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Our approach to bone marrow biopsies in cytopenia

Quentin Le Clef[a,b], Thomas Menter[a], Alexandar Tzankov[a,*]

[a] Institute of Pathology and Medical Genetics, University Hospital Basel, Switzerland
[b] Department of Pathology, Maastricht University Medical Center (MUMC+), Maastricht, the Netherlands

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ABSTRACT

Unexplained cytopenia is one of the most common indications for performing trephine bone marrow (BM) biopsy (BMB). The histopathological examination in this regard must be seen in the broader context of a multimodal approach in order to reach an as entity-specific as possible diagnosis, considering medical history, physical examination, laboratory data, peripheral blood morphology, BM aspiration smear, flow cytometry results and, if indicated, cytogenetics and molecular genetics. The particular irreplaceability of the histopathological work-up and the expectations to the BMB lie especially in the detection of fibrosing and/or focal processes (e.g. localized islets of blasts) and disorders extrinsic to the BM such as e.g. metastases, thrombotic microangiopathies, granulomatous myelitides etc. We propose a systematic combined histopathological pattern-based and blood count-based approach that can be applied in such circumstances to achieve a precise diagnosis or, at least, a clinically useful differential diagnosis, particularly taking into consideration specific morphologic pitfalls and application of ancillary techniques. Constitutional BM failure syndromes will not be profoundly addressed.

1. Introduction

Unexplained cytopenia is – apart from the demands on staging and follow-up – one of the most common indications for performing trephine bone marrow (BM) biopsy (BMB). The histopathological examination in this regard must be seen in the broader context of a multimodal approach in order to establish an as entity-specific as possible diagnosis, taking into account medical history, physical examination, laboratory data, peripheral blood morphology, BM aspiration smear results, flow cytometry (FCM) results and, if indicated, cytogenetics and molecular genetics. Indeed, the particular irreplaceability of the histopathological work-up and the expectations to the BMB lie especially in the detection of fibrosing and/or focal processes (e.g. localized islets of blasts) and disorders extrinsic to the BM such as e.g. metastases, thrombotic microangiopathies, granulomatous myelitides etc. [1,2]. Particularly in the case of a fibrosing process with a dry tap, the BMB will be the most important diagnostic specimen available, both for histomorphological assessment and – if proper decalcification methods are applied [3] - molecular analysis as touch preparations of the biopsy core for cytological examination are not always routinely performed and only in some instances genetic aberrations will be detectable in the peripheral blood. A systematic combined histopathological pattern-based and blood count-based approach – as listed below – taking into consideration specific morphologic pitfalls can be applied in such circumstances to achieve a precise diagnosis or, at least, clinically useful differential diagnosis. Constitutional BM failures will not be addressed here and, in that respect, we refer to other excellent reviews [4–7].

As stated above, a definitive diagnosis, or at least a differential diagnosis with respect to cytopenia, can in most cases only be made by integrating input from various specialties. Although this multimodal approach is acknowledged and encouraged by hematopathologists, clinicians are – in our experience – less aware of it. In this regard, the pathologist should not only receive the basic laboratory results, but also has to expect relevant information regarding medical history and clinical examination. Is there a suspicion of an underlying congenital or genetic disease? Have any previous chemotherapy, radiotherapy or potentially myelotoxic drugs been applied? Are there signs of a systemic infection or (auto-)immune disease? What is the duration of the complaints and of the abnormal blood values noticed? Are there signs of concurrent renal, hepatic or pulmonary disease? Relevant features of the clinical examination are fever (whether or not of unknown origin), other signs of infection, hepatomegaly, splenomegaly, lymphadenopathy and skin changes. The peripheral blood examination, constituted of blood cell counts and a hemogram, should also include blood levels of iron, copper (often forgotten/underestimated), folate and vitamin

* Corresponding author at: Institute of Pathology, Schönbeinstrasse 40, 4031, Basel, Switzerland.
E-mail address: alexandar.tzankov@usb.ch (A. Tzankov).

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B12. Finally, a complete and thorough list of medication and – if applicable – other exposed toxic agents should be added. Indeed, toxic myelopathies due to drugs, though common, are largely underestimated and are hardly diagnosable as long as one is unaware of the patient's medication.

This need for a multilateral approach will only grow, especially dealing with new entities and emerging concepts, such as "pre-myelodysplastic" conditions. These conditions presenting with cytopenias, namely idiopathic cytopenias of unknown significance (ICUS) and clonal cytopenias of unknown significance (CCUS), per definition do not (yet) meet the criteria for myelodysplastic syndromes (MDS) and, thus, require that MDS have been ruled out thoroughly (BM smear, BMB, cytogenetics, molecular studies) [8,9]. Having these concepts in mind, in order to establish the correct diagnosis, each such a case will have to be viewed and discussed by the treating physician, the laboratory hematologist, the cytogeneticist, the molecular geneticist and the hematopathologist.

2. Different types of cytopenias

2.1. Pancytopenia

Scenario 1: Pancytopenia with BM hypo-/aplasia is the typical presentation of aplastic anemia (AA). By definition (Table 1), the cellularity of the BM should be less than 25% of the expected cellularity for the given age (calculated by the formula 100-age = expected % cellularity, for ages between 20 and 80) or hematopoiesis should be less than 30% in a background BM with 25–50% cellularity for the given age [10]. Usually, the BMB contains plasma cells, mast cells, histiocytes and lymphocytes as well as some erythropoietic "hot spots" (i. e. circumscript aggregates of residual erythroid precursors), which can be easily visualized e.g. by an E-cadherin staining, but particularly myelopoiesis appears highly hypoplastic (Fig. 1). Those erythropoietic hot spots may show megaloblastic changes; however, no overt signs of dysplasia should be found. Normally, there are mostly no stromal changes, except some (proteinaceous) edema.

