in a tissue section by a thinning of the trabeculae. Trabecular thickness varies individually and unless the condition is severe it is not that easy to identify osteoporosis in a routine trephine.



Osteoporosis: Typical, thin, easily fractured trabeculae. Note the osteoid seams are relatively normal (arrow). Giemsa.

Renal Osteodystrophy

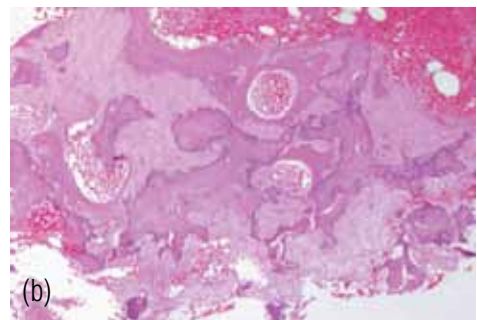
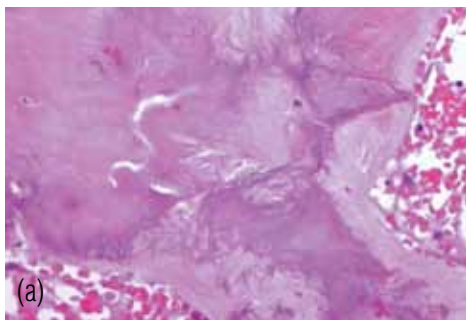
Renal osteodystrophy or renal bone disease is caused by the metabolic changes associated with chronic renal failure.

Bone marrow biopsies from patients with chronic renal failure may show a number of histological changes in the bones including osteomalacia and osteoporosis. The marrow itself is unremarkable or shows erythroid hyperplasia and there may be increased hemosiderin from multiple blood transfusions for anemia.

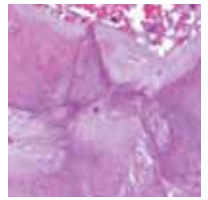
Paget's Disease

The histological features are:

1. Increased osteoclastic activity in the early stages.
2. Bone hyperplasia resulting in thickened trabeculae with disjointed, multidirectional cement lines: The so-called mosaic pattern.
3. An increase in vascularity, which, if extensive, can act as an arterio-venous shunt resulting occasionally in high-output cardiac failure.



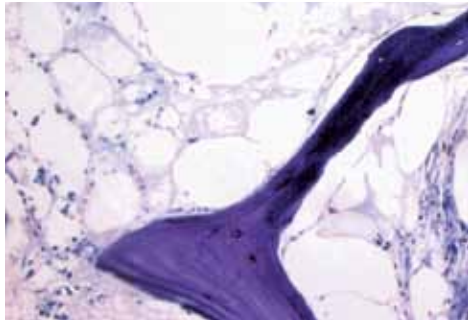
Paget's disease. (a and b) Mosaic bone pattern. H&E.



Serous Atrophy

This has also been termed gelatinous transformation and serous degeneration. It was originally described in patients with anorexia nervosa, but may be seen in anyone with rapid marked loss in body weight (AIDS and terminal cancer). The changes are usually focal with the marrow fat cells being surrounded and gradually replaced by an extracellular homogeneous fibrillary pale material which is rich in hyaluronic acid.

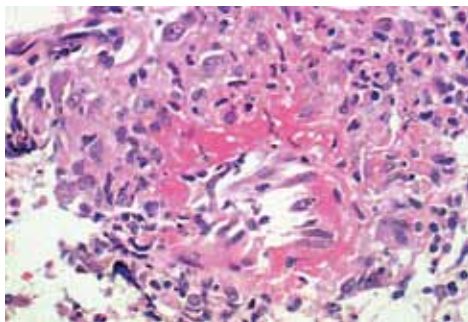
On H&E stained sections it has a smoky lilac color, sometimes with brighter red aggregations which should be distinguished from amyloid.



Serous atrophy in bone marrow. Giemsa.

Vasculitis

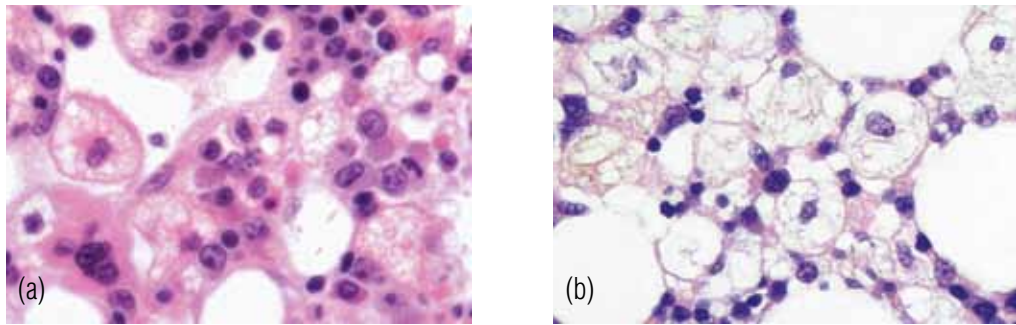
The marrow has a rich blood supply and contains a range of blood vessel types, including small arteries, which are susceptible to the same diseases that affect blood vessels elsewhere in the body. It is not particularly well endowed with the medium to large vessels which are more commonly affected by the major forms of vasculitis so the trephine rarely yields a positive diagnosis. Occasionally it can be helpful or even diagnostic in conditions such as polyarteritis nodosa.



Polyarteritis nodosa affecting arteriole in bone marrow. H&E.

Foamy Macrophages

Foamy macrophages are seen in a number of different conditions. These include severe sepsis, inherited lipid storage disorders (Gaucher's, Niemann–Pick or Fabry's disease). In certain hematological malignancies, particularly chronic myeloid leukemia, cells resembling Gaucher's cells ('pseudo-Gaucher cells') have been described.

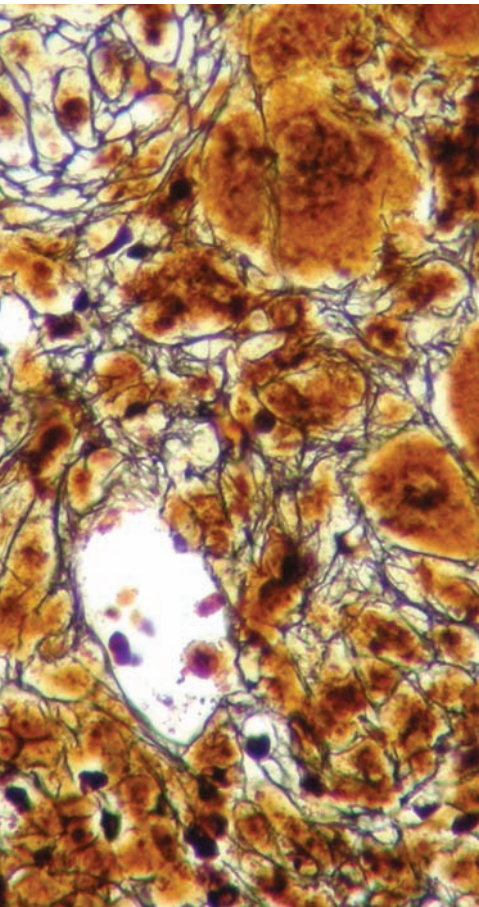


Foamy macrophages in various conditions: (a) Sepsis. H&E. (b) Gaucher's. Giemsa.

The Effects of Drugs and Chemicals

Drugs and chemicals have a variety of effects on the marrow most of which are non-specific. Others are more dramatic such as the striking aplasia that can be induced by solvent exposure or the pseudo-lymphomatous changes of phenytoin.

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