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Hematological Diagnosis

Recommendations from the society for diagnosis and therapy of haematological and oncological diseases

DGHO DEUTSCHE GESELLSCHAFT FÜR HÄMATOLOGIE UND MEDIZINISCHE ONKOLOGIE

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1 Summary

Hematological diagnostics include the proper assessment of the overriding entity according to the current internationally valid classification, the most accurate subtyping, the determination of relevant predictive prognostic parameters and the monitoring of the therapy response as well as the characterization of acquired therapy resistance.

The subsequent explanations are intended for treating physicians and aim to describe currently valid standards of different diagnostic procedures, outlining their possibilities and limits.

2 Introduction

An accurate diagnosis is an indispensable prerequisite for an appropriate therapy decision in hematology. This includes not only the correct assessment of the overriding entity according to the currently valid WHO classification [16], but also the most precise subtyping, because also a subtype entity may impact on the treatment strategy.

Furthermore, and this is of particular importance before the use of targeted therapies, it must be ensured that the biological target structure is present on or in the concerned cells (e.g. CD20, CD22, CD30, CD52, BCR-ABL1, JAK2 V617F, PML-RARA).

Another important contribution of hematological diagnostics is to provide prognostic parameters for the individual diseases. Thus, based on this information, treatment within an entity can significantly change (eg, TP53 mutations in chronic lymphocytic leukemia). This applies not only to the initial assessment, but also during the course of the disease, since a series of mechanisms may influence resistance to treatment. (e.g. BCR-ABL1 mutations in chronic myeloid leukemia).

Finally, molecular detection methods provide particular important information on the treatment response and the depth of remission reached after a specific treatment. For a curative treatment approach, the evidence of persistent minimal residual disease (MRD) is meaningful for the prognosis [15]. Meanwhile, it could be demonstrated that even in non-curable diseases a good therapeutic response beyond the microscopic assessment also correlates with the expected length of remission.

The subsequent explanations are intended to briefly describe to the users of the various diagnostic tools the current valid standards, and to outline for the treating colleagues [3, 17] the possibilities and also the limits of this laboratory information [6, 7, 8].

3 Cytomorphology

3.1 Indications

- Unexplained abnormalities in the automated cell counter.
- Suspected primary bone marrow diseases (leukemia, lymphoma, etc.)
- Follow-up of remission status of hematological diseases
- Suspicion of neoplastic infiltration in non-hematopoietic organs or compartments (leptomeningeal space, peritoneum, etc.)

3.2 Methodological background

Evaluation of cytomorphology of peripheral blood and bone marrow is a rapid and inexpensive method to obtain relevant diagnostic information about hematological diseases [1]. Particularly with the acquisition of newer and more expensive procedures, cytomorphology has a pivotal role guiding the next diagnostic steps selecting the subsequent most meaningful and economic tests to perform [5].

The most important characteristics of cytomorphology are:

- Duration of the examination: The duration of the examination for a panoptic staining is approx. 15-30 minutes. In case of special staining or cytochemical staining, the duration of the examination depends greatly on the type of staining used. Ultimately, the cytomorphology can be completed within a day. This is useful to quickly guide the need of subsequent tests to perform.
- Advantages and disadvantages: The advantages of the method are its nearly ubiquitous availability, quick feasibility and the low costs. The main disadvantages are the low sensitivity of the method (approx. 1: 100) and the requirement of a high level of experience by the examiner.

3.3 Preanalytics

Peripheral blood, bone marrow aspirate/trephine biopsy and liquid puncture materials (CSF, ascites, pleural fluid, etc.) are all material suitable for cytomorphology examination. All of them can be well evaluated in a quickly prepared native state. However, they can also be mixed with the anticoagulant ethylenediaminetetraacetate acid (EDTA), which allows later preparations of comparable quality. Anticoagulated samples should be prepared and analyzed within 24 hours, this applies to both, the numerical and the differential blood count. Heparin is not a suitable anticoagulant for cytomorphology examinations because induces morphologic artifacts. Bone marrow smears should be dried for at least 30 minutes before staining. This also applies if unstained smears have to be sent, before they are packed in the shipping container.

A prerequisite for an informative cytomorphology exam is the collection of representative material and a professional preparation. Therefore, for any puncture, the accurate selection of the anatomic area is mandatory. The first sample of aspirate must be selected for cytomorphology analysis and this is the one that will be evaluated in the visual inspection under the microscope.