A first pitfall to consider is pseudohypoplasia, which applies to the first 2–4 subcortical BM spaces (the biopsy must be large and deep enough). Some authors advocate a BMB of at least 1,5–2 cm [11]. In case of being confronted with a small, less representative biopsy, one should keep some reservation with regard to a diagnosis of AA. A second pitfall are hypoplastic neoplastic disorders such as MDS, acute myeloid or lymphoblastic leukemias (AML or ALL) as well as lymphoid malignancies that may mimic AA (Fig. 2). Missing the latter can and must be avoided by the proper use of ancillary *in situ* techniques for blast- (CD34, TdT) and/or lymphoma-detection (CD3, CD5, CD20, CD79a, kappa/lambda light chains) as well as proper integration of FCM-, BM aspiration-, cytogenetic- and molecular data. Especially hairy cell leukemia (CD20+, CD103+, cyclin D1+, $BRAF^{V600E}$+) is a known mimicker of AA, and can be very subtle and hardly noticeable on the H&E-slide. Reactive lymphoid/lymphoplasmacytic aggregates are

Table 1
Diagnostic criteria for severe aplastic anemia (SAA) [10].

1	Bone marrow cellularity < 25% (or 25-50% if < 30% of residual cells are hematopoietic)
2	At least two of the following:
	Peripheral blood absolute neutrophil count (ANC) < 500/μL (< 0.5×10^9/L)
	Peripheral blood platelet count < 20.000/μL
	Peripheral blood reticulocyte count < 20.000/μL
Very severe AA (VSAA)	
1	Hypocellular bone marrow (as described for SAA)
2	ANC is < 200/μL
Non-severe AA	
1	Hypocellular bone marrow (as described for SAA)
2	Peripheral blood cytopenias not fulfilling criteria for SAA or VSAA (see above)

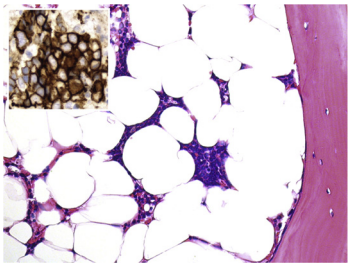

Fig. 1. Aplastic anemia: hypocellular bone marrow with marked hypoplasia of the myeloid lineage and presence of erythroid hot spots (H&E). The precursors of the erythroid lineage can be highlighted using an E-cadherin stain (inlet).

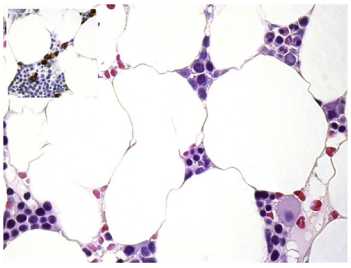

Fig. 2. Hypoplastic myelodysplastic syndrome: Hypocellular bone marrow with megaloblastoid changes of the erythropoiesis, one dysplastic monolobated megakaryocyte (right lower part) and an increase of CD34+ blasts forming atypically located immature precursor clusters (ALIPs) (H&E; inlet CD34). An increase in blasts and the formation of ALIPs as well as the presence of monolobated megakaryocytes is incompatible with the diagnosis of aplastic anemia.

common in AA, and even if their benign/polyclonal nature has been established by immunohistochemistry, one must still be attentive that these aggregates do not mask an underlying (also myeloid) neoplasm. An increased amount of blasts – either in the BMB (defined as > 5% of BM cellularity) or in the peripheral blood (any) – almost excludes the diagnosis of AA, and indicates a neoplastic process such as MDS, AML or ALL. As mentioned before, a mild dyserythropoiesis can be expected in AA, yet there should be no signs of myeloid dysplasia or dysmegakaryopoiesis.

AA is further subdivided in primary or idiopathic AA and secondary AA, as in the latter an underlying trigger can be identified [12]. Common triggers are drugs [e.g. chloramphenicol (Table 2)], radiation, chemical agents (e.g. benzene derivatives, pesticides), viral infections (hepatitis), eosinophilic fasciitis, autoimmune diseases (especially systemic lupus erythematosus), paroxysmal nocturnal hemoglobinuria (PNH) and pregnancy. For obvious reasons establishing a diagnosis of secondary AA can only be achieved in an integrative manner.

Looking for a PNH-clone by FCM (fluorescently labelled aerolysin or FLAER), is mandatory in the work-up of AA patients [13]. AA puts the hematopoietic stem cell under severe proliferative stress and survival pressure and, thus, fosters the dominance of clones with specific advantages, among others PNH clones. This explains the high prevalence of clonal hematopoiesis in up to 50% of AA patients. PNH is a rare disease caused in most instances by mutation of *PIG-A* in a hematopoietic stem cell, resulting in a deficiency of the GPI-anchored complement inhibitors CD55 and CD59 on the surface of erythrocytes that makes them highly prone to complement-mediated hemolysis [14]. Although subtle differences are described (higher cellularity and more prominent erythropoiesis in classical PNH), there are no significant histomorphological differences between AA with or without PNH clones [15].

As to be anticipated from the existence of primary and secondary forms, AA does not have specific unifying molecular features, yet mutational analysis might be occasionally important to rule out differential

Table 2
Common drugs and toxins involved in reactive cytopenias.

