Diagnosis

A Guide for Nurses

Leukaemia Care
Nurse Academy

Introduction

The diagnosis of leukaemia can begin with a patient presenting with non-specific symptoms that are consequences of pancytopenia. However, many types of leukaemia show no obvious symptoms early in disease and may be diagnosed incidentally during a physical examination or as a result of a routine blood test. Suspicion of either ALL or AML constitutes a medical emergency: assessment and a management plan should be instituted promptly.

In general, the diagnostic work-up for leukaemia includes:

- history and physical examination
- blood tests
- bone marrow aspiration and biopsy
- integrated review of flow cytometric, genetic and morphological results.

If you would like any information on the sources used for this booklet, please email **communications@leukaemiacare.org.uk** for a list of references.

In this booklet

About Leukaemia Care

Leukaemia Care is a national charity dedicated to ensuring that people affected by blood cancer have access to the right information, advice and support.

Our services

Helpline

Our helpline is available 9:00am – 5:00pm Monday - Friday and 7:00pm – 10:00pm on Thursdays and Fridays. If you need someone to talk to, call **08088 010 444**.

Alternatively, you can send a message via WhatsApp on **07500068065** on weekdays 9:00am – 5:00pm.

Nurse service

We have two trained nurses on hand to answer your questions and offer advice and support, whether it be through emailing **nurse@leukaemiacare.org.uk** or over the phone on **08088 010 444**.

Patient Information Booklets

We have a number of patient information booklets like this available to anyone who has been affected by a blood cancer. A full list of titles – both disease specific and general information titles – can be found on our website at **www.leukaemiacare.org.uk/support-and-information/help-and-resources/information-booklets/**

Support Groups

Our nationwide support groups are a chance to meet and talk to other people who are going through a similar experience. For more information about a support group local to your area, go to **www.leukaemiacare.org.uk/support-and-information/support-for-you/find-a-support-group/**

Buddy Support

We offer one-to-one phone support with volunteers who have had blood cancer themselves or been affected by it in some

way. You can speak to someone who knows what you are going through. For more information on how to get a buddy call **08088 010 444** or email **support@leukaemiacare.org.uk**

Online Forum

Our online forum, **www.healthunlocked.com/leukaemia-care**, is a place for people to ask questions anonymously or to join in the discussion with other people in a similar situation.

Webinars

Our webinars provide an opportunity to ask questions and listen to patient speakers and medical professionals who can provide valuable information and support. For information on upcoming webinars, go to **www.leukaemiacare.org.uk/support-and-information/support-for-you/onlinewebinars/**

Website

You can access up-to-date information on our website, **www.leukaemiacare.org.uk**.

Campaigning and Advocacy

Leukaemia Care is involved in campaigning for patient well-being, NHS funding and drug and treatment availability. If you would like an update on any of the work we are currently doing or want to know how to get involved, email **advocacy@leukaemiacare.org.uk**

Patient magazine

Our magazine includes inspirational patient and carer stories as well as informative articles by medical professionals: **www.leukaemiacare.org.uk/communication-preferences/**

Clinical presentation

TABLE 4.1

Signs and symptoms of bone marrow failure and hyperleucocytosis*

Anaemia	Neutropenia	Thrombocytopenia	Hyperleucocytosis
• Fatigue • Pallor • Dyspnoea on exertion • Dizziness • Angina • Cardiac flow murmur • Myocardial infarction	• Fever and infection, including both bacterial and atypical organisms (e.g. a fungal source)	• Bleeding gums • Petechiae and purpura • Other serious internal bleeding (e.g. GI tract and intracranial)	• Respiratory distress • Altered mental state • Bone pain

*The clinical manifestations of AML are variable. GI, gastrointestinal.

Acute myeloid leukaemia

Acute myeloid leukaemia typically presents with signs and symptoms of bone marrow failure (Table 4.1). Patients may have symptoms of anaemia, such as dyspnoea or angina. Thrombocytopenia may result in bruising and bleeding. Neutropenia may present as sepsis or fever. Symptom onset is usually no more than a few months before diagnosis. Conversely, patients may be asymptomatic, with AML being diagnosed from a full blood count performed for other reasons.

Extramedullary disease

AML can also present as a soft-tissue swelling, known variably as extramedullary disease, granulocytic sarcoma or chloroma. Extramedullary disease can present without bone marrow involvement and is associated

with certain cytogenetic changes such as the t(8;21) chromosome translocation.1 Treatment for extramedullary disease is similar to that for AML that is confined to the bone marrow.

Leucocytosis

Infiltration of leukaemia cells can cause gum swelling and bone pain due to increased bone marrow pressure. The accumulation of AML blasts in the skin can also cause a rash known as leukaemia cutis. Hyperleucocytosis (>100,000/L leukaemia cells) can present with symptoms of leucostasis, such as respiratory distress and altered mental status, and should be treated as an emergency.

Acute promyelocytic leukaemia

Acute promyelocytic leukaemia (APML) is a specific subtype of AML that results from the t(15;17) translocation. It produces the fusion protein PML-RARA. This specific subtype of AML commonly presents with coagulopathies, resulting in haemorrhage, including in the central nervous system (CNS).

Acute lymphoblastic leukaemia

Acute lymphoblastic leukaemia presents with similar symptoms of bone marrow failure as those described for AML above. Patients with ALL, particularly T-cell ALL, may also present with lymphadenopathy and hepatosplenomegaly. Dyspnoea may occur as a result of hilar lymphadenopathy due to a mediastinal mass. Patients with ALL may also present with bone pain. ALL commonly affects the CNS and CNS-directed treatment is required. Despite this, the CNS may provide a reservoir of ALL in patients and may drive relapsed disease, resulting in symptoms such as seizures or headaches.

Chronic myeloid leukaemia

The signs and symptoms of CML are usually non-specific and insidious, often occurring long after the onset of disease. Indeed, both CML and CLL, especially chronic-phase CML, may be diagnosed in asymptomatic patients following blood tests for other indications. CML in the accelerated phase or blast

Clinical presentation (cont.)

phase is rarer at diagnosis but may present with more severe symptoms.