The preparation of the material can be processed immediately on the native material or later on anticoagulated material (EDTA or citrate). To guarantee a correct morphological evaluation of peripheral blood smears, it must be ensured that a decreasing thickness gradient, a feathered edge, is created on the glass slide.

Bone marrow preparations are made without delay after aspiration (within a few seconds because the aspirate can clot very quickly) or within 24 hours when the syringe contains EDTA. It is important to ensure that enough bone marrow fragments are spread on the slide. In case of frustrating aspiration (dry tap), touch preparations from a native trephine biopsy should be prepared. In principle, it is ideal to prepare at least 5 blood and bone marrows films. Other puncture materials should preferably be brought quickly, unmanipulated, to the laboratory, as they often need special processing there (e.g. cytocentrifugation). In case of doubt, the responsible of the laboratory should be consulted.

3.4 Analytics

The preparations should air-dried for at least 15 minutes (30 minutes for cell-rich bone marrow smears), especially if they need to be shipped. It is mandatory to carry out a panoptic staining (e.g. according to Pappenheim), which allows a good overview of the different cell fractions. All other colorations are summarize in Table 1.

Table 1: Cytomorphology Staining.

Staining	Indication
Pappenheim-Staining (Giemsa- & May-Grünwald-Staining)	Panoptic/panchromatic staining
Alpha-naphtyl acetate esterase staining (ANAE)	Detection of monocyte differentiated cells
Prussian blue reaction	Detection of extracellular (marrow macrophages) and intracellular (e.g. sideroblasts) iron
Brilliant cresyl blue	Detection of the reticulofilamentous pattern of ribosomes characteristically precipitated in reticulocytes
Periodic Acid Schiff Reaction (PAS)	Detection of glycogen-containing cells (e.g. in erythroleukemia)
Peroxidase Reaction (POX)	Detection of granulocyte differentiated cells
Acid Phosphatase (SP)	Characterization of neoplastic lymphoid cells (e.g. T-ALL)

The examination of the prepared slides is performed using a high-quality light microscope. The objective equipment should include an overview magnification (10x or 20x), a medium magnification (40x or 63x) and a high-resolution magnification (100x). The examination starts with an orientation about the quality of the material and a quantitative analysis of the cell distribution (10X objective without oil). Then, at least 100 nucleated cells (differential blood count) and the erythrocyte morphology are assessed in the peripheral blood smear. In the bone marrow at least 200 nucleated cells (myelogram) are categorized. Any deviation from the norm, e.g. in the case of pronounced hematopoietic hypoplasia, must be documented. With the exception of CSF, the analysis of other puncture materials does not follow a fixed principle, but depends primarily on the problem to address.

In addition to the quantitative analysis, an accurate description of qualitative cell characteristics deviating from the norm should be done. Negative findings (e.g. absence of foreign cells to the bone marrow like tumor cells) can also be helpful for the final assessment. The latter should be formulated as specifically as possible and can also contain references to complementary diagnostic methods.

3.4.1 Peripheral blood (differential blood count)

Nuclear blood cells are classified into at least the following categories (with exemplary standard ranges), see Table 2.

Table 2: Cytomorphology – classification and distribution of nuclear cells of peripheral blood

	Reference Range	Units
Band neutrophils	3-5	%
Segmented neutrophils	40-70	%
Eosinophils	2-4	%
Basophils	0-1	%
Monocytes	3-7	%
Lymphocytes	20-40	%

If necessary (e.g. reactive or pathological left-shift), following categories can be added: blasts, promyelocytes, myelocytes and metamyelocytes. Other hematopoietic cells such as erythroblasts, plasma cells, etc. are quantified separately.

The so-called nuclear shadows can be assigned to the lymphocyte fraction, but it can also be specified quantitatively (% of the leukocytes) or semi-quantitatively in the comments to the findings: few (+), some (++) or many (+++)].

Findings on lymphocyte morphology (stimulated and activated lymphocytes, LGL cells, mantle cells, etc.) require usually an interpretation and should therefore be included in the comments to the findings. Experienced examiners can, according to the DGHO recommendations, classify them as “typical lymphocytes”, “atypical lymphocytes, presumably reactive” and “atypical lymphocytes, presumably neoplastic”, and make a quantitative statement (% of the leukocytes) [2].

