

Diagnosis

When a patient presents with suspected chronic myeloid leukemia (CML) appropriate assessments are needed to confirm the diagnosis and stage of disease, and to assign a risk score to that patient.

Diagnostic laboratory tests

Blood picture and biochemistry

Most chronic phase CML patients present with a characteristic blood picture with increased and left-shifted granulopoiesis, and a predominance of neutrophils and myelocytes (Figure 2.1). There is also an increase in eosinophils and basophils.

A variant presentation of chronic phase CML is marked thrombocytosis with little or no neutrophilia, mimicking essential thrombocythemia. Another rare presentation mimics chronic myelomonocytic leukemia with predominant monocytosis: such cases may express p190 breakpoint cluster region–Abelson (*BCR–ABL*) oncogene [1]. Biochemical correlates of myeloid hyperplasia include increased uric acid and lactate dehydrogenase.

Bone marrow morphology

The bone marrow is markedly hypercellular with granulocytic and variable megakaryocytic hyperplasia, and relatively depressed erythropoiesis (Figures 2.2 and 2.3).

The differential counts resemble those in the peripheral blood with left-shift eosinophilia and basophilia. The megakaryocytes have

a typical morphology with a marked increase in small, hypolobated forms. Dysplastic features are unusual. The cytoplasm of debris-laden macrophages can have a characteristic deep blue (sea blue histiocytes) or crinkled tissue paper appearance (pseudo-Gaucher cells), reflecting increased cell turnover. Reticulin fibrosis is not usually seen, but a minority of CML cases can have significant fibrosis, resulting in features that may resemble primary myelofibrosis. The presence of marrow fibrosis in CML has been reported as an adverse prognostic factor, and can be associated with disease progression [2].

The blast crisis bone marrow shows features that would be expected in *de novo* acute myeloid leukemia (AML) or acute lymphoblastic leukemia (ALL), but there may be morphological clues to the origin of the leukemia, such as eosinophilia or basophilia. In the accelerated phase there are features intermediate between the chronic and blast crisis phases. Diagnostic criteria for CML disease phase are summarized in Table 2.1.

The criteria of Kantarjian and colleagues have been widely used in clinical trials [3]. Accelerated phase CML can be defined solely on the basis of karyotypic clonal evolution, with blood and marrow morphology

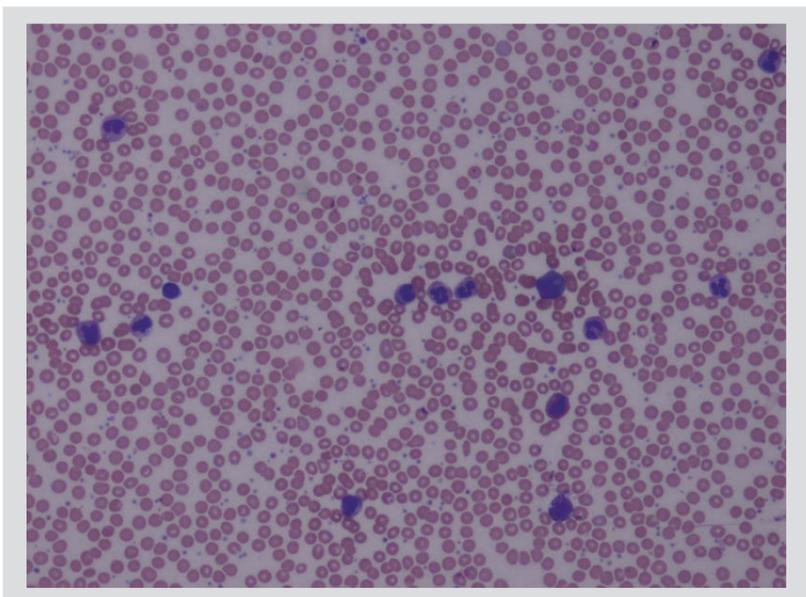


Figure 2.1 Blood film x200 magnification of chronic phase chronic myeloid leukemia. Note the bimodal differential count with peaks in the neutrophils and myelocytes.

consistent with ongoing chronic phase. Accelerated phase patients, defined solely by cytogenetic clonal evolution, may have a better prognosis than those with hematological acceleration [4,5].