Pancytopenia
- Cytostatic drugs of all kind
- Chloramphenicol
- Benzene derivatives
Anemia
- Penicillins
- Cephalosporins
- α-methyldopa
- Non-steroidal anti-inflammatory drugs (NSAID)
- Lead
- Zinc containing preparations
Neutropenia
- Clozapine
- Olanzapine
- Sulfasalazine
- Trimethoprim/sulfamethoxazole
- Ticlopidine
- Levamisole
- Rituximab
Trombocytopenia
- Heparin
- Anti-platelet drugs (abciximab and tirofiban)

diagnoses. The most commonly mutant genes in AA are *PIG-A, BCOR/BCORL1, DNMT3A* and *ASXL1* [16]. The first two genes are considerably more commonly affected in AA compared to myeloid neoplasms, while the latter two overlap with mutations seen in pre-malignant and neoplastic myeloid BM disorders and might be more a reflection of clonal hematopoiesis due to proliferation and survival pressure on the stem cells because of the AA rather than of direct causality. In contrast, mutations in *TET2, TP53, RUNX1* and splicing factors are much rarer in AA than in MDS/AML, and their detection should seriously question the diagnosis of AA. Cytogenetic abnormalities similar to those in MDS, can be found in AA, including +8 and -7 (the latter particularly in high-risk AA), whereas +6 and +15 are found in AA but rarely occur in MDS [17,18].

Scenario 2: Pancytopenia with increase of histiocytes (with hemophagocytosis) often accompanies hemophagocytic lymphohistiocytosis (HLH), also known as hemophagocytic syndrome (Fig. 3). The gnosticdia criteria for hemophagocytic lymphohistiocytosis are listed in Table 3. Yet, hemophagocytosis on its own can be observed in patients without any systemic disease as well as in patients, who have received blood transfusions. In addition, patients suffering from HLH may not display frank histologically detectable hemophagocytosis, and the amount of hemophagocytosis is of poor predictive value for the severity of HLH [19]. In fact, in HLH, hemophagocytosis is far less observed in BMB than it is in the spleen or lymph nodes. Consequently, HLH is an integrative clinico-pathological diagnosis and is established based on specific criteria (Table 3) [20]. As those patients are generally very ill and HLH evolves rapidly into a life-threatening situation, establishing the diagnosis as soon as possible is of paramount importance.

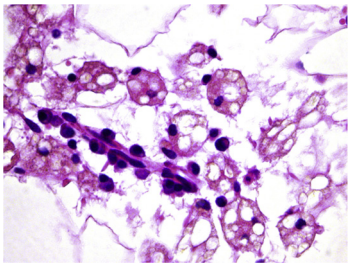

Fig. 3. Hemophagocytosis: hypocellular bone marrow with an increase of macrophages, showing a vacuolated “strawberry-like” cytoplasm containing ingested erythrocytes and nucleated blood cells in a patient suffering from secondary hemophagocytic lymphohistiocytosis associated with Epstein-Barr virus infection; note the reactive, perisinusoidal plasma cell population (H&E).

Table 3
Diagnostic criteria for hemophagocytic lymphohistiocytosis [20].

A. Molecular diagnosis consistent with HLH: pathologic mutations of *PRF1*, *UNC13D*, *MUNC18-2*, *RAB27A*, *STX11*, *SH2D1A*, or *BIRC4*
or
B. Five of the 8 criteria listed below are fulfilled:
1. Fever ≥38.5 °C
2. Splenomegaly
3. Cytopenias (affecting at least 2 of 3 lineages in the peripheral blood)
Hemoglobin < 9 g/dL (in infants < 4 weeks: hemoglobin < 10 g/dL)
Platelets < 100.000/μL
Neutrophils < 1.000/μL
4. Hypertriglyceridemia (fasting, > 265 mg/dL) and/or hypofibrinogenemia (< 150 mg/dL)
5. Hemophagocytosis in bone marrow, spleen, lymph nodes, or liver
6. Low or absent NK-cell activity
7. Ferritin > 500 ng/mL
8. Elevated sCD25 (α-chain of sIL-2 receptor)

Primary or familial HLH is a rare, autosomal recessive disorder that arises at infant age - mostly before the age of one year - and is frequently triggered by infections, particularly Epstein-Barr virus (EBV). In most cases, point mutations can be found in *PRF1* (coding for perforin), *UNC13D* or *STX11*, which as a net effect perturb the cytotoxic effector functions of T-cells forcing cell signaling to hyperactivate histiocytes [19]. HLH also occurs in patients with inherited immune deficiencies, such as Griscelli syndrome 2 and Chédiak-Higashi syndrome, which are also linked to cytotoxic granule dysfunction [19,21].

Most of the secondary HLH cases are initiated by an underlying, either infectious (mostly EBV, more rarely histoplasmosis), neoplastic (mostly T-cell lymphomas or histiocytic neoplasias) or chronic inflammatory trigger (systemic lupus erythematosus or rheumatoid arthritis), often in patients with respective immunogenetic susceptibility [21]. As this obviously influences both prognosis and treatment, identifying the underlying condition is one of the primary goals of the BMB. In cases with virus-infection-associated (particularly EBV) HLH, which represent approximately half of the HLH, variable numbers of atypical, particularly EBV-infected T-lymphocytes can be encountered (Fig. 4). Apart from the EBV-association in secondary HLH in the setting of EBV-driven T-cell lymphomas (NK/T nasal type lymphoma, systemic EBV-positive T-cell lymphoma of childhood, etc.), as mentioned, most primary HLH will be associated with EBV-infection too. Therefore (since a substantial proportion of patients with HLH suffer from either EBV-infection, or EBV-driven malignancies or both), careful examination of the BM for respective evidence by means of EBER, EBER-CD3/-CD4/-CD8 and EBER-CD20/-CD79a double-stains, CD56, cytotoxic markers and additional B- and T-cell markers is warranted (Fig. 4). Further (immuno-)histochemical stains should be performed in order to exclude other infectious cause, such as histoplasmosis or CMV, as well.

An important clinical differential diagnosis that can be histopathologically verified/falsified is leishmaniosis, which is accompanied by swollen macrophages containing Donovan inclusions that can be highlighted in the Giemsa staining (Fig. 5) [22].