Further qualitative changes of the leukocytes (e.g. toxic granulations, pseudo-Pelger forms), erythrocytes (e.g. anisocytosis, poikilocytosis, polychromasia, ruleaux formations) or thrombocytes (e.g. giant platelets) can also be indicated semiquantitatively by classifying them into light (+), medium (++) or strong (+++).

The proportion of fragmentocytes is given in percent (%) or per thousand (‰) of the erythrocytes [4]. To determine the value, at least 5 fields of a 100x power magnification (corresponds to approx. 200 erythrocytes / field) must be counted.

3.4.2 Bone marrow

The evaluation of the bone marrow preparations should also initially contain a description on the cellular content, thereafter a quantification of the nucleated cells (myelogram), containing at least the following categories (with reference ranges), see Table 3:

Table 3: Cytomorphology: classification and distribution of nucleated cells in the bone marrow

	Reference range	Units
Myeloblasts	0-3	%
Promyelocytes	2-5	%
Myelocytes	8-17	%
Metamyelocytes	10-25	%
Band neutrophils	8-20	%
Segmented neutrophils	8-16	%
Eosinophils	2-6	%
Basophils	0-1	%
Monocytes	0-3	%
Proerythroblasts	0-2	%
Basophilic erythroblasts	1-4	%
Polychromatophilic erythroblasts	12-24	%
Orthochromatic erythroblasts	2-24	%
Lymphocytes	10-20	%
Plasma cells	0-3	%
Megakaryocytes	0-1	%

It is also useful to quantify the proportion between granulocytopoiesis and erythrocytopoiesis (G: E ratio). All other qualitative observations are described in the results report. For MDS and AML, the percentages of dysplasia in granulopoiesis, erythropoiesis, and megakaryopoiesis should be reported. It should be noted that in MDS, we speak of dysplasia when more ≥10% of nucleated cells of a specific cell line are affected by dysplastic changes; in AML, dysplasia can only be retained when ≥50% of nucleated cells of a cell-line are affected. The morphological criteria of "dysplasia" are described in the MDS and AML chapters in the 2017 WHO classification.

3.5 Quality standards

The quantitative (cell differentiation) and qualitative analysis (e.g. dysplasia, erythrocyte morphology) can be carried out by an experienced and medically authorized laboratory specialist. An evaluative analysis (interpretation of findings) has to be performed by an internist with specialization in hematology and oncology, a specialist in pathology, or a laboratory medicine specialist. The report must be signed or validated by an electronic signature by a physician. Regular participation to external quality controls is required, a certification / accreditation of the laboratory according is recommended (Germany: ISO 15189). National guidelines must be followed (Germany: RiliBÄK).

4 Flow cytometry

4.1 Indications

- Unclear deviations in automated or microscopic blood counts [14]
- Suspicion of primary bone marrow diseases (leukemia, lymphoma, etc.)

- Evaluation of remission status of hematological diseases
- Evidence of minimal residual disease (MRD)
- Suspicion of neoplastic infiltration of non-hematopoietic compartments (cerebrospinal fluid (CSF), peritoneum, pleural cavity, etc.).
- Suspicion of other clonal hematopoietic diseases (e.g. PNH)
- Suspected cellular immunodeficiency (e.g. HIV, common variable immunodeficiency, CVID).
- Lymphocyte immunphenotyping from bronchoalveolar lavage (BAL).

4.2 Methodological background

Characterization of leukocytes by flow cytometry is an important technique which allows measuring populations of physiological cells (for example, quantification of CD4-positive helper cells) and for the diagnostic classification of lymphatic neoplasms [12]. This method has also been established to identify myeloid cells based on normal or pathological granulation patterns or the type of antigens expressed. Also changes of the erythrocyte cells (e.g. paroxysmal hemoglobinuria, spherocytosis) can be detected.

The most important characteristics of this method are:

- Duration of the examination: The duration of the examination is approx. 60 minutes for a standard measurement. It can require more time for special applications.
- Advantages and disadvantages: The advantages of the method are its rapid implementation, it's good reproducibility and its higher sensitivity of up to 1: 10^5. The main disadvantage is the limited availability and their costs for equipment and antibodies, which is due to the necessary degree of technology.
- Due to the increasing use in MDS and in particular to detect MRD in AML, ALL and lymphoma, good technological equipment (number of colors) and a great expertise on the part of the operators is required.