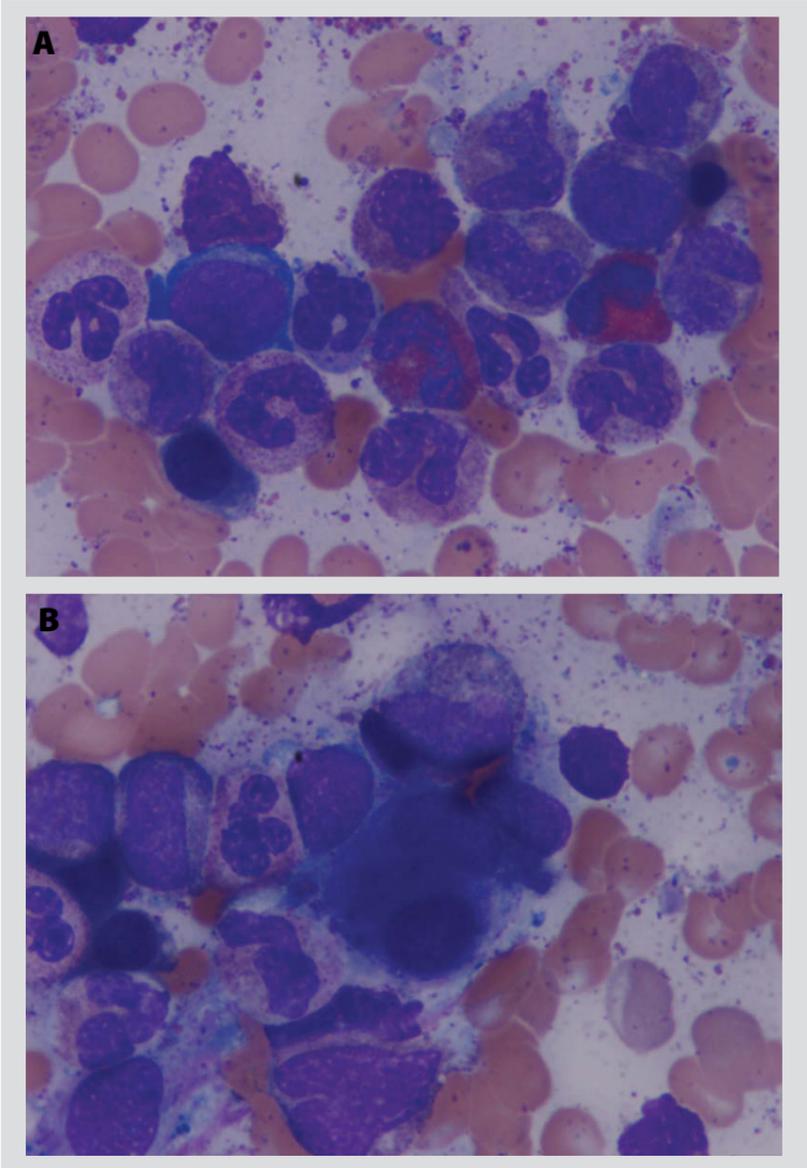


Figure 2.2 Bone marrow aspirate x1000 magnification of chronic phase chronic myeloid leukemia. Note **A**, the prominent eosinophils and **B**, micromegakaryocyte.

Immunophenotyping

Immunophenotyping is not required to diagnose CML in the chronic or accelerated phase. In blast crisis the immunophenotype is helpful in confirming the lineage of the leukemia, which is myeloid in approximately two-thirds of cases and B-lymphoid in approximately one-third of cases; cases with a T-cell lineage are rare. Aberrant expression of lineage-associated markers is commonly observed, and biphenotypic leukemia is seen in a small proportion of cases [6,7].

