1 Diagnosis and classification of leukaemias

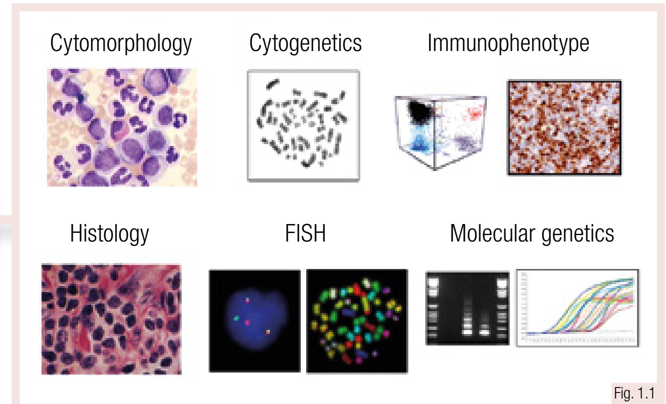
Diagnosis of leukaemias – conventional techniques

An abnormal complete blood count (CBC) raises the suspicion of acute myeloid/lymphoblastic leukaemia (AML/ALL), chronic myeloid leukaemia (CML) or myelodysplastic syndrome (MDS).

In leukaemia patients, white blood cell counts can be either elevated or depleted.

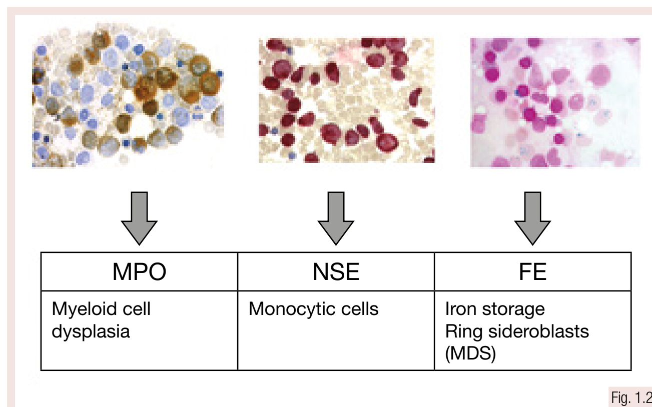
Bone marrow aspirate and histology are mandatory to establish the diagnosis.

Diagnostic tests include cytomorphology, immunophenotyping, cytogenetics and molecular analyses



FISH, Fluorescent *in situ* hybridisation.

Typical cytochemistry staining includes myeloperoxidase (MPO) to differentiate myeloid from lymphoid cells, nonspecific esterase (NSE) to detect monocytic cells and iron staining (FE) to assess iron storage



MPO	NSE	FE
Myeloid cell dysplasia	Monocytic cells	Iron storage Ring sideroblasts (MDS)

MDS, Myelodysplastic syndrome.

Cytomorphology is a rapid but observer-dependent technique that allows the diagnosis of most AML and MDS cases.

Morphology is used to quantify blasts in peripheral blood and bone marrow, where ≥20% is the World Health Organization (WHO) cut off to diagnose acute leukaemia.

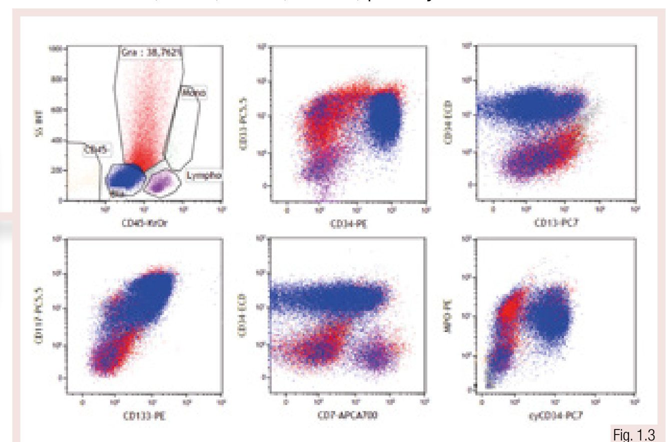
Cytochemistry is used to subspecify cells and to assess the iron storage, which is especially helpful in discriminating MDS subtypes.

Flow cytometry using fluorochrome antibody conjugates identifies blast cells and is a valuable tool to differentiate AML from ALL.