Presenting symptoms in patients with CML include fatigue and dyspnoea. Splenomegaly of variable size is also a feature; the spleen may be massive. The liver may also be enlarged but usually not to the same extent. The spleen size is important in all major prognostic scoring systems, including the Sokal and EUTOS scores (www.thecalculator.co/health/Sokal-Score-For-CML-Calculator-994.html, last accessed 12 January 2021).

Very high white blood cell (WBC) counts in CML may result in leucostasis. In these cases, symptoms may be due to the sluggish flow of blood through the vasculature. They include visual disturbances due to the poor blood flow through the retinal vessels, resulting in haemorrhages.

Chronic lymphocytic leukaemia

Early-stage CLL is often asymptomatic, whereas advanced-stage CLL, or CLL that has transformed to high-grade disease, may present with worse symptoms. Cytopenias due to bone marrow infiltration or autoimmune disease may cause symptomatic anaemia, and bruising and bleeding from thrombocytopenia. CLL can be associated with B symptoms (fever, weight loss and night sweats). Symptoms may also arise as a result of local mass effect from lymphadenopathy and hepatosplenomegaly. Not infrequently, patients with CLL demonstrate profound fatigue and frequent infections, which may be a result of hypogammaglobulinaemia. Recurrent infections in patients with hypogammaglobulinaemia may be reduced by regular intravenous immunoglobulin therapy.

The symptoms, as described above, are non-specific and seen in all B-cell lymphoproliferative disorders. CLL is considered to be identical (that is, one disease with different manifestations) to the mature (peripheral) B-cell neoplasm SLL. CLL is the likely diagnosis when the disease is seen in the blood and bone marrow, whereas SLL is mainly nodal.

B-cell prolymphocytic leukaemia

B-cell prolymphocytic leukaemia is a rare B-cell neoplasm characterised by high concentrations of prolymphocytes in the peripheral blood, bone marrow and spleen. It is most common in elderly white individuals. Patients usually present with a rapidly rising WBC count (>100,000/μL) and splenomegaly, with or without B symptoms (fever, weight loss and night sweats). Peripheral lymphadenopathy may be present, but it is not usually prominent. Very little information is available about prognostic markers, but in general B-PLL has a poor prognosis, particularly in older patients and those with anaemia and/or thrombocytopenia.

Large granular lymphocytic leukaemia

Large granular lymphocytic leukaemia is a rare chronic leukaemia of T-cell or natural killer (NK)-cell lineage. It usually presents in the sixth decade of life. Approximately one-third of patients are asymptomatic and diagnosed with cytopenia (most commonly neutropenia) on a routine blood test. The most common symptoms are neutropenia, anaemia and thrombocytopenia, which occur in approximately 85%, 50% and 20% of patients at diagnosis, respectively. Symptoms include fever and recurrent bacterial infections related to neutropenia; 20–30% of patients present with the typical B symptoms of lymphoma (fever, weight loss and night sweats). Splenomegaly is common, while lymphadenopathy and skin involvement are rare. About 40% of patients have an associated condition, most commonly rheumatoid arthritis or an autoimmune cytopenia.

The cause of T-cell LGL leukaemia is not known but is postulated to be due to exposure to a common antigen.

Hairy cell leukaemia

Hairy cell leukaemia is another relatively uncommon chronic B-cell leukaemia. The most common symptoms are weakness and fatigue due to anaemia, bleeding from thrombocytopenia, and fever and infections due to neutropenia.

Differential diagnoses

As described above, many bone marrow disorders present with pancytopenia and its attendant signs and symptoms. The main diagnoses to consider in the differential diagnosis of pancytopenia due to AML or ALL are other haematological cancers with marrow infiltration and bone marrow failure syndromes, such as aplastic anaemia. Myeloproliferative disorders with bone marrow fibrosis can also present similarly.6 Rarely, pancytopenia caused by the displacement of haematopoietic bone marrow tissue by fibrosis, tumour or granuloma (called myelophthisis) may mimic an acute leukaemia. Examples include solid organ cancers with extensive bone marrow involvement and granulomatous disease such as tuberculosis.

Morphological, immunophenotypical (or cytochemical), cytogenetic and molecular studies must be performed in every case of suspected leukaemia. Information from these studies is important for the correct diagnosis and classification of the leukaemia, as it informs the selection of treatment and determines prognosis. Morphological and immunophenotypical analyses remain the foundation of rapid initial assessment, from which specific cytogenetic and molecular