Cytogenetics

CML is associated with the classical Philadelphia (Ph) chromosome, an abnormally shortened chromosome 22 due to $t(9;22)(q22;q34)$ seen on G-banded karyotypic examination in at least 90–95% of cases (Figure 2.4).

A variant Ph-rearrangement, often involving other chromosomes in addition to chromosomes 9 and 22, may be identified in a further 5% of cases. A cytogenetically cryptic Ph rearrangement is observed in rare

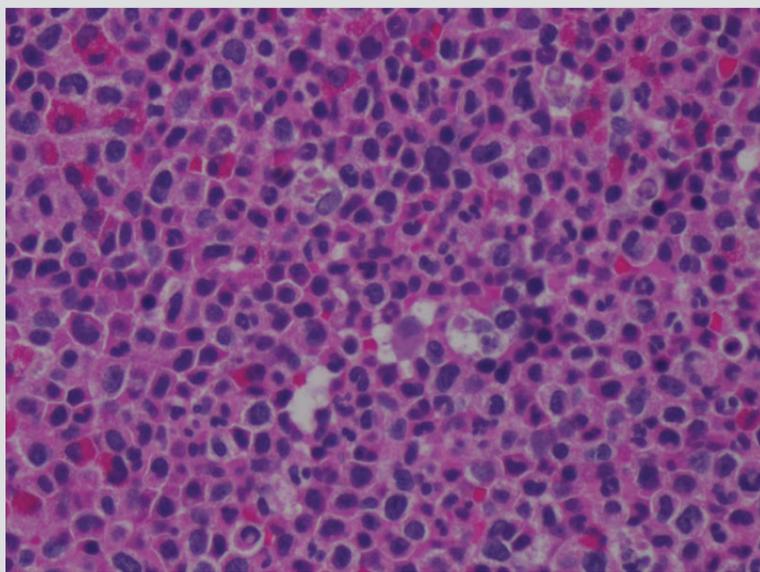


Figure 2.3 Bone marrow trephine. Note the near-complete obliteration of fat spaces and proliferation of granulocyte and megakaryocyte lineages. Micromegakaryocytes and debris-laden macrophages can be seen.

| | Chronic phase | | Accelerated phase | | Blast crisis | |
|--|----------------------|----------------|---|---------------------------|----------------------|----------------|
| | All of the following | | Any of the following | | Any of the following | |
| | WHO [8] | Kantarjian [3] | WHO [8] | Kantarjian [3] | WHO [8] | Kantarjian [3] |
| Blasts in either PB or BM (%) | <10 | <15 | 10–19 | 15–29 | ≥20 | ≥30 |
| Blasts + promyelocytes in either PB or BM (%) | NR [3] | <30 | NR | ≥30 | NR | NR |
| Basophils in PB (%) | <20 | <20 | ≥20 | ≥20 | NR | NR |
| Platelet count (x 10 ⁹ /L) | ≥ 100 & ≤ 1000 | ≥ 100 | <100 unrelated to therapy or > 1000 unresponsive to therapy | <100 unrelated to therapy | NR | NR |
| WHO classification only | | | | | | |
| Increasing spleen size and/or leukocytosis unresponsive to therapy | Absent | | Present | | NR | |
| Acquired cytogenetic clonal evolution | Absent | | Present | | NR | |
| Extramedullary or focal BM blast proliferation (chloroma) | Absent | | Absent | | Present | |

Table 2.1 Criteria for disease phase in chronic myeloid leukemia. BM, bone marrow; NR, not required; PB, peripheral blood; WHO, World Health Organization. Reproduced with permission from © Wiley, 1988. All rights reserved. Kantarjian et al [3]. Reproduced with permission from © IARC, 2008. All rights reserved. Swerdlow et al [8].