Typically, AML blasts have low side scatter (SSlow), show low expression of CD45 (CD45low), express CD34, CD13, CD117, CD133, MPO (myeloperoxidase) and can have aberrant expression of CD2, CD5, CD7, CD56, CD11b and CD15.

The leukaemia-associated (aberrant) immunophenotype (LAIP) is a valuable tool to detect minimal residual disease (MRD) following treatment.

Typical immunophenotype of an AML sample. Here SSlow and CD45low blast cells (gate in blue) express CD34, CD13, CD117, CD133, partially CD7 and MPO



REVISION QUESTIONS

1. What is the first diagnostic test used for leukaemia or MDS?
2. Does a white blood cell count of 1000/µL rule out leukaemia?
3. What is the main indication for flow cytometry in leukaemia diagnosis?

Diagnosis of leukaemias – cytogenetic techniques

Following a short period of culturing the diagnostic sample, metaphase chromosomes are analysed to establish the karyotype. This assay requires a fresh heparinised bone marrow or blood sample.

Giemsa-banded metaphase after capture by an automated microscope reveals a classical *t(9;22)* translocation, as in CML (A).

Complex karyotypes are hard to decipher by standard banding, and 24-colour fluorescent *in situ* hybridisation (FISH) on the identical metaphase helps to resolve complex rearrangements (B).

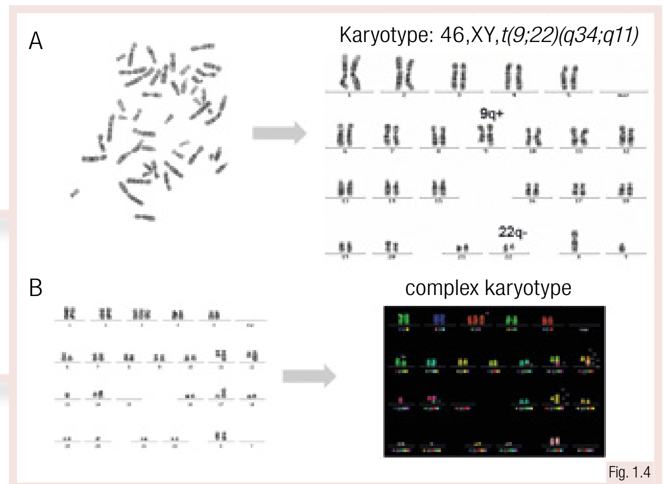


Fig. 1.4

Workflow for AML diagnosis: A. Cytomorphology showing typical blast cells with Auer rods. B. Immunophenotype with aberrant expression of CD56 and CD19. C. Karyogram showing translocation *t(8;21)*

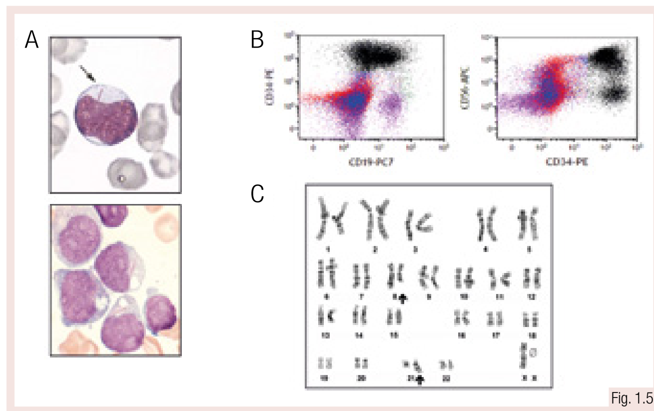


Fig. 1.5

In a routine workflow, cytomorphology and flow cytometry are rapid techniques that usually yield results within a few hours.

The typical morphology and immunophenotype can raise suspicion for certain subtypes of AML, which need further specification.

A conventional karyogram then returns the final diagnosis, e.g. an AML with a recurrent cytogenetic aberration: a *t(8;21)* translocation.

FISH is a tool to detect specific chromosomal aberrations. It can be applied to interphase nucleoli or metaphases after cell culture.

Probes are designed to bind specific genomic regions and allow the detection of trisomy (A), deletions (B) and translocations (C).

FISH is more sensitive than karyotyping and, in cases of specific translocations, can detect 1 in 200 cells (0.5%).