Peripheral blood tests

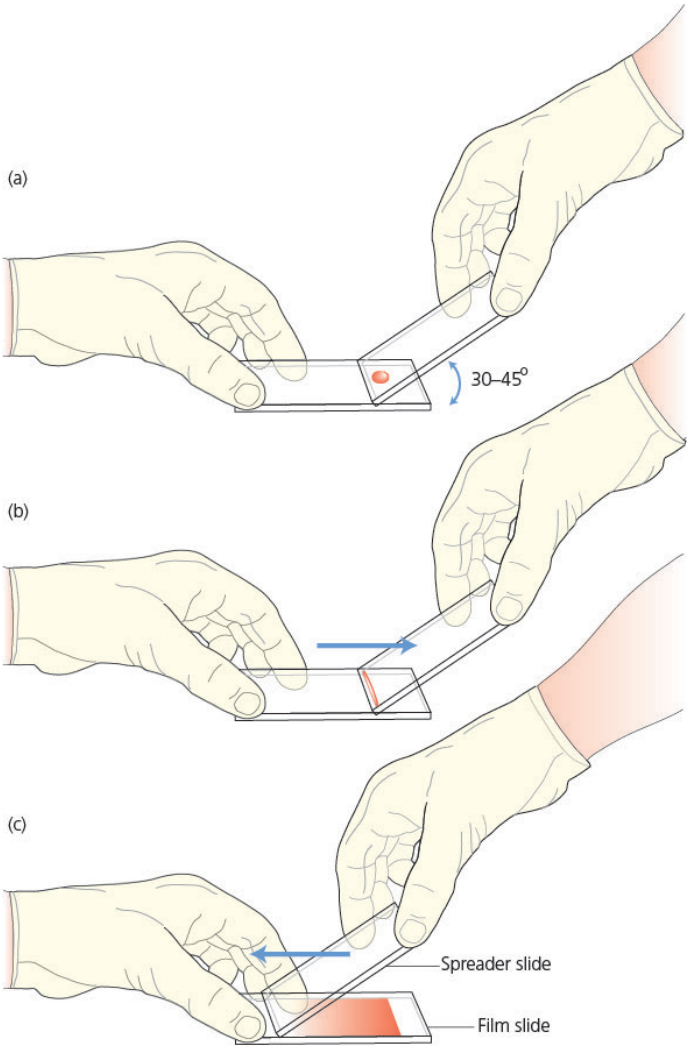

Figure 4.1 Preparation of peripheral blood smear using the wedge technique. (a) A drop of blood is placed at one end of the film slide. (b) The spreader slide is then drawn back into the drop of blood such that the blood spreads across the width of the slide. (c) It is then pushed to the end of the slide to create a wedge film.

Peripheral blood tests (cont.)

Examination of the peripheral blood smear provides rapid and reliable haematological information. It is an inexpensive test that provides key information about the functional status of the bone marrow. The precise disease classification may rely upon evaluation of abnormal circulating cells (for example, the presence of Auer rods in the blasts of patients with AML, see below).

In most cases, peripheral blood smears are prepared using a wedge technique, either manually or via an automated process (Figure 4.1). Smears should be made from one small drop of blood that has not been allowed to clot. If the blood comes from an anticoagulated blood sample that has been allowed to settle for a while, then it should be completely mixed first.

Normal haematological findings

A normal peripheral smear should contain mature leucocytes, including lymphocytes, neutrophils and monocytes. Small lymphocytes comprise 30–40% of the circulating WBCs. They have clumped nuclear chromatin and a scant rim of deep blue cytoplasm. LGLs are morphologically distinct lymphoid cells, comprising 10–15% of normal peripheral blood mononuclear cells. They are approximately twice the size of red blood cells (RBCs), with abundant cytoplasm, a round to oval nucleus and a small number of azurophilic cytoplasmic granules.

Abnormal haematological findings

Certain abnormalities should never be found on the normal peripheral smear; if present, they always signify a pathological process. While it is normal to identify a range of early WBCs in pregnancy or during a leukaemoid reaction, it is never normal to see blasts (lymphoblasts or myeloblasts) on the peripheral smear. The presence of blasts or tumour cells warrants further evaluation (review of the peripheral smear, haematological consultation and bone marrow

examination).

Myeloblasts

The presence of myeloblasts – immature cells with large nuclei and nucleoli, and a scant rim of dark blue cytoplasm – suggests an underlying malignant haematological disorder. Blasts containing Auer rods (a rod-like conglomeration of granules in the cytoplasm) are pathognomonic for AML.

Lymphoblasts

The lymphoblasts of ALL vary from small cells with scant cytoplasm, condensed nuclear chromatin and indistinct nucleoli, to larger cells with moderate amounts of cytoplasm, dispersed chromatin and multiple nucleoli. A few azurophilic cytoplasmic granules may be present. Auer rods are absent.

Other abnormal lymphoid cells

Lymphoid cells with ragged or 'hairy' cytoplasm may be seen in HCL. Lymphoid cells with hyperlobulated nuclei (clover leaf or flower cells) may be seen in patients with adult T-cell leukaemia/lymphoma.

Bone marrow aspiration and biopsy

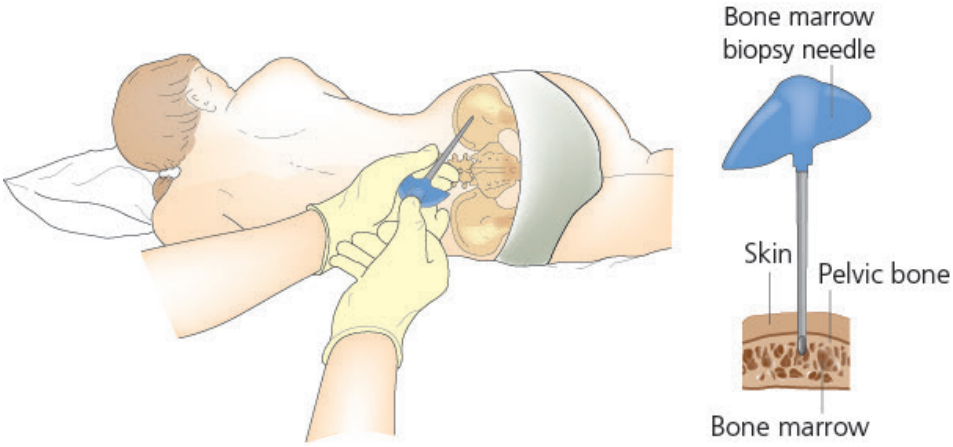

Figure 4.2 Bone marrow aspiration and biopsy. After local anaesthesia, a bone marrow needle is inserted into the pelvic bone and samples of blood, bone and bone marrow are removed for analysis.

Bone marrow aspiration and biopsy are used for the diagnosis, confirmation and staging of haematological disease. These procedures are also used to diagnose malignancies and non-haematological disorders, such as storage disease or systemic infection. They are performed in an ambulatory procedure with local anaesthesia; morbidity is low (Figure 4.2).

Acute myeloid leukaemia

Acute myeloid leukaemia is diagnosed by bone marrow biopsy using morphological, immunophenotypical and cytogenetic/molecular analysis.