4.3 Preanalytics

Any native and anticoagulated liquid material is suitable as test material for flow cytometry. During transport, samples should be kept at room temperature. In the case of anticoagulated materials, the interval between collection and analysis of the material should generally not exceed 48 hours, native fluids (e.g., CSF, etc.) should be examined within 6 hours after collection. Special transport tubes for CSF should be considered.

4.4 Analytics

Samples preparation should be performed according to standard procedures. A relevant aspect in this preparation is the incubation of the cell suspension with fluorochrome labeled antibodies or other fluorescent dyes. The specificity is determined by the type of antibody or by the fluorochrome used.

Regarding the type of antigen or target structures to investigate, there are a number of international recommendations that can assist as a reference. Thus, the European LeukemiaNet recommendations [15] and those from the EuroFlow Consortium [12] are good examples.

4.5 Quality standards

The analysis can be performed by an experienced and medically authorized laboratory specialist. It is important to point out that the generation of the data is highly dependent on the instrument settings and requires a high degree of instrument-specific expertise. For this reason, a daily internal quality control and, if necessary, adjustment of the fluorescence calibration must be done.

The interpretation of results is highly dependent on data acquisition (the so-called gating strategy) and should be performed by an internist with specialization in hematology and oncology, a specialist in pathology, or a laboratory medicine specialist. The report must be signed or validated by an electronic signature by a physician. Regular participation to external quality controls is required, a certification / accreditation of the laboratory is recommended.

5 Cytogenetics

5.1 Indication

- Detection or exclusion of hematologic diseases with recurrent chromosomal abnormalities
- Diagnosis and classification of diseases with specific cytogenetic changes
- Assessment of predictive factors for myelodysplasias, leukemias and lymphomas
- Control of remission in hematologic diseases

5.2 Methodological background

Cytogenetic examination techniques are well- established in the diagnosis and prognosis of hematologic diseases. According to the current WHO classification, many genetic abnormalities are associated with distinctive clinicopathological features [16]. In addition, cytogenetics can provide crucial prognostic information. In general the complete karyotype evaluating metaphases (according to ISCN, twenty metaphases must be examined), has to be distinguished from the assessment of specific loci analyzed by fluorescence-in-situ-hybridization (FISH), which is usually performed on interphases, however analysis on metaphases, i.e. the so-called chromosome painting, or also the 24-color FISH technic is also possible.

The karyotype can provide comprehensive information about structural (e.g. translocations) and numerical (aneuploidy) anomalies, but is, as a method relatively insensitive (approx. 1: 25-50). FISH examinations, on the other hand, only provide information on the examined chromosome locus, but are usually somewhat more sensitive (approx. 1: 200-500).

The most important characteristics of this method are:

• Duration of the examination: The duration of the examination for a classic chromosome analysis is 3-15 days. FISH analyzes can be performed within 1-2 days or within a few hours as part of urgent direct preparations (e.g. for PML-RARA).

• Advantages and disadvantages: The advantages of the procedure consist of its very well established diagnostic and prognostic value. The disadvantages consist of the need for viable malignant cells for the cultures of cytogenetics, the examination which is time-consuming, the low sensitivity (1:20 to 1: 500) and also its dependence of the examiner’s skills and experience.

5.3 Preanalytics

Classical chromosome analysis requires living cells as starting material; therefore the use of correct anticoagulant and the rapid sample transport are of particular significance. In principle, the examination is exclusively possible on heparin-anticoagulated material. To keep the death rate of the cells as low as possible during transport, the samples should be brought to the cytogenetics laboratory at room temperature within 24 (at least less than 48) hours. In general, the shorter the transport times, the more reliable the evaluation of cytogenetics. FISH analyzes on interphase nuclei can also be performed on EDTA anticoagulated material, but for reasons of flexibility and simplicity it is advisable to perform them from the same material as for classical chromosomal analysis. The material must be collected in a sterile container. Usually 2-5 ml of bone marrow or 10-20 ml of peripheral blood are required, but larger amounts greatly increase the sensitivity and the informative value.