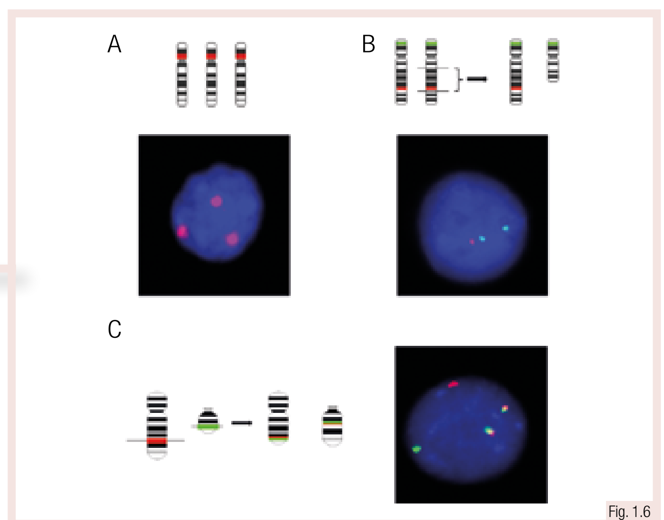


Fig. 1.6

REVISION QUESTIONS

1. What is the purpose of conventional cytogenetics?
2. What material (i.e. fresh or fixed) can be used for a cytogenetic workup?
3. What is the lower sensitivity level of FISH?

Diagnosis of leukaemias – molecular techniques

Polymerase chain reaction (PCR) is a method used for the detection of specific gene regions, e.g. specifically after rearrangement.

After gel electrophoresis, PCR products are visualised by DNA staining. Different PCR products of 9 patients of the rearranged fusion gene *BCR-ABL1* in CML discriminate the breakpoint (A).

Quantitative PCR (qPCR) (B) is a highly sensitive method to detect even low levels of tumour burden. In certain AML and CML, qPCR is validated for MRD detection.

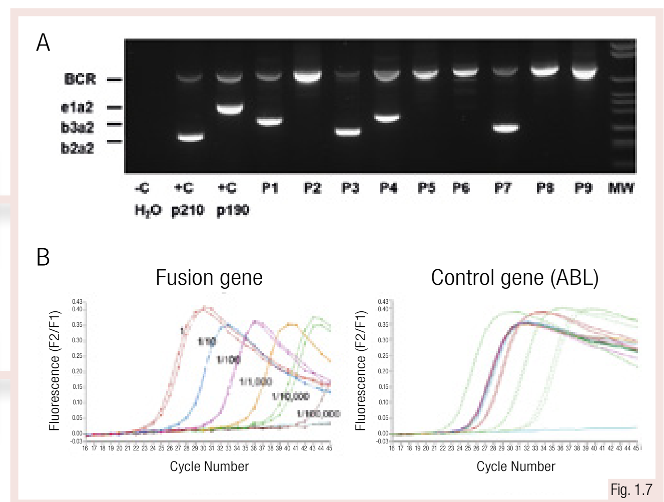


Fig. 1.7

Overview of NGS (next generation sequencing) instruments launched since 2005 from Roche(454), Illumina, Ion Torrent and Qiagen; illustrating the development of NGS with increasing sequencing capacities

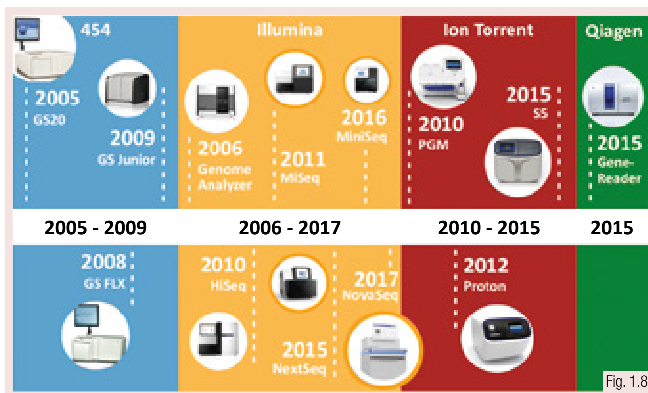


Fig. 1.8

Molecular sequencing techniques have enabled fast and accurate analysis from single genes to whole genomes.

The first cancer genome reported was an AML genome published in *Nature* in 2008.