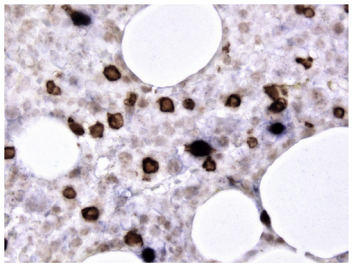

Fig. 4. Epstein-Barr virus infection of T-cells in a patient with hemophagocytic lymphohistiocytosis accompanying chronic active Epstein-Barr virus infection (CD3/EBER doublestain).

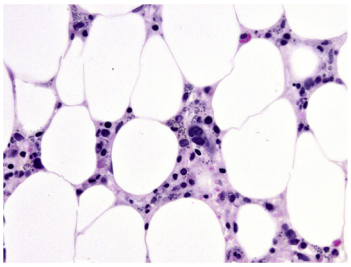

Fig. 5. Leishmaniosis: increased number of interstitial bone marrow macrophages with abundant cytoplasms containing numerous Donovan bodies (Giemsa).

Morphological differential diagnoses are Langerhans cell histiocytosis (S100 +, CD1a +, langerin +, CD68-, in 50% $BRAF^{V600E}$ +), Rosai-Dorfman disease (S100 +, CD1a-, CD68-, $BRAF^{V600E}$-), Erdheim-Chester disease (S100-, CD1a-, langerin-, CD68 +, in 50% $BRAF^{V600E}$ +) [23]. In the latter, hemophagocytic activity may be occasionally observed and some more advanced disease forms may be accompanied by HLH. Malignant histiocytic lesions, such as histiocytic sarcoma, can – although rarely – exhibit an overt hemophagocytosis as well. However, malignant histiocytic disorders show a considerable degree of cellular atypia. The presence of crystal-storing histiocytes in kappa-clonal plasma cell myeloma can be a pitfall too, which can be avoided by looking at the PAS stain, highlighting the crystalloid structures in the cytoplasm of histiocytes. An additional pitfall is the accumulation of histiocytes after previous myelo-ablative therapy. Lastly, one should keep in mind the possibility of a lysosomal storage disease (e.g. Gaucher disease, Niemann-Pick disease, sea blue histiocytosis, Tangier disease, etc.), which can occasionally present unexpectedly late in life as pancytopenia with a marked - either patchy or diffuse - increase of histiocytes [24]. As these histiocytes phagocyte/retain the accumulating substrate, they tend to have an enlarged, variably diastase-PAS positive cytoplasm with some entity-specific features (Gaucher disease: voluminous, eosinophilic, striated cytoplasm; Niemann-Pick disease: voluminous cytoplasm composed of uniform vacuoles; sea blue histiocytosis: cytoplasmic deep blue granules) [25].

2.2. Bicytopenia

Scenario 3: Bicytopenia (anemia and thrombopenia) with BM necrosis and microangiopathic changes can result from widespread endothelial damage seen in hemolytic-uremic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP), transplant-associated thrombotic microangiopathy (Fig. 6), and dissemination of (mucinous) carcinomas (especially of the stomach and the breast, occasionally of the prostate). A BMB is very rarely obtained in such conditions, as most cases can be solved clinically (e.g. demonstrating antibodies against ADAMTS13 in acquired TTP or *ADAMTS13* mutations in primary genetic forms) [26]; nevertheless, awareness of their existence and morphological appearance with hyaline thrombi, intravascular goblet cells or marrow necrosis is important to properly deal with these findings in unexpected circumstances. An additional very helpful hint can be the presence of fragmented red cells (schistocytes) in the peripheral blood film, caused by the fragmentation of erythrocytes passing through the almost occluded blood vessels.

Scenario 4: Bicytopenia (especially anemia and neutropenia) with dysplastic features should always raise the suspicion of an underlying copper deficiency (defined by decreased serum copper, decreased ceruloplasmin or decreased 24 -h urine copper excretion) [27]. There are multiple causes of copper deficiency: decreased intake (malnutrition, parenteral nutrition, excessive zinc substitution/ingestion), increased demand (pregnancy), inadequate absorption/uptake (inflammatory bowel diseases, coeliac disease, gastric surgery) or hereditary disorders (Wilson's disease, Menkes disease). Morphologically the BM can show extensive dysplastic changes, especially in the erythroid lineage, with presence of ring sideroblasts in the smear [27]. One important clue to this diagnosis is the presence - in the smear – of cytoplasmic vacuolization within erythroid and myeloid precursors. Timely recognizing an underlying copper deficiency is of importance, as protraction can lead to serious – yet at early stage, mostly reversible – neurological damage (optic neuropathy, demyelination of the central nervous system and motor neuron disease).

2.3. Anemia

Anemia is defined as a reduction of one or more red blood cell values, namely hemoglobin concentration, hematocrit or red blood cell count. Although the WHO has set the value for the hemoglobin concentration at 13.0 g/dL for men and 12.0 g/dL for women, the normal values may be adjustable for specific conditions and circumstances such as pregnancy, ethnicity, age and the employed laboratory methodology [28].