5.4 Analytics

According to the current WHO classification, a large number of hematologic diseases are defined by recurrent chromosomal abnormalities. A selection of some of these entities are summarized in Table 4:

Table 4: Cytogenetic – disease-defining chromosomal abnormalities

Cytogenetic	Clinical entities
t(6;9)(p23;q34)	Acute myeloid leukemia with *DEK-NUP214*
inv(16)(p13;q23)	Acute myeloid leukemia with *CBFB-MYH11*-fusion
t(15;17)(q24;q21)	Acute promyelocytic leukemia with *PML-RARA*
t(1;22)(p13;q13)	Acute myeloid leukemia (megakaryoblastic) with *RBM15-MLK1*
t(9;22)(q34;q11.2)	Chronic myeloid leukemia (CML) or a Philadelphia chromosome-positive (Ph^+) acute lymphoblastic leukemia (ALL) or Ph^+ acute myeloid leukemia (AML)
Isolated del(5q)	Myelodysplastic syndrome with isolated del(5q)
t(v;11)(v;q23)	*KMT2A*- rearranged acute leukemia
t(1:19)(q23;p13.3)	B-ALL with *E2A-PBX1*
Hyper- or hypodiploidy	B-ALL with hyper- or hypodiploidy

In addition to these disease-defining chromosomal abnormalities, there are numerous cytogenetic findings which, in addition to other laboratory examinations, provide key information for the diagnostic process. A selection of some of these entities are summarized in Table 5:

Table 5: Cytogenetic– entities with further cytogenetic abnormalities as main diagnostic finding

Cytogenetic	Clinical entities
t(11;14)(q13;q32)	Mantle cell lymphoma (MCL)
t(14;18)(q32;q21)	Follicular lymphoma (FL)
t(8;14)(q24;q32)	Burkitt lymphoma/leukemia (BL)
inv(14)(q11q32) or t(14;14)(q11;q32)	T-cell prolymphocytic leukemia (T-PLL)

Finally, it has been shown in recent years that cytogenetic findings are of paramount importance in the determination of the prognosis of hematological entities. This report does not provide a detailed information on this topic.

5.5 Quality standards

In chromosome analysis the analysis of at least 20 metaphases should be evaluated. If a major clonal aberration is detected, an examination of at least 10 metaphases is sufficient. In the FISH analysis at least 100 interphase nuclei should be examined. The reporting of the results of the chromosome and the FISH analysis will be elaborated according to the current valid version of the international nomenclature (international system of human cytogenetic nomenclature, ISCN) [10].

Cytogenetic analysis can be performed by an experienced and medically authorized laboratory technician. The control of the chromosome findings and its interpretation should be performed by a specialist in human genetics, a physician in human genetics or a similarly qualified person. The report must be signed or contain a validated electronic signature from a physician. Regular participation in external quality controls (round robin tests) is required, a certification / accreditation of the laboratory is recommended.

6 Molecular genetics

6.1 Indications

- Detection or exclusion of hematologic diseases with known recurrent chromosomal abnormalities
- Assessment of predictive factors for myelodysplasias, leukemias and lymphomas
- Control of remission in hematologic diseases, particularly, detection of minimal residual disease (MRD).
- Monitoring of hematopoietic chimerism after allogeneic stem cell transplantation.

6.2 Methodological background

Molecular genetic examination techniques are now very well established for the diagnosis, prognosis and monitoring of hematological diseases [13]. The methods used include conventional polymerase chain reactions (PCR), and increasingly, modern genomic methods which allow the simultaneous quantitative analysis of a large number of genes, even offering the possibility of the examination of the complete genome, exomes, transcriptomes, etc. (NGS = next generation sequencing).

Due to the enormous spectrum of analytical possibilities, the indication to investigate various parameters must be done very carefully, based on clinical data, and the data provided by cytomorphology and flow cytometry.

The most important characteristics of this method are:

• Duration of the examination: Once the sample has been prepared, the duration of the examination of a conventional PCR requires 2-4 hours. Procedures that technically include DNA sequencing can take 24 hours (short DNA/RNA sequence) to one to two weeks (genome analysis). Furthermore, the time required for the bioinformatics interpretation of the data must be taken into account. This laborious step can be time-consuming depending on the amount of data to analyze.

• Advantages and disadvantages: The advantages of the procedure consist of its unexcelled specificity and sensitivity. The main disadvantages consist of the extraordinary high degree of

specialization required, the, need of collaboration between bioinformatics, molecular biologists and doctors for the use of NGS approaches, and the costs.