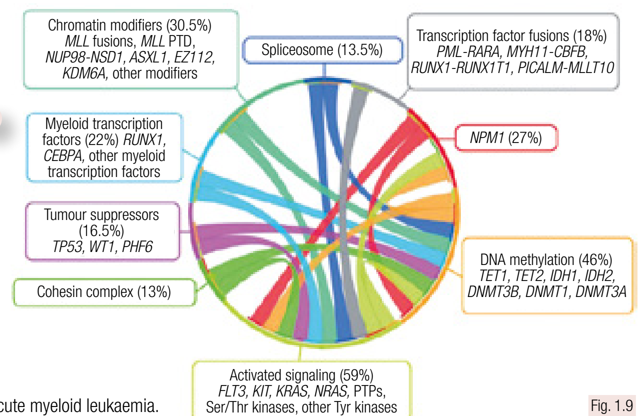
The Cancer Genome Atlas project has added numerous AML genomes, identifying driver and passenger mutations.

Those analyses have shown that AML is a disease with only few recurrent mutations.

Genetic events in AML occur in 9 different functional classes. Some mutations are strongly associated with each other, while others are mutually exclusive (Chen et al. 2013).

Gene panel sequencing is on its way to becoming a routine measure in the diagnosis of leukaemias and MDS, and is of diagnostic and prognostic value.

Circos plot showing genetic events leading to AML. Ribbons connecting distinct categories reflect the associations between mutations. Mutual exclusive alterations are not connected



AML, Acute myeloid leukaemia.

Fig. 1.9

REVISION QUESTIONS

1. Which molecular techniques are used in leukaemia diagnosis?
2. What is the role of real-time PCR in molecular diagnostics?
3. What is the role of gene sequencing in establishing the diagnosis?

Classification of AML and CML

The French-American-British (FAB) classification for AML was based on cytomorphologic features and has been replaced by the WHO 2001/2008 and 2016 classifications.

The European LeukemiaNet (ELN) defines 3 risk groups according to genetic abnormalities (Döhner et al. 2017).

Certain AML subgroups such as acute promyelocytic leukaemia (APL) (*PML-RARA*, *t[15;17]*) benefit from targeted treatment and have an excellent prognosis.

2017 ELN risk stratification by genetics

Risk category	Genetic abnormality
Favourable	*t(8;21)(q22;q22.1); RUNX1-RUNX1T1* *inv(16)(p13.1q22)* or *t(16;16)(p13.1;q22); CBFB-MYH11* Mutated *NPM1* without *FLT3*-ITD or with *FLT3*-ITDlow* Biallelic mutated *CEBPA*
Intermediate	Mutated *NPM1* and *FLT3*-ITDhigh* Wild-type NPM1 without *FLT3*-ITD or with *FLT3*-ITDlow* (without adverse-risk genetic lesion) *t(9;11)(p21.3;q23.3); MLLT3-KMT2A* Cytogenetic abnormalities not classified as favourable or adverse
Adverse	*t(6;9)(p23;q34.1); DEK-NUP214* *t(v;11q23.3); KMT2A* rearranged *t(9;22)(q34.1;q11.2); BCR-ABL1* *inv(3)(q21.3q26.2)* or *t(3;3)(q21.3;q26.2); GATA2,MECOM(EVI1)* *−5* or *del(5q); −7; −17/abn(17p)* Complex karyotype**, monosomal karyotype*** Wild-type *NPM1* and *FLT3*-ITDhigh Mutated *RUNX1* Mutated *ASXL1* Mutated *TP53*

Fig. 1.10

*Low, low allelic ratio (<0.5), high, high allelic ratio (≥0.5)
**Three or more unrelated chromosome abnormalities in the absence of WHO-designated recurring translocations
***Defined by the presence of 1 single monosomy (excluding loss of X or Y) in association with at least 1 additional monosomy or structural chromosome abnormality
ELN, European LeukemiaNet; ITD, internal tandem duplication; WHO, World Health Organization.

Proposed new AML classification scheme discriminates 13 subgroups

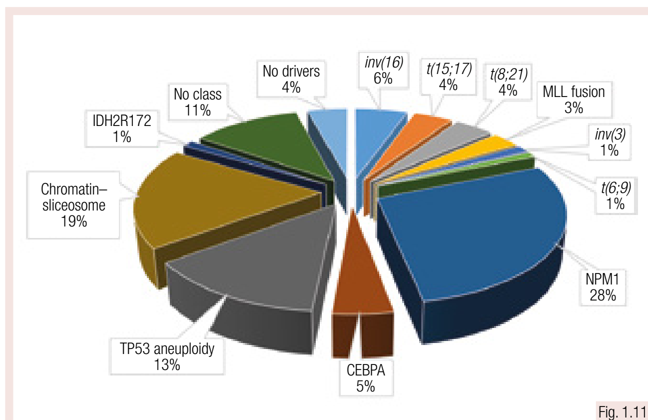


Fig. 1.11

AML, Acute myeloid leukaemia.