Scenario 5: Anemia with erythroblastopenia or pure red cell aplasia can be idiopathic (autoimmune; especially in collagen vascular diseases), drug-induced (e.g. phenytoin; Table 2), paraneoplastic [accompanying thymoma or large granular T-lymphocyte leukemia (T-LGL); see later], linked to parvovirus B19 infection or alloimmune in cases of allogeneic BM transplantation over the AB0-barrier [29]. Histopathological examination showing profound decrease to complete lack of erythroid precursors can be enhanced by stainings for E-cadherin or glyocophorin to visualize erythroblastopenia (Fig. 7) [30]. Myelopoiesis and megakaryopoiesis are mostly unremarkable. The diagnosis of parvovirus B19 infection is easy if the typical enlarged, infected proerythroblasts with characteristic nuclear inclusions (so-called lampion cells) (Fig. 8), which can be verified by application of immunohistochemistry, are detected; at occasions, these cells are scarce and must be dedicatedly sought for. MDS must be sufficiently falsified by means of histomorphological analysis and CD34 staining as well as

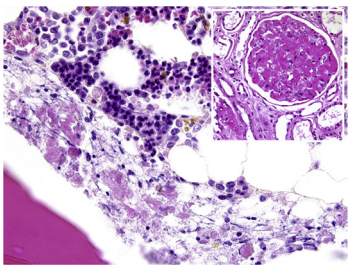

Fig. 6. Microangiopathic changes in the bone marrow of a patient suffering from transplant-related thrombotic microangiopathy with partial, ischemic-type necrosis and with presence of a fibrin-thrombus in one sinus in the lower middle part of the figure. The patient also had renal thrombotic microangiopathy (inlet) with numerous fibrin thrombi in the glomerular capillaries (H&E).

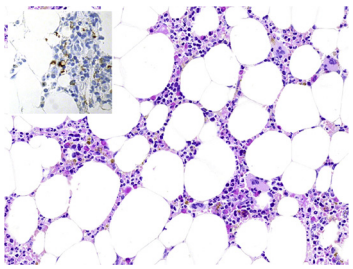

Fig. 7. Pure red cell aplasia in a patient transplanted across the ABO-barrier: bone marrow with normal maturation of the megakaryocytic and myeloid lineage, which can mask the decrease or absence of the erythroid lineage. However, staining for glycophorin A highlights the hypoplastic to absent erythroid lineage (H&E; inlet: glycophorin A).

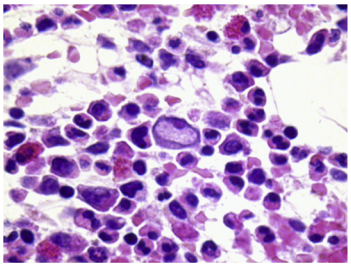

Fig. 8. Red cell hypoplasia caused by parvovirus B19 infection: an enlarged, infected proerythroblast with a viral inclusion ("lampion cells") scattered between myeloid precursors and erythroblasts with slight dyserythropoiesis is observable (H&E).

karyotypic and genotypic correlation.

Scenario 6: Anemias with increased erythroid precursors are either due to intrinsic (e.g. hemoglobinopathies, thalassemias, membrane defects, enzyme defects) or acquired [(e.g. vitamin B12-, folic acid- and/or iron deficiency, autoimmune hemolytic anemias (AIHA) or (thrombotic) microangiopathies] erythroid survival and maturation defects. For obvious reasons, a specific diagnosis based on histomorphological analysis is hardly ever possible in such conditions. In spite, integration of laboratory tests, germline genetic profiling, FCM data (e.g. for detection of PNH clones) is of unmet importance. Very prominent erythroid hyperplasia with left-shift and mild dyserythropoiesis in chronic hemolytic diseases (Fig. 9), complete lack of stainable iron in the BMB in pure iron deficiency anemias, or severe megaloblastic changes with hypersegmentation of mature granulocytes in vitamin B12 or folate deficiency (Fig. 10; see next paragraph) might help establishing a proper, at least, differential diagnosis. However, they can be hardly ever considered pathognomonic, especially given the fact that e.g. iron stains performed on EDTA-decalcified material, should be regarded with caution. Other distinct features such as ring sideroblasts, which might accompany toxic BM failure, are not visible on BMB. Nevertheless, histological examination of a BMB can certainly help to narrow further the differential diagnosis and especially to exclude extrinsic processes. In addition the peripheral hemogram, which must be mandatorily available for the hematopathologist, is particularly helpful, as it allows further subdivision of the anemias according mean cellular volume, chromasy etc. (Table 4).

Megaloblastic changes with nuclear-cytoplasmic asynchrony, dyserythropoietic changes, presence of giant metamyelocytes and hypersegmentation of granulocytes are due to an impaired DNA synthesis, and are characteristic of vitamin B12 or folic acid deficiency (as well as of congenital dyserythropoietic anemias) (Fig. 10). The BM is generally hypercellular with a marked increase of the erythroid lineage, resulting in a reversed M:E ratio. Both an inadequate intake (veganism, infestation by parasites) as well as an inadequate absorption (pernicious anemia/chronic atrophic gastritis), can be the underlying cause [31]. The usual presentation is that of an isolated macrocytic anemia, at times

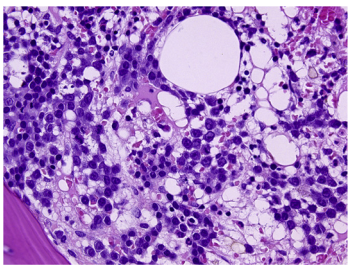

Fig. 9. Hemolytic anemia: synchronous erythroid hyperplasia with megaloblastoid changes and increased mitotic activity. Variable degree of dyserythropoiesis is often present, with irregular, angulated nuclear contours of the erythroblasts (H&E).

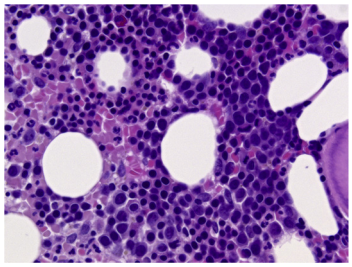

Fig. 10. Vitamin B12 deficiency: hyperplasia of the erythroid lineage with left-shift and megaloblastic changes; note the hypersegmentation of the neutrophilic granulocytes, as well as the presence of a giant metamyelocyte in the upper central part of the figure (H&E).

Table 4
Histopathological differential diagnosis of erythroid hyperplasia.