Morphology

The blasts must be identified as cells of myeloid lineage (that is, distinct from lymphoid cells). These myeloblasts must account for at least 20% of the total cellularity of the bone marrow biopsy sample; exceptions include leukaemias with certain genetic abnormalities and myeloid sarcoma, which are considered

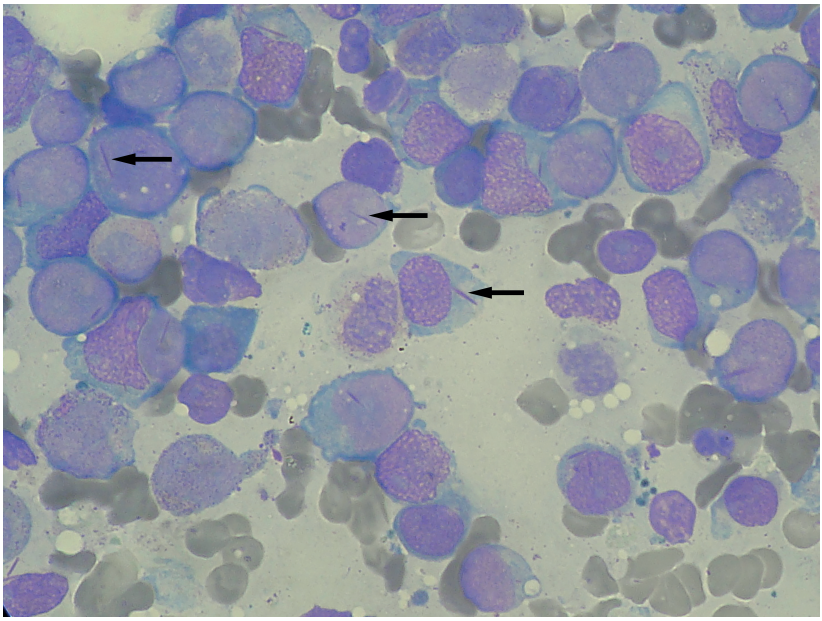

Figure 4.3 Bone marrow aspirate showing myeloblasts with Auer rods (arrowed), pathognomonic of AML. Reproduced courtesy of Paulo Henrique Orlandi Mourao (own work) CC BY-SA 3.0 (creativecommons.org/licenses/by-sa/3.0).

diagnostic of AML regardless of blast count. Blasts containing Auer rods are pathognomonic of AML (Figure 4.3).

Immunophenotyping

AML can be distinguished from ALL, and in some cases classified further into subtypes, by the expression of specific markers. For example, immunophenotypical analysis of AML with minimal differentiation is crucial to determine the cell lineage and rule out a leukaemic presentation of a mature lymphoma process.

Acute lymphoblastic leukaemia

Morphology

In tissue sections, the tumour cells are small- to medium-sized, with scant cytoplasm, round, oval or convoluted nuclei, fine chromatin, and indistinct or small nucleoli (Figure 4.4). Occasional cases have larger cells.

Immunophenotyping

Immunophenotyping by flow

Bone marrow aspiration and biopsy (cont.)

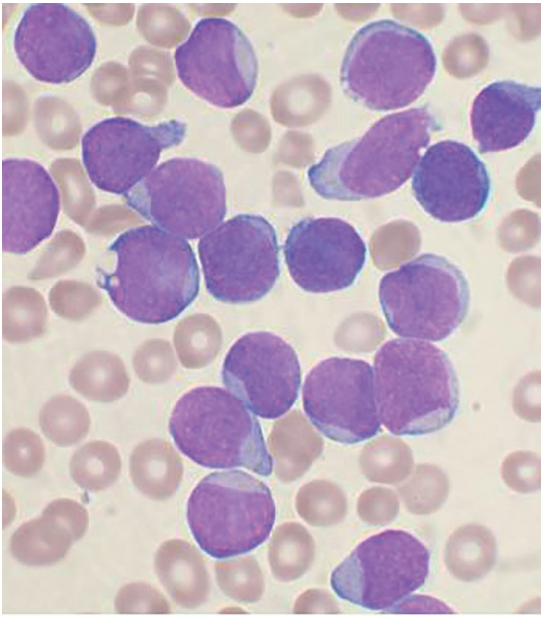

Figure 4.4 Bone marrow aspirate showing lymphoblasts of B-cell ALL with scant cytoplasm. Wright's stain. Reproduced courtesy of VashiDonsk CC-BY-SA-3.0 (creativecommons.org/licenses/by-sa/3.0/).

cytometry differentiates precursor B-cell ALL from precursor T-cell ALL, by demonstrating B-cell antigens and the absence of T-cell antigens. Precursor B- and T-cell ALL are differentiated from AML by positivity for terminal deoxynucleotidyl transferase and lack of staining for myeloperoxidase.

Chronic myeloid leukaemia

While CML is definitively diagnosed by the chromosome translocation t(9;22), also known as the Philadelphia chromosome (see below), examination of the peripheral blood and bone marrow helps to characterise the patient's disease phase (chronic stable, accelerated or blast phase).

Morphology

The bone marrow in CML is most often hypercellular, with a markedly increased myeloid to erythroid cell ratio due to an absolute increase in myeloid series cells. The most abundant cells are usually myelocytes, metamyelocytes and mature neutrophils, with myeloblasts usually representing less than 5% of all myeloid series cells (without transformation). Auer rods are not seen unless the disease has transformed into an AML. There is often an increase in basophils in the peripheral blood.

Immunophenotyping

Immunophenotyping has a limited role in diagnosing CML, but it may be used to identify atypical blasts and/or maturing myeloid cells in the blood and bone marrow.

Chronic lymphocytic leukaemia

Chronic lymphocytic leukaemia is characterised by a progressive accumulation of functionally incompetent lymphocytes, usually monoclonal.

Morphology

The bone marrow smear in patients with CLL and its variant B-PLL is hypercellular with monotonous areas of small round cells containing a thin rim of cytoplasm. On higher magnification the lymphocytes can be clearly distinguished from erythroid precursors. The mature lymphocytes of CLL are small round cells with pyknotic nuclei and scant blue cytoplasm. In contrast, the most mature normoblasts have a more clumped nuclear chromatin along with a greater amount of cytoplasm, which is either bluish-red (polychromatophilic normoblast) or reddish (orthochromatic normoblast).