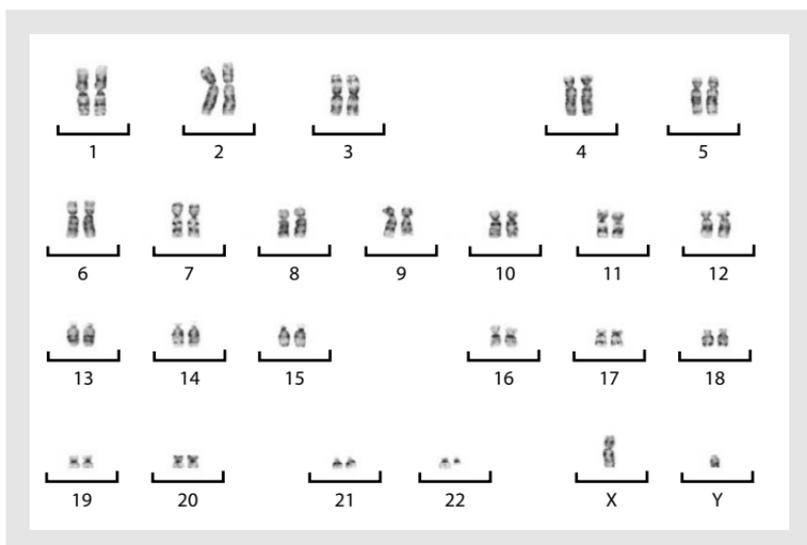


Figure 2.4 Metaphase karyotype of a male patient with chronic phase chronic myeloid leukemia. One long arm of chromosome 9 contains additional material derived from one of the long arms of chromosome 22, resulting in the shortened Philadelphia chromosome. *BCR-ABL* is formed on chromosome 22 and the reciprocal *ABL-BCR* gene is formed on chromosome 9.

cases, which can be detected only by fluorescence in situ hybridization (FISH) or polymerase chain reaction (PCR) [8]. Variant Ph rearrangements do not seem to influence prognosis in patients treated with imatinib [9,10].

In addition to the Ph rearrangement, other chromosomal abnormalities can be observed at diagnosis in around 10% of patients [11]. Selected high-risk additional chromosomal abnormalities (Ph duplication, isochromosome 17q, and trisomy 8) are referred to as ‘major route’ abnormalities and are associated with a significantly inferior response to treatment [10]. Other additional chromosomal abnormalities (including loss of the Y chromosome) do not seem to have an influence on prognosis.

Molecular studies

Qualitative reverse transcriptase (RT)-PCR for *BCR-ABL* can be used to confirm the presence of the translocation and this method may be the only means of confirming a molecular diagnosis in cases with a cryptic Ph rearrangement. The use of long template PCR with a forward primer in *BCR* exon 1 and a reverse primer in *ABL1* exon 3 enables the detection of not only the typical e13a2 and e14a2 *BCR-ABL* transcripts, but also

rarer *BCR-ABL* variants, such as e1a2 (p190 *BCR-ABL*) and e19a2 (p230 *BCR-ABL*), which may otherwise cause diagnostic difficulties [12]. A multiplex RT-PCR assay has been widely used and incorporates primers for e13a2, e14a2 and e1a2 [13]. It is important to be aware of what method is being used in the laboratory in order to be certain that the rarer molecular variants of *BCR-ABL* have been excluded.

The typical p210 *BCR-ABL* protein that is expressed in CML can be associated with either e13a2 or e14a2 mRNA transcripts. The majority of patients (~60%) express e14a2 only and ~30% of patients express e13a2 only [14–16]; the remainder express both transcripts because of the presence of a polymorphic splice acceptor site that results in splicing out of the 75 bases of *BCR* exon 14 and, consequently, a proportion of the e14a2 being processed to e13a2 [17,18]. While some authors have reported prognostic relevance of the transcript type, there is no convincing evidence of a difference in outcome between patients with p210 CML according to *BCR* breakpoint and mRNA transcript type [15,16,19].

In rare cases, Ph-positive patients express an e19a2 *BCR-ABL* mRNA transcript that results in a p230 *BCR-ABL* protein. In comparison with classical CML, the resulting syndrome of neutrophilic CML is characterized by less marked leukocytosis, frequent thrombocytosis, the absence of splenomegaly, and a more indolent disease course [20]. However, clonal evolution can be associated with acceleration in patients with neutrophilic CML, and progression to blast crisis may occur. It is important to distinguish between neutrophilic CML and chronic neutrophilic leukemia, a rare *BCR-ABL*-negative myeloproliferative syndrome with prominent neutrophilia and hepatosplenomegaly, which, in around half of cases, is associated with activating mutations in the receptor for colony-stimulating factor 3, and which may be sensitive to SRC or Janus kinase (JAK) inhibition [21].