Erythroid hyperplasia associated with normocytic anemia
- Hemorrhage
- Hemolytic anemias
- Intrinsic bone marrow disease (aplastic anemia, malignancies)
- Anemia of chronic disease
Erythroid hyperplasia associated with macrocytic anemia
- Megaloblastic anemia (e.g. vitamin deficiency)
- Myelodysplastic syndromes and other neoplastisc myeloid disorders
- Severe hemolytic anemia
- Alcohol
- Liver disease
- Cytotoxic drugs
- Hypothyroidism
Erythroid hyperplasia associated with microcytic anemia
- Iron deficiency
- Lead intoxication
- Thalassemia

accompanied by thrombocytopenia. Occasionally, particularly if the diagnostic pathologist is not informed on or is not paying attention to the peripheral blood findings, the rapidly expanding macroblastic erythroid precursors may raise suspicion of an underlying MDS. In order to rule out MDS, the presence of dysplasia in other cell lines (BM smear) should be assessed in conjunction of vitamin levels in the blood. The presence of non-CHIP typical genetic (numeric/structural or single nucleotide) abnormalities (rev. in [32]) would seriously challenge the diagnosis of isolated vitamin deficiency as the sole cause of symptoms.

Similar histopathological changes (as those mntioned above) accompanied by dysmegakaryopoiesis with presence of small pyknotic and hypo-/alobated forms are observed in patients taking a specific subset of drugs, which have in common either being metabolized by or inhibiting the enzyme thiopurine-S-methyltransferase (TPMT), resulting in a disturbed purine metabolism [33]. This subset includes widely used drugs, given for a variety of diseases, such as azathioprine (autoimmune diseases), mercaptopurine (ALL, inflammatory bowel disease), thioguanine (ALL/AML) or allopurinol (gout). Analogously, (low dose) methotrexate – used in autoimmune diseases – inhibits the enzyme dihydrofolate reductase and as such impairs the synthesis of purines, too. The proper function of TPMP itself varies in the population, with approximatively 10% of individuals having a reduced TPMT function and approximatively 0.5% having a genuine deficiency of TPMP [34]. Especially the latter group is at great risk of life-threatening BM-toxicity with conventional drug dosages, particularly in cases with concomitant use of allopurinol.

Patients with AIHA often display splenomegaly and rheological complications and importantly, a number of them – especially those with cold agglutinin disease – suffers from an underlying lymphoid malignancy, which also represents the primary indication for performing a BMB. Therefore, this differential diagnosis must be addressed, irrespective of the very prominent erythroid hyperplasia with

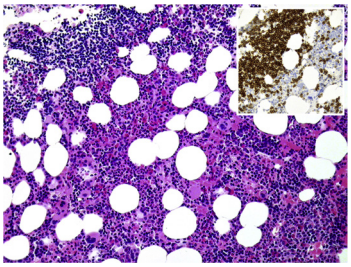

Fig. 11. Autoimmune hemolytic anemia accompanying chronic lymphocytic leukemia: nodular and interstitial infiltrates of mature small B-cells; the residual hematopoiesis shows disorganized and slightly increased erythropoiesis and interstitial eosinophilia (H&E; inlet: CD20).

left-shift and mild dyserythropoiesis that will be observed in the BMB, by ancillary immunohistochemistry for B- and T-cell markers (Fig. 11) [35]. In addition, a number of drugs can cause AIHA by inducing oxidative stress (dapsone, primaquine, sulfanilamide, nitrofurantoin and rasburicase), which can be particularly pronounced in patients suffering from glucose-6-phosphate dehydrogenase deficiency, while other drugs cause AIHA by immunologic mechanisms acting as haptens (e.g. penicillins, cephalosporins, α-methyldopa, some non-steroidal anti-inflammatory drugs) [36]. For obvious reasons, the latter conditions can only be integratively diagnosed.

Anemia of aging and anemia of chronic disease - as seen in patients with malignancies, infections, autoimmune disorders, chronic rejection after transplantation, as well as in kidney and liver disorders - is a definitive example of an integrative clinico-pathological diagnosis. BMB usually show some degree of hypercellularity with an increase of stainable iron and polytypic plasmacytosis (Fig. 12). Yet, histopathological examination can yield useful key features of significant underlying diseases such as renal osteopathy/hyperparathyroidism, chronic infections (e.g. Whipple's disease, different granulomatous myelitides etc.), myelofibrosis (e.g. accompanying autoimmune myelofibrosis), occult neoplasms, or even Paget's disease of bone, all of which should be actively sought for (Fig. 13). The underlying, complex pathophysiology of anemia of chronic disease has been more and more unraveled in recent years. The bottom-line is that in various chronic conditions monocytes produce cytokines, amongst them interleukin-6, which increase hepcidin- and tumor necrosis factor-α production, finally leading to hypochromic normocytic anemia [37].

2.4. Neutropenia

2.4.1. Isolated neutropenia

When confronted with neutropenia (defined as less than 1500 granulocytes/μL), it is first warranted looking for the presence of other concurring cytopenias, since their presence will definitely broaden the differential diagnosis to include MDS, BM failure

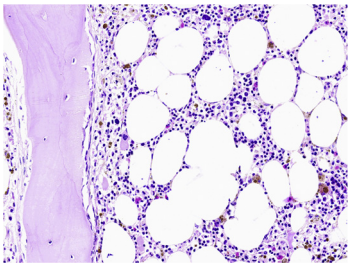

Fig. 12. Renal osteodystrophy and anemia of chronic disease in a patient suffering from chronic renal failure: hypercellular bone marrow with an increase of histiocytes and polytypic plasma cells, as well as prominent iron deposition. The bony trabeculae are very irregular, cuffed by reticulin fibers and a decreased number of osteoblasts (H&E).

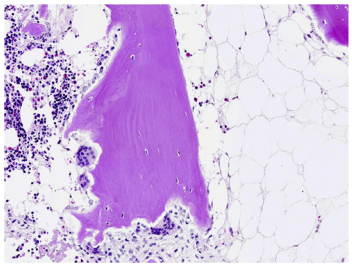

Fig. 13. Incidental Paget disease in a patient suffering from anemia: broadened irregular bony trabeculae with prominent osteoclastic activity and irregular cement lines (H&E).

syndromes and nutritional deficiencies. It is important to realize that all these conditions can – at an early stage – also present with isolated neutropenia. The difference between neutropenia and agranulocytosis is solely defined by blood cell counts; agranulocytosis (defined as less than 500 granulocytes/μL) is regarded as the extreme end of the spectrum of neutropenia. The age of the patient should be taken in account too. An isolated neutropenia in children is (particularly if accompanied by lymphocytosis) mostly caused by (mainly viral) infections, yet constitutional/hereditary disorders should certainly be considered, especially in the presence of somatic anomalies and in the neonatal population (e.g. severe congenital neutropenia, Shwachman-Diamond syndrome) [4–7]. In the adult population, the most common causes of an isolated neutropenia are drug toxicity, infections, underlying lymphoid malignancies and autoimmune diseases.

Scenario 7: Isolated neutropenia with lymphocytosis in an adult should raise the suspicion of an underlying T-LGL. T-LGL express Fas-ligand that binds to the Fas-receptor of the neutrophils and triggers apoptosis of the latter. As in anemia with erythroblastopenia, an underlying T-LGL must always be considered in the differential diagnosis of neutropenia in adults and actively sought for by means of immunohistochemistry (CD3 + /CD5-/CD8 + /CD57 + /TIA1 + and often pSTAT3 +) that will highlight a characteristic infiltration pattern in the BM, which is usually patchy and often sinus-associated (Fig. 14); the positive nuclear staining for pSTAT3 reflecting presence of activating mutations of *STAT3* in about 40–70% of patients [38]. According to the current WHO classification, to establish the diagnosis, a persistent increase of T-LGL in the peripheral blood – with a count of 2–20 G/L – should be found for a period of at least 6 months [11]. In addition, large granular T-cell lymphocytoses have been associated with prior autologous stem cell transplantation and autoimmune diseases (especially those accompanied by Felty syndrome). Vice versa, patients with T-LGL and a somatic *STAT3* mutation are more prone to develop rheumatoid arthritis [39]. In our experience, particular harm is caused by occasional misclassifying T-LGL (and overtreating the patients), either by missing the infiltrating lymphocytes and diagnosing a possible low-blast count MDS, or by misinterpreting the immunophenotype and classifying T-LGL as BM infiltration by other systemic T-cell lymphomas.

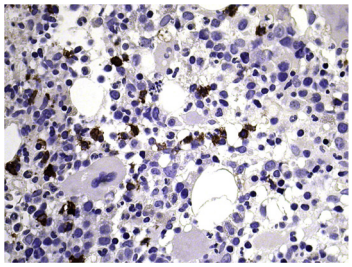

Fig. 14. T-large granular lymphocyte leukemia s in a patient suffering from neutropenia: subtle intrasinusoidal spread of CD57-positive lymphocytes, which gives it a certain linear aspect.

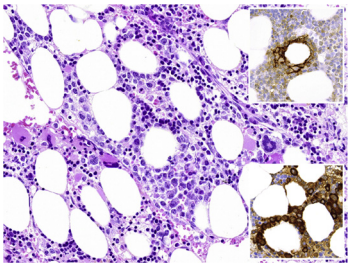

Fig. 15. Drug-induced myelopathy: hypercellular bone marrow with left-shifted myelopoiesis showing a maturation arrest (H&E) and presence of lipogranulomas, which are highlighted by CD11c (upper insert); the typical appearance of the regenerating myelopoiesis is highlighted by a myeloperoxidase staining in the lower insert.

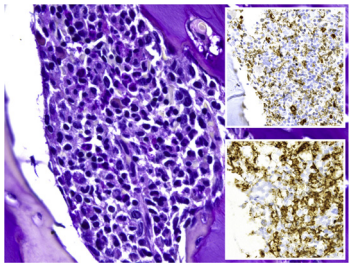

Fig. 16. Anti-CD16b-mediated neutropenia in a child: dominant, not left-shifted myelopoiesis with maturation (PAS); the lack of myelopoietic left-shift is also perceptible comparing the CD15 stain (upper insert) - highlighting the more mature myeloid cells - with the myeloperoxidase stain (lower insert).

Scenario 8: Neutropenia with myeloid hypoplasia or maturation disturbance (Fig. 15) is mostly due to drug toxicity (Table 2), infections (e.g. CMV), or accompanies autoimmune diseases.

A fair amount of idiosyncratic drug reactions presents as agranulocytosis, which is linked to significant morbidity and mortality due to secondary infections. Those drugs commonly either contain functional groups that are oxidized to reactive metabolites by the myeloperoxidase (e.g. arylamine-containing drugs) in the myelopoietic cells or lead to cytokine release by activating inflammosomes [40]. However, like in most hypersensitivity reactions, patients do often harbor multiple predisposing factors that make them susceptible for developing neutropenia. These factors are age, intake of multiple medications, distinct *HLA* haplotypes, genetic polymorphisms in immunoregulatory genes such as e.g. the *TNF* loci or in genes encoding for neutrophilic enzymes that either lead to decrease drug clearance or increase toxic metabolite production [40]. Usually in the BMB, a so called "maturation arrest" of myelopoiesis at the level of pro- or metamyelocytes is observable, which is explained by the increased apoptosis, or rapid release into the blood of the more mature series and the increased effects of (endo- or – if applicated - exogenous) G-CSF. At occasions, a significant T-cell lymphocytosis with lots of CD4 (and, often, ^{dim}PD1) expressing cells, lipogranulomas and/or eosinophilia may be observed (Fig. 15).