Immunophenotyping

Immunophenotyping will confirm the presence of circulating clonal B lymphocytes, with specific surface markers distinguishing CLL from other B-cell neoplasms such as B-PLL.

In B-PLL, prolymphocytes must exceed 55% of lymphoid cells in the peripheral blood. These cells express pan-B-cell markers and high levels of surface immunoglobulin; they are CD5-negative in two-thirds of cases and almost always negative for CD10. Cases of mantle cell lymphoma masquerading as B-PLL must be excluded, particularly in suspected B-PLL

Bone marrow aspiration and biopsy (cont.)

cases that express CD5. The differential diagnosis of B-PLL includes other chronic lymphoid neoplasms with a leukaemic presentation.

Large granular lymphocytic leukaemia

Large granular lymphocytic leukaemia is characterised by infiltration of peripheral blood and bone marrow by LGLs, with T-cell or NK-cell lineage.

Morphology

LGLs are large (15–18 µm), approximately twice the size of a normal RBC. They are characterised by abundant cytoplasm, containing fine or coarse azurophilic granules, and a reniform or round nucleus with a high nuclear to cytoplasmic ratio. LGLs comprise 10–15% of normal peripheral blood mononuclear cells. The absolute number of LGLs in the peripheral blood of normal subjects is 200–400/µL. The diagnosis can usually be made based on a morphological and immunophenotypical analysis of the peripheral blood, demonstrating increased numbers of clonal LGLs of T-cell lineage. Bone marrow aspirate and/or biopsy may be required to confirm the diagnosis in some cases, especially those with low absolute numbers of circulating LGLs.

Immunophenotyping

T-cell LGL leukaemia cells typically express CD3, CD8, CD16, CD57 and the α/β T-cell receptor (TCR), but do not usually express CD4, CD56 or CD28. The differential diagnosis of T-cell LGL leukaemia includes several lymphomatous and leukaemic conditions affecting T cells. In particular, T-cell LGL leukaemia must be differentiated from reactive LGL expansions, chronic lymphoproliferative disorders of NK cells and aggressive NK-cell leukaemia.

Cytogenetics and molecular genetics

TABLE 4.2

Key reasons for cytogenetic and molecular genetic testing
• To establish the specific diagnosis (e.g. the Philadelphia chromosome in CML)
• To distinguish between benign reactive lymphoid or myeloid hyperplasia and a monoclonal malignant proliferation
• To better understand the pathogenesis of the disease
• To identify genes that control cell growth and leukaemogenesis
• To help plan treatment or select a targeted therapy, as some chromosome changes predict response to specific therapies
• To gain prognostic insight from recurring or new karyotypic abnormalities or clonal heterogeneity/evolution. These often signal a change in the pace of the disease, usually to a more aggressive course (e.g. disease transformation)

Cytogenetics examines the number, structure and functional changes of chromosomes using microscopic analysis; it has an important role in leukaemia classification risk stratification and treatment decisions (Table 4.2). Morphologically and clinically distinct subsets of leukaemia are associated with specific chromosome abnormalities, providing important prognostic information. Given the ease with which blood and bone marrow samples can be obtained, there is now a lot of information available about the association between cytogenetic abnormalities and the pathogenesis and natural history of the leukaemias, more so than for any other malignancy.

Molecular genetics analysis can identify gene mutations from the structure and function of the DNA. The identification of many molecular abnormalities has helped to inform prognosis and predict response to therapy.

Cytogenetics and molecular genetics (cont.)

Sample collection

Cytogenetic studies require samples that contain viable dividing cells; samples of blood, bone marrow or tumour in preservatives or fixatives cannot be used. Samples of blood or bone marrow (5 mL) should be aspirated into a syringe coated with preservative-free heparin and transferred to a tube containing an appropriate culture medium. Anticoagulants that chelate calcium (for example, ethylenediaminetetraacetic acid) should be avoided in the collection medium. The specimen must be transported immediately at room temperature to the cytogenetics laboratory. Other methods, such as fluorescence in situ hybridisation (FISH) or reverse transcription polymerase chain reaction (RT-PCR), can be performed on fixed samples or blood or bone marrow smears if karyotyping indicates an aberration.

Cytogenetic and molecular genetic methods include:

- conventional metaphase cytogenetic analysis (karyotyping)
- FISH analysis
- RT-PCR
- microarray-based gene expression profiling
- genomic copy number analysis
- DNA or RNA sequencing.

The detection of specific chromosome translocations/gene fusions enables a precise diagnosis of leukaemia. In contrast, the detection of recurring gene mutations in AML and ALL provides important genetic information regarding prognosis and informs the selection of targeted therapies. Combining conventional cytogenetic analysis, FISH, microarray and NGS offers an informative genetic landscape of leukaemia and enables personalised genetic-based treatment. Molecular methods, such as RT-PCR or NGS, assess the entire cell population, whereas FISH provides analysis at the single-cell level.

Fluorescence in situ hybridisation

In FISH analysis, labelled DNA probes are hybridised to either metaphase chromosomes or interphase nuclei, and the hybridised probes are detected with fluorochromes. It is a rapid and sensitive technique for detecting recurring numerical and structural abnormalities.

In some cases, FISH is more sensitive than morphological and conventional cytogenetic analyses. It can be used to detect small chromosome deletions and duplications that are not visible, or to confirm rearrangements suspected from microscopic analysis.

FISH is most useful when the analysis is targeted towards abnormalities known to be associated with a particular malignancy or disease. Thus, in the clinical setting, complete cytogenetic analysis, performed at the time of diagnosis, can be used to identify the chromosome abnormalities in a particular patient's malignant cells. FISH can be used with the appropriate probes to evaluate the efficacy of treatment or to detect MRD or early relapse.

FISH can be used as the primary method of confirming the diagnosis of haematological malignancies with distinctive morphology, such as APML or CML. However, additional abnormalities or multiple clones will not be detected by this method alone. Therefore, FISH serves in most clinical cases as a complement to conventional cytogenetic analysis.