A new classification scheme was proposed, including karyotype and somatic mutations, and defines 13 AML subgroups (Papaemmanuil et al. 2016).

Specific chromosomal aberrations such as *t(8;21)*, *inv(16)*, *t(15;17)* are disease-defining, irrespective of the quantified blast count.

In the future, diagnosis of AML might rely solely on genetic findings.

CML is characterised by leukocytosis with myeloid progenitors in the peripheral blood termed ‘left shift’. The disease is driven by the Philadelphia chromosome *t(9;22)*, which produces the constitutive active fusion protein BCR-ABL1.

CML is classified into chronic phase, accelerated phase and blast phase, according to the blast cell count.

Therapy monitoring is performed using highly sensitive real-time PCR to detect BCR-ABL1.

Typical peripheral blood (A) and bone marrow (B) smear of CML patient, with hypercellularity and left shift. (C) FISH detects the *t(9;22)* translocation

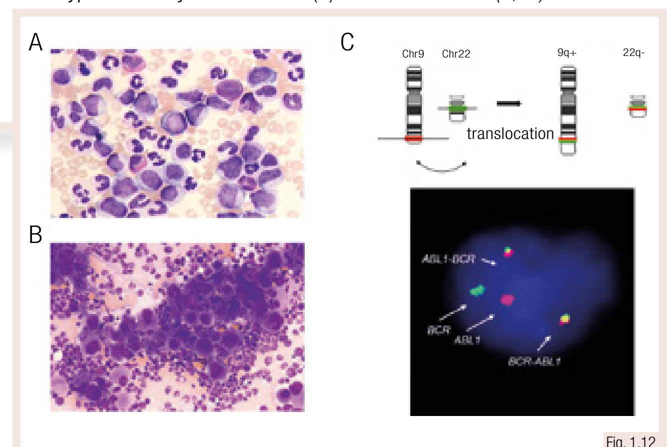


Fig. 1.12

CML, Chronic myeloid leukaemia; FISH, fluorescent *in situ* hybridisation.

REVISION QUESTIONS

1. What is the basis for the WHO 2016 AML classification?
2. Which aberrations and mutations are diagnostic for AML without a need for ≥20% blasts?
3. What is the genetic basis of CML?

Classification of ALL and MDS

The FAB classification for ALL is no longer in use. The European Group for the Immunological Characterization of Leukaemias (EGIL) classification is based on the immunophenotype according to maturation markers.

The EGIL subgroups are informative about prognosis and guide treatment, e.g. early allogeneic transplantation.

The WHO 2016 classification defines genetic ALL subtypes. There is a special focus on *BCR-ABL1*-positive and Philadelphia-like ALL, which require targeted treatment.

Immunophenotype as basis for EGIL classification: ALL subtypes include B and T cell lineages and different maturation stages

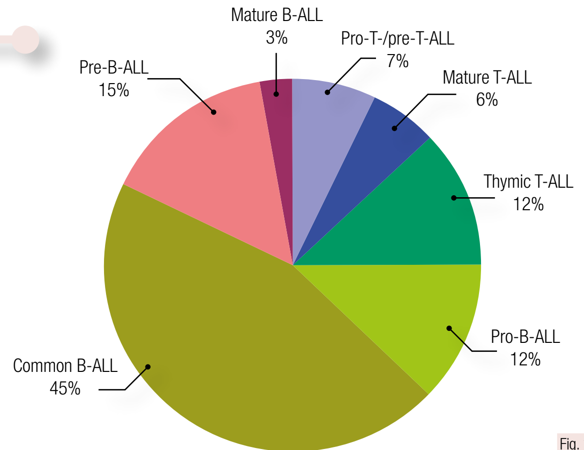


Fig. 1.13

ALL, Acute lymphoblastic leukaemia; EGIL, European Group for the Immunological Characterization of Leukaemias.