6.3 Preanalytics

In principle, any cell-containing body fluid, particularly peripheral blood, bone marrow aspirates and punctures, is suitable as test material. Ethylenediaminetetraacetate acid (EDTA) is the anticoagulant of choice for coagulable fluids, but other anticoagulants such as heparin can also be used. Due to the short half-life of ribonucleic acid (RNA), rapid transport (within 24 (-48h)) at room temperature to the testing laboratory must be ensured. Alternatively, special transport tubes with stabilizer additives can be used, but their use has not yet been established for all used methods.

While the targeted examination of pathogenic somatic (acquired) genetic aberrations is not subject to any particular regulation, germline analysis requires according to the German Genetic Diagnostics Act (GenDG) invariably a separate information and the patient's written consent.

6.4 Analytics

Many molecular genetic detection methods are based on the principle of the polymerase chain reaction (PCR). The obtained amplified material can be revealed directly using electrophoretic or fluorescence-assisted methods. Fluorescence methods also allow accurate quantification of the number of copies of the starting sample. Methodologically, there are two areas of problems in molecular genetics: false-negative results due to nucleic acid degradation and false-positive results due to cross-contamination. In order to reduce false-negative results, it is mandatory to perform in parallel a sample-specific positive control (internal control). This can also be used as a quantitative reference. Cross contamination must be avoided by strict room and areas separation for the preparative and analytical steps. An additional possibility is to eliminate uracil-containing contaminating amplicons by digesting them using uracil-DNA-glycosylase.

Sequencing techniques are increasingly used today. Some of these are still based on the Sanger chain termination method. In principle, double-stranded sequencing is preferred, which reduces the probability of errors by this method. The comparison of the determined nucleic acid sequence (now called variants) with public databases requires special expertise, especially when it concerns variable gene regions (e.g. immunoglobulin chains).

A new development within molecular genetics is the use of genomic high-throughput methods that enable the simultaneous (parallel) sequencing of a large number of gene segments or genes or even the entire genome (NGS). Although these procedures are increasingly affordable, methodologically simpler and faster, they are still a method that involves a very complex data analysis. Furthermore, the clinical significance, particularly the prognosis of many new molecular aberrations has not yet been clarified, therefore, an important task in the near future will be to further clarify the diagnostic and prognostic value of individual aberrations and define methodological requirements (for example, sensitivity). Each laboratory has to fix its own sensitivity of the Sanger sequencing (sensitivity approx. 10-20%) or NGS (sensitivity 2-5%) methods, based on his own experience, the usual quality controls, round robin testings and, if necessary, accreditation of the methods. Among a number of the advantages that NGS offers, the ability to accurately describe tumor burden (variant allelic frequency = VAF) is clearly on of them.

The number of molecular genetic abnormalities that are of diagnostic or prognostic significance is steadily increasing. However, not all of them have already been clinically adequately validated. A selection of some molecular abnormalities with a defined clinical significance are summarized in Table 6.

Table 6: Molecular biology - mutations with established clinical significance

Mutations	Clinical entities
BCR-ABL1	Chronic myeloid leukemia (CML) or a Philadelphia chromosome-positive (Ph^+) acute lymphoblastic leukemia (ALL) or Ph^+ acute myeloid leukemia (AML)- MRD detection
JAK2 V617F-exon 14 *JAK2* - exon 12 *MPL* W515 *CALR*	Confirm or exclude a myeloid disease, mainly a myeloproliferative neoplasms.
RUNX1-RUNX1T1	Acute myeloid leukemia with recurrent genetic abnormality. AML with t(8;21)(q22;q22.1) – MRD detection.
PML-RARA	Acute myeloid leukemia with recurrent genetic abnormality. Acute promyelocytic leukemia with PML-RARA – MRD detection.
CBFB-MYH11	Acute myeloid leukemia with recurrent genetic abnormality. AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22) – MRD detection.
NPM1-mutation	Acute myeloid leukemia with gene mutation: AML with mutated NPM1 (new entity included in the classification of WHO 2017). Prognostic factor- MRD detection.
CEBPA-mutation	Acute myeloid leukemia with gene mutation: AML with biallelic mutation of CEBPA (new entity included in the classification of WHO 2017). Prognostic factor- MRD detection.
PDGFRA/PDGFRB- rearrangement	Confirm or exclude a myeloid/lymphoid neoplasms with eosinophilia and gene rearrangement
P53- mutations (exons 4-10)	Usually in the presence of a high-risk constellation (e.g. CLL, AML, MDS)

6.5 Quality standards

The molecular analysis can be performed by an experienced and medically authorized laboratory technician. The control of the findings and its interpretation should be performed by a geneticist in human genetics, a physician in human genetics or a similarly qualified person (e.g. molecular biologist or molecular pathologist, if necessary with the support of a bioinformatician). The report must be signed or validated by an electronic signature by a physician. Regular participation in external quality controls (round robin test) is required, certification / accreditation of the laboratory is highly recommended.

7 Histopathology (Bone marrow)

7.1 Indications

If in the diagnostic workup of a hematologic disease, a bone marrow investigation is indicated, performing a bone marrow aspiration for cytology together with a trephine bone marrow biopsy is recommended. These both methods provide complementary information allowing thus a more complete understanding about bone marrow involvement of the disease [11]. Some relevant indications for obtaining a trephine bone marrow biopsy for histology are:

- Extent of infiltration of the bone marrow in leukemia and MDS [9]. Extent of infiltration and topography in lymphoma and multiple myeloma, at first diagnosis or during follow-up to evaluate the grade of remission after therapy and assessment of the residual hematopoiesis.
- Diagnostic of myeloproliferative neoplasms, especially with dry tap (assessment of the evidence and degree of fibrosis, e.g. in prefibrotic and primary myelofibrosis, hairy cell leukemia, etc.)
- Infiltrates of a classic Hodgkin lymphoma

- Diagnostic of focal processes in the bone marrow (metastases of solid tumors, evidence of a granulomatous disease, etc.).

7.2 Methodological background

Histopathological examinations of the bone marrow and solid tissues, especially of lymph nodes, are still the diagnostic gold standard for many hematological entities, especially for malignant lymphomas. The procedure has been established since many years and for the examination of solid tissues is practically unequaled. Nowadays it is supplemented by immunohistochemistry and molecular pathological techniques. It is the only technic discussed in this chapter which allows to detect tissue alterations. In the current updated WHO classification, there are many hematological entities that are very well characterized by the histopathology.

The most important characteristics of histopathology are:

- Duration of the examination: The duration of the examination varies, depending on the material and the clinical problem to address; thus, it can take few hours (so-called frozen section) to several days (e.g. complex immunohistochemistry and molecular examinations of the bone marrow and lymph nodes).
- Advantages and disadvantages: The advantages of the procedure are the long experience and, in particular, the very good specificity. The main disadvantages are the high dependence on the level of experience of the examiner, as well as the resulting limitations for standardization.

7.3 Preanalytics

A prerequisite for a meaningful histological examination of a bone marrow trephine biopsy is the collection of representative material. The biopsy cylinder is usually obtained in the area of the postero-superior iliac crest, the length of the biopsy cylinder should be at least 1.0 cm, but if possible 2.0 cm. By sending the bone marrow biopsy to an institute for pathology, all relevant clinical information (blood count, bone marrow cytology, previous illnesses, etc.) and, in particular, a formulation of the clinical question should be transmitted. Neutral-buffered formalin is usually used for fixation, followed by paraffin embedding in pathology, which first requires a decalcification procedure. This processing leads, in contrast to embedding in synthetic resins for example, to good preservation of the surface antigens of the bone marrow cells, which is important for frequent requested immunohistochemistry investigations.

7.4 Analytics

Bone marrow sections should be routinely stained with hematoxylin-eosin (HE -), possibly also with Giemsa staining, which allow a good overview of the various cell populations in the bone marrow. The examination is performed on a high-quality light microscope by a physician for pathology who has special experience in hematopathology diagnostics. The report of the bone marrow findings should contain information on cellularity, the topographical distribution of the cellular elements, and assessments of the cell morphology and, of course, describe pathological changes / infiltrates. For many clinical questions (e.g. subclassification of a lymphoma infiltrate) immunohistochemistry examinations are required, occasionally molecular examinations (e.g. clonality analysis in the case of discrete bone marrow infiltration by a lymphoma) must be carried out.

7.5 Quality standards

Preanalytic steps can be performed by an experienced and medically licensed laboratory technician. Assessment and interpretation of findings should be done by a medical specialist in pathology. The report must be signed or validated by an electronic signature by a physician. Regular participation in external quality controls (round robin test) is required, certification / accreditation of the laboratory is recommended.

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15 Disclosure of Potential Conflicts of Interest

according to the rules of the responsible Medical Societies.