Polymerase chain reaction (PCR) assay

Polymerase chain reaction (PCR) assay can markedly increase the sensitivity of detection of chromosome abnormalities. PCR can detect abnormalities in bone marrow or blood samples at a level of 1 in 105 to 1 in 106 normal cells. The high sensitivity of PCR means that patients in clinical remission can be tested to detect MRD.

Cytogenetics and molecular genetics (cont.)

Microarray-based genomic copy number analysis

Microarray-based genomic copy number analysis is beginning to play a major role in diagnosing and managing haematological disorders. Microarray technology has enabled high-resolution genome-wide genotyping using single nucleotide polymorphisms. Several studies have validated this technology's diagnostic utility, and many laboratories offer this test as an adjunct to cytogenetic and FISH analysis for genomic profiling. Genomic analysis of the clonal origins of relapsed ALL has shown that genomic abnormalities contributing to ALL relapse are selected for during initial treatment. This highlights potential targets for new therapeutic interventions.

NGS techniques

NGS techniques such as targeted, whole-genome or whole-exome sequencing can be incorporated into the diagnostic evaluation of leukaemia to detect, with high sensitivity, recurring leukaemia-specific gene fusion transcripts or novel gene fusions that define specific leukaemia entities (for example, BCR-ABL1-like B-cell ALL). NGS analysis of multiple genes using panels of targeted exon mutations has become part of routine diagnostic procedures in clinical laboratories for haematological malignancies, allowing better risk stratification and selection of targeted therapies.

Acute myeloid leukaemia

Cytogenetic analysis of metaphase cells is a key component in evaluating all patients with newly diagnosed or suspected AML. Specific cytogenetic abnormalities are closely, and sometimes uniquely, associated with morphologically and clinically distinct subsets of the disease. Using standard banding techniques, 50–60% of patients with newly diagnosed AML have abnormal karyotypes. The most common chromosome abnormalities are:

- t(8;21)
- t(15;17)
- inv(16)/t(16;16)
- 11q rearrangements
- trisomy 8.

t(8;21) is seen in approximately 7% of adults with newly diagnosed AML and results in the creation of the RUNX1–RUNX1T1 fusion gene. It is the most frequent abnormality in children with AML. Cases of AML with this translocation have a morphologically distinct phenotype. It is associated with a favourable prognosis in adults, but a poor prognosis in children.

t(15;17) creates the PML-RARA fusion gene, which is highly specific for APML. APML represents a medical emergency, with a high early death rate, often due to haemorrhage from disseminated intravascular coagulation (DIC). Fast intervention is critical as soon as the diagnosis is suspected from the morphological findings alone, before cytogenetic confirmation. Patients with APML who receive prompt treatment have an excellent prognosis.

inv(16) is found in approximately 5% of newly diagnosed AML cases. Monocytic and granulocytic differentiation with abnormal eosinophils is usually present in the bone marrow. Patients with inv(16) generally have a good response to intensive chemotherapy.

t(9;11) involves the KMT2A and MLLT3 (AF9) genes. Patients with this translocation tend to have an intermediate response to standard therapy.

t(6;9) is seen in approximately 1% of patients with newly diagnosed AML, and cases with this translocation are characterised by basophilia, pancytopenia and dysplasia. Patients with this abnormality have a poor response to standard therapy, which may result from the lesion itself or its association with FMS-like tyrosine kinase (FLT) 3 internal tandem duplication (ITD), which is also known to convey a poor prognosis (see below).

Trisomy 8 is the most common trisomy in newly diagnosed AML. It can be the sole abnormality detected, part of

Cytogenetics and molecular genetics (cont.)

TABLE 4.3

Most common class I and II mutations in AML22

Gene	Estimated proportion of cases with mutation (%)*
Class I	
FLT3	28
NRAS or KRAS	12
TP53	8
Class II	
NPM1	27
CEBPA	6

a complex karyotype or the second aberration to a primary rearrangement such as t(8;21), inv(16)/t(16;16) or t(9;11). The prognosis of patients with trisomy 8 depends on the presence and nature of the abnormalities that accompany it.

Other chromosome abnormalities. t(3;3) and inv(3) account for approximately 1% of AML cases and are associated with a poor response to therapy. AML with t(1;22) is also rare. It is typically a megakaryoblastic process in infants, although it is not seen in patients with Down's syndrome. The prognostic significance of t(1;22) with modern therapy is unclear.

Damage from alkylating agents, causing abnormalities in chromosomes 5 and/or 7, is the most common therapy-related MDS/AML. AML associated with chemotherapeutic drugs that inhibit DNA topoisomerase II (etoposide, teniposide, doxorubicin, mitoxantrone, epirubicin and dexrazoxane) is the next most common form of treatment-related AML. This AML

subtype often features balanced translocations involving the KMT2A gene at 11q23.3 or the RUNX1 gene at 21q22.1.

Molecular genetic abnormalities. Similar to other malignancies, the genetic alterations in AML include the mutation of oncogenes and the loss of tumour suppressor genes. Mutations associated with AML are found in genes that encode enzymes involved in DNA methylation (for example, DNMT3A) and DNA demethylation (for example, IDH and TET2).

The two-step leukaemogenesis model offers a conceptual framework for classifying gene mutations associated with AML. According to this model, a class I mutation conferring a proliferative and/or survival advantage to haematopoietic progenitors must occur in conjunction with a class II mutation that impairs differentiation and subsequent apoptosis for leukaemia to develop. Common class I and II genes are shown in Table 4.3. Mutations in genes involved in epigenetic regulation have also been discovered, including DNMT3A, TET2, IDH1 and IDH2. These are found in more than 40% of cases. RUNX1 mutations were introduced as part of the 2016 revised WHO classification of AML; these mutations have been found in 10% of patients.

As Table 4.3 shows, mutations in FLT3 and NPM1 represent the most frequent genetic mutations in AML and are important diagnostic and prognostic indicators.

FLT3 encodes the transmembrane tyrosine kinase receptor FLT3, which stimulates cell proliferation when activated. There are two main types of FLT3 mutations. An ITD of the juxtamembrane domain is the most common. It results in activation of the FLT3 receptor in the absence of ligand and no proliferative signal. Point mutations, resulting in amino acid substitutions within the activation loop of the tyrosine kinase domain of FLT3, occur in 5–10% of patients. In patients with a normal karyotype, FLT3 ITD is associated with a poor prognosis, whereas FLT3 ITD with t(8;21), inv(16) or t(16;16) may be associated with a favourable prognosis.

NPM1 encodes nucleophosmin 1 (NPM1), a ubiquitously expressed phosphoprotein that normally

Cytogenetics and molecular genetics (cont.)

shuttles between the nucleus and cytoplasm. It is involved in ribosomal protein assembly and transport and regulates the tumour suppressor ARF pathway (cyclin-dependent kinase inhibitor 2A). Mutation of the NPM1 gene in AML impairs transport of the NPM1 protein to the nucleus and it is retained in the cytoplasm.

NPM1 mutations (in the absence of FLT3 mutations) are associated with better outcomes in both adults and children with AML, although the mechanism for increased chemosensitivity is not known.

Acute lymphoblastic leukaemia

The most useful prognostic indicators in ALL are age, WBC count, immunophenotype, MRD detection and karyotype. Recurring chromosome abnormalities, including translocations, inversions and deletions, occur in approximately 80% of B-cell ALL cases. Approximately 60% of children with T-cell ALL have recurring chromosome abnormalities involving TCR and non-TCR gene loci.

The frequencies of recurring chromosome abnormalities in children and adults with ALL differ substantially. Up to 30% of B-cell ALL is not classified within any of the existing WHO subgroups; these cases do not have recurring chromosome abnormalities, kinase-activating gene fusions or BCR-ABL1-like B-cell ALL [see below]). However, several recurring cytogenetic abnormalities are associated with distinct immunological phenotypes of ALL and characteristic outcomes. Certain abnormalities, such as t(4;11) and t(9;22), are associated with treatment failure even when using intensive chemotherapy. Conversely, t(12;21), t(1;19) and hyperdiploidy (50–60 chromosomes) are associated with more favourable outcomes.

t(12;21), which results in the ETV6-RUNX1 fusion gene, is the most common chromosome abnormality in paediatric B-cell ALL: it is found in about 25% of children with B-cell ALL, compared with 3% of adults. It is not easily detected by conventional cytogenetic analysis because of the similarity in size and

banding patterns of 12p and 21q. As a result, RT-PCR or FISH analysis is required to detect this rearrangement. Patients with ALL and t(12;21) generally have a favourable prognosis.

t(8;14) occurs in approximately 1% of adults with ALL. It is associated with lymphoblasts with deeply basophilic cytoplasm and prominent vacuoles. Patients with t(8;14) have a high incidence of CNS involvement at diagnosis and a poorer prognosis than any other group of patients classified by chromosome abnormalities.

t(4;11), which results in the KMT2A-AFF1 fusion gene, is present in up to 60% of infants with ALL younger than 12 months, but is rarely observed in adults with ALL. Common features of ALL with t(4;11) are raised leucocyte counts, an immature immunophenotype, B-cell lineage and frequent co-expression of myeloid antigens.

KMT2A encodes a histone methyltransferase that regulates gene transcription via chromatin remodelling. Leukaemias with KMT2A translocations have a characteristic and distinctive gene expression profile. ALL in infants and children with KMT2A translocations is associated with a high rate of early treatment failure and a very poor outcome.

t(9;22), which produces the Philadelphia chromosome, is observed in 2–5% of children and about 30% of adults with ALL. It is associated with a poor prognosis. Treatment of patients with t(9;22) includes TKIs, which target the BCR-ABL1 protein.

t(1;19), which results in the fusion gene TCF3-PBX1, occurs in approximately 30% of patients with childhood precursor B-cell ALL and less commonly in other types of B-cell ALL in children and adults. In the past, patients with t(1;19) typically had early treatment failure. However, more intensive chemotherapy can overcome this adverse prognosis, such that t(1;19) is now associated with a favourable prognosis.

Translocations involving the CRLF2 gene or mutations/rearrangements of other tyrosine kinase targets, such as ABL1, ABL2, JAK2 or PDGFRB, activate ABL1, JAK-STAT or Ras signalling pathways that are involved in B-cell development, proliferation

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and differentiation. Some of these cases of ALL are sensitive to TKIs or JAK inhibitors.

Hyperdiploidy. Patients with hyperdiploid ALL with more than 50 chromosomes often have a good prognosis. Individual structural abnormalities do not appear to influence the outcome in patients with hyperdiploidy, except for the t(9;22) translocation, which is associated with a poor prognosis.

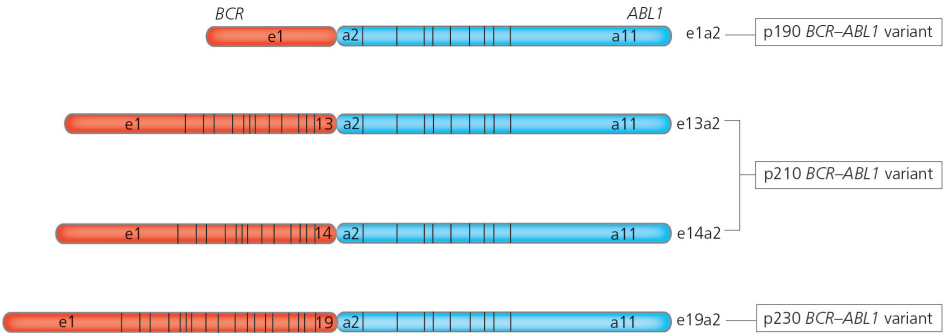
Hypodiploidy. Regardless of age, 5–6% of patients with ALL have hypodiploidy (45 chromosomes or less). These patients generally have a poor prognosis, especially those with near-haploid and low-hypodiploid clones. Similarly, the deletion of chromosome 9p is an adverse risk factor associated with a high relapse rate in precursor B-cell ALL in children.

Molecular genetic abnormalities. Genome-wide profiling of genetic abnormalities in ALL has identified additional subtypes of ALL in cases that fail to demonstrate chromosome rearrangements or hyperdiploidy/hypodiploidy on cytogenetic analysis.

BCR-ABL1-like B-cell ALL (also known as Philadelphia-like B-cell ALL) has a gene expression profile that resembles B-cell ALL. The t(9;22) translocation/BCR-ABL1 fusion gene is present but the fusion oncogene is not. In total, 10–15% of children and up to 28% of adults with B-cell ALL have this phenotype. It is resistant to standard chemotherapy and is associated with a poor prognosis.

Mutation of IKZF1 is a hallmark of BCR-ABL1 and BCR-ABL1-like B-cell ALL. IKZF1 encodes IKAROS family zinc finger protein 1, a transcription factor that is indispensable in the induction of B-lineage differentiation in HSCs. IKZF1 alterations are associated with a poor outcome.

Mutations and deletions in T-cell ALL involve genes that regulate T-cell development and tumour suppressor pathways. More than 50% of patients with T-cell ALL have activating mutations of the NOTCH1 gene; other mutations affect FBXW7, PTEN and RB1.



p190 *BRC–ABL1* – present in two-thirds of patients with Ph+ B-cell ALL and a minority of patients with CML
p210 *BCR–ABL1* – present in most patients with CML and one-third of patients with Ph+ B-cell ALL
p230 *BCR–ABL1* – present in some patients with chronic neutrophilic leukaemia

Figure 4.5 In creating the BCR-ABL1 fusion gene, the BCR may break at one of four exons (e1, e13, e14 or e19), before joining with the ABL1 oncogene at exon a2 to produce one of four possible transcript products: e1a2, e13a2, e14a2 or e19a2. These transcript products correspond to three common variants of BCR-ABL1: p210 BCR-ABL1, p190 BCR-ABL1 and p230 BCR-ABL1. p210 BCR-ABL1 is present in most patients with CML. Ph+, Philadelphia chromosome-positive.

Chronic myeloid leukaemia

Chronic myeloid leukaemia is a clonal myeloproliferative neoplasm in which myeloid cells that have acquired the BCR-ABL1 fusion gene undergo inappropriate clonal expansion. This fusion gene is the product of a balanced translocation between the long arms of chromosomes 9 and 22, denoted as t(9;22)(q34.1;q11.21). An abnormally small chromosome, known as the Philadelphia chromosome, is formed from the juxtaposition of a 5′ segment of a breakpoint cluster region (BCR) at 22q11 with a 3′ segment of the ABL1 oncogene at 9q34, resulting in an abnormally small chromosome known as the Philadelphia chromosome. There are three common variants of BCR-ABL1 (Figure 4.5): most patients with CML have the p210 BCR-ABL1 variant.

The 2016 WHO diagnostic criteria for CML require detection of the Philadelphia chromosome or its products, the BCR-ABL1 fusion mRNA and the BCR-ABL1 protein. This can be accomplished

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through conventional cytogenetic analysis (karyotyping), FISH analysis or RT-PCR.

Chronic lymphocytic leukaemia

The molecular pathogenesis of CLL is a complex multistep process leading to the replication of a malignant clone of B-cell origin. Some steps in this pathway are now known, but many are not.

Chromosome abnormalities are detected in up to 80% of patients with CLL. Although not necessary for the diagnosis or staging of CLL, pretreatment assessment should include cytogenetic evaluation of the peripheral blood using FISH for del(17p), del(11q), trisomy 12 and del(13q), all of which have a known prognostic value. The result of FISH for del(17p) will affect the choice of initial treatment.

Chromosome 11q contains the ATM gene, which encodes ATM serine/threonine kinase. Historically, patients with del(11q) have been at high risk of not responding to initial treatment or relapsing soon after achieving remission.

The prognosis of patients with del(11q) has improved following treatment with the combination of fludarabine, cyclophosphamide and rituximab (FCR). The del(17p) mutation is also associated with a worse prognosis.

Monoallelic or biallelic 13q abnormalities, when they are the only abnormalities present, appear to be associated with a favourable outcome. Similarly, the presence of trisomy 12 as the sole abnormality or with normal genetics confers a favourable outcome.

Molecular genetic abnormalities. Patients with mutations of the TP53 tumour suppressor gene (which is located on the short [p] arm of chromosome 17) or those with del(17p) often do not respond to standard treatment or have a high risk of relapse soon after remission. For this reason, they usually require different treatment to other patients with CLL, including novel agents such as ibrutinib.

TP53 mutations are also associated with a poor prognosis in B-PLL.

Key points – diagnosis

- Diagnosis of leukaemia is based on an integration of clinical, morphological, immunophenotypical and genetic findings.
- Many types of leukaemia, including CML and CLL, show no specific symptoms in the early stages and may be diagnosed incidentally during a physical examination or routine blood test.
- Both AML and ALL can present with signs and symptoms of bone marrow failure, including those related to anaemia, thrombocytopenia and neutropenia.
- The presence of blasts in a peripheral blood smear suggests an underlying haematological disorder.
- Myeloblasts containing Auer rods are pathognomonic for AML.
- Examination of bone marrow aspirate can confirm the diagnosis of leukaemia.
- Flow cytometry can differentiate between ALL and AML based on different cell surface markers.
- On immunophenotypical analysis, the presence of B-cell antigens in the absence of T-cell antigens differentiates B-cell ALL from T-cell ALL.
- CML is diagnosed by the presence of the chromosome translocation t(9;22), also known as the Philadelphia chromosome. The t(9;22) mutation also occurs in some cases of ALL.
- Certain cytogenetic abnormalities are associated with favourable responses to treatment, while others are associated with treatment failure.
- Detection of recurring gene mutations in AML and ALL provides important genetic information about prognosis and informs the selection of targeted therapies.

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www.leukaemiacare.org.uk

support@leukaemiacare.org.uk

Leukaemia Care,
One Birch Court,
Blackpole East,
Worcester,
WR3 8SG

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