Clonal haematopoiesis and evolution to overt AML: There is a mutational continuum from pre-MDS to MDS and full-blown AML

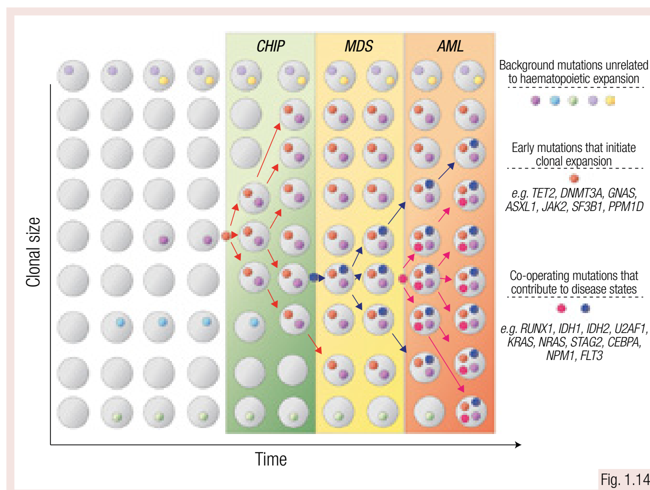


Fig. 1.14

AML, acute myeloid leukaemia; CHIP, clonal haematopoiesis of indeterminate potential; MDS, myelodysplastic syndrome.

MDS is a heterogeneous disease characterised by cytopaenia and single- or multilineage dysplasia.

Cytomorphologic diagnosis on bone marrow smears is gold standard. Certain genetic abnormalities such as *5q* deletion are associated with good prognosis and respond to targeted treatment.

Analysis of somatic mutations revealed the continuum from healthy individuals to MDS and AML in the pathogenesis of disease (Steensma et al. 2015).

Patients with cytopaenia and certain somatic mutations can be diagnosed with CCUS – clonal cytopaenia of undetermined significance.

Spliceosome mutations or co-mutations with *ASXL1*, *TET2* and *DNMT3A* in a patient with unexplained cytopaenia are highly predictive of a haematological malignancy.

The Revised International Prognostic Scoring System (IPSS-R) score is used for risk stratification: it defines risk groups according to karyotype, haemoglobin level and percentage of blast cells, platelet and neutrophil counts (Greenberg et al. 2012).

The IPSS-R score uses diagnostic parameters at initial presentation to define the patient's risk for progression and death

Subgroup	0	0.5	1	1.5	2	3	4
Cytogenetics	Very good	–	Good	–	Intermediate	Poor	Very poor
BM blast, %	≤2	–	>2–<5	–	5–10	>10	
Haemoglobin	≥10	–	8–<10	<8	–	–	–
Platelets	≥100	50–100	<50	–	–	–	–
Neutrophils	≥0.8	<0.8	–	–	–	–	–

Risk category: very low ≤1.5, low >1.5–3, intermediate >3–4.5, high >4.5–6, very high >6
BM, Bone marrow; IPSS-R, Revised International Prognostic Scoring System.

Fig. 1.15

REVISION QUESTIONS

1. What is the basis for the EGIL ALL classification?
2. What is the role of somatic mutations in a patient with cytopaenia?
3. Which parameters are needed for risk stratification of MDS?

Summary: Diagnosis and classification of leukaemias

- The diagnostic material for AML, ALL, CML and MDS is peripheral blood, bone marrow aspirate and histology
- Cytomorphology and cytochemistry are cheap and fast and can accurately diagnose leukaemias and MDS
- Flow cytometry is used to differentiate AML from ALL and defines ALL subgroups
- A LAIP can be used for MRD monitoring
- Cytogenetic evaluation by karyotyping and FISH is a diagnostic tool that also yields prognostic information
- The WHO 2016 classification of haematological neoplasms recognises the importance of genetic aberrations and somatic mutations
- The Philadelphia chromosome *t(9;22)* generates the fusion protein BCR-ABL1, which drives CML
- *BCR-ABL1*+ or Ph+ ALL is a specific ALL subgroup that needs specific targeted treatment
- Somatic mutations define clonal haematopoiesis. In a patient with cytopaenia, this results in the diagnosis of CCUS
- Specific mutations (spliceosome) or mutational patterns (co-mutations with *ASXL1*, *TET2*, *DNMT3A*) might become disease-defining or diagnostic in the future

Further Reading

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