One important diagnostic pitfall is to distinguish this morphological appearance from acute promyelocytic leukemia, which at occasions may require even e.g. FISH studies to detect *PML-RARA* rearrangements. A second pitfall is linked to the fact that the removal of the causative agent – especially in drug-induced neutropenia - will not immediately lead to a normalization of the neutrophilic count, as it usually needs one to two weeks to do so. So, while the patient is still agranulocytic, the regeneration can be overwhelming with a considerable and – at first sight very worrisome – increase (even > 20%) of mainly peritrabecular blasts, so that the differential diagnosis of AML can be raised. Additional molecular studies, as well as follow-up biopsies to monitor the gradual shift of the morphological changes, may be required to exclude AML.

Direct infection of the granulocytic progenitor cells that will finally lead to cell death and neutropenia is caused by some viruses, in particular CMV and HIV. The preferential binding of CMV to myeloid associated antigens such as CD13 and the expression of viral receptors in CD34-positive myeloid progenitor cells in HIV can partly explain the infection of those cell populations [24]. In bacterial infections, neutropenia will be caused by various mechanisms such as activation of the complement cascade, sequestration in the spleen, increased peripheral demand or hemophagocytosis, and such instances will more commonly present with scenario 9.

Neutropenia in systemic autoimmune diseases – especially rheumatoid arthritis and systemic lupus erythematosus – and in some cases of drug-induced neutropenia is mainly caused by autoantibodies directed against the neutrophils that lead to decreased half-life of the latter. These antineutrophilic antibodies – such as anti-Fc gamma RIIIb (CD16b) antibodies [41] – should not be confused with the more commonly known antinuclear antibodies (ANA). In patients with Felty syndrome (trias of rheumatoid arthritis, splenomegaly and neutropenia) destruction of neutrophilic granulocytes is only to a part due to antineutrophilic antibodies, in addition Fas-ligand mediated apoptosis and inhibition of the myelopoiesis by cytokines also play a role and explain the accompanying myeloid hypoplasia. Since T-LGL is also strongly associated with rheumatoid arthritis and neutropenia, in any case of suspect Felty syndrome the differential diagnosis of T-LGL must be sufficiently falsified as suggested above (see Scenario 7).

Scenario 9: Neutropenia with granulocytic hyperplasia is due to increased consumption of mature granulocytes in the periphery in, most commonly, septic conditions (rare indication for BMB). Especially in infants, autoimmune neutropenias (of childhood) due to neutrophilic destruction by e.g. anti-CD16b antibodies (anti-Fc gamma RIIIb antibodies) should be considered in such circumstances as well (Fig. 16) [41]. Although the peak incidence lies around the age of 1 year, presentations at adult age are occasionally observable. Mostly blood counts normalize with time, as these antibodies disappear. In contrast to neutropenia associated with systemic lupus erythematosus, in which anti-Fc gamma RIIIb also play a role, the myeloid hyperplasia in the former condition is right-shifted, rather than left-shifted. One hypothesis to explain this discrepancy is that in autoimmune neutropenia associated with rheumatic diseases, the more mature myeloid stages are already destroyed intramedullary before entering circulation and that mechanisms related to the malfunction of large granular T-cells and cytokine signaling do also play a role (see above), thus leading to a more profound maturational disturbance of the myeloid lineage.

2.5. Thrombocytopenia

Thrombocytopenia is defined as a platelet count of less than 150.000/μL and is one of the most common reasons for referral to the hematologist. The etiology of thrombocytopenia is variable [24]. Most of the cases being resolved clinically, a BMB is only needed in cases that are long-lasting, severe, irresponsive to therapy or remain inexplicable (see later).

Scenario 10: Thrombocytopenia with decreased megakaryocytes is usually seen in AA, toxic BM injuries (e.g. chemotherapy; Table 2), in virus infections or, rarely, accompanying T-LGL [24]; exceptional autoimmune disorders with anti-C-MPL antibodies have been described as well [42]. Since MDS is one of the most important differential diagnoses in all cytopenic states, it must be always sufficiently falsified also in a-/hypomegakaryocytic thrombopenia (Fig. 17); staining for CD34 to quantify blasts and uncover ALIPs as well as careful examination of the PAS or – better - CD42b or CD61 stained slides for pathologic micromegakaryocytic forms (see below) can be helpful in such occasions.

Scenario 11: Thrombocytopenia with increased megakaryocytes ("megakaryocytic thrombopenia") is prototypic of immune thrombocytopenia (ITP) (Fig. 18). The hyperplastic megakaryocytic

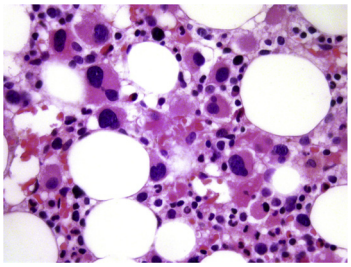

Fig. 17. Myelodysplastic syndrome with del(5q): increased number of megakaryocytes with hypo-/monolobated nuclei, some of them having a cloudy, naked appearance, and erythroid precursors with irregular, angulated contours, suggestive for dyserythropoiesis (H&E).

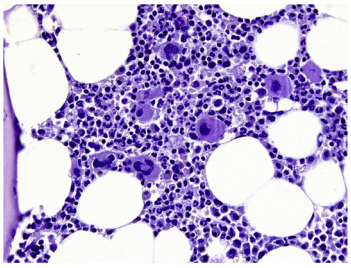

Fig. 18. Immune thrombocytopenia: increased megakaryopoiesis consisting of relatively small left-shifted forms with normal nuclear lobulation of the nucleus. There are no micromegakaryocytes (PAS).