Differential diagnosis

In most cases the diagnosis of CML is uncomplicated, with a typical blood picture and confirmatory cytogenetic or molecular tests. Other hematological malignancies and reactive conditions have features that overlap with CML, and some conditions that may mimic CML are discussed here.

Reactive conditions

'Leukemoid reaction' is a term that is used to describe reactive leukocytosis with predominant neutrophilia that may resemble CML. Cytochemical staining for neutrophil alkaline phosphatase was used to distinguish between leukemoid reaction and CML, but is now obsolete where PCR for *BCR-ABL* is readily available. Immune-mediated disorders such as vasculitis, allergy, and parasitic infection may cause leukocytosis with eosinophilia, but are rarely confused with CML.

Other hematological neoplasms

Chronic Ph-negative myeloproliferative neoplasms such as primary myelofibrosis may present with leukocytosis with eosinophilia or splenomegaly, as may rare cases of myelodysplasia, or the myelodysplastic and myeloproliferative overlap syndromes. Chronic myeloproliferative neoplasms associated with tyrosine kinase fusion genes other than *BCR-ABL* may resemble CML, but are usually evident on karyotyping. These include *ETV6-ABL* with t(9;12), *ETV6-PDGFRB* with t(5;12), and fibroblast growth factor receptor (FGFR) fusions with abnormalities of chromosome 8p. Chronic eosinophilic leukemia with the *FIP1L1-PDGFR* fusion is cytogenetically cryptic, and can be identified only by FISH or PCR [22].

Eosinophilia and splenomegaly may occur in lymphoproliferative conditions (especially Hodgkin lymphoma and T-cell lymphoma) in which eosinophilia is thought to be driven by abnormal production of cytokines such as interleukin 3 (IL-3), IL-5, and granulocyte-macrophage colony-stimulating factor (GM-CSF) related to the lymphoid clone [8]. Acute leukemia may mimic blast crisis CML, particularly in conditions such as AML with eosinophilia (eg inv[16]), and AML with basophilia (eg t[6;9]). Clinical features and karyotyping may not reliably distinguish between lymphoid blast crisis CML and Ph-positive ALL if there is no prior history of CML.

Clinical risk scores

Clinical risk scores were developed in the 1980s and 1990s, driven by the availability of allogeneic stem cell transplantation as a potentially curative treatment for CML patients. For example, Sokal and colleagues [23] developed a scoring system that divided patients with CML into three risk

groups: high (median survival 32 months), intermediate (median survival approximately 45 months), and low (median survival 60 months). This enabled the selection of patients for whom the risk–benefit ratio of the allograft procedure was most favorable. The Sokal score is calculated at diagnosis from the age of the patient, palpable spleen size (in centimeters below the costal margin), platelet count, and blast percentage in the peripheral blood. Elements of the Sokal score overlap with those that are used to define the accelerated phase, so that there is a continuum between high-risk chronic phase and accelerated phase disease, and the definitions that are used to classify patients are somewhat arbitrary. The Sokal score was developed for patients treated with hydroxyurea or busulphan but it is also useful for predicting the outcome for patients treated with imatinib de novo [24,25].

Other scoring systems have been used, including the Hasford score, which was developed in interferon-treated patients. This score incorporates the same four variables as the Sokal score, in addition to the eosinophil count and basophil count in the peripheral blood at the time of diagnosis [26,27]. The more recent European Treatment and Outcome Study for CML (EUTOS) score was developed in imatinib-treated patients and uses only the spleen size and percentage of basophils in the peripheral blood [28]. In an independent series of over 1000 patients this score was predictive of progression-free and overall survival, but not all studies have confirmed these findings [29,30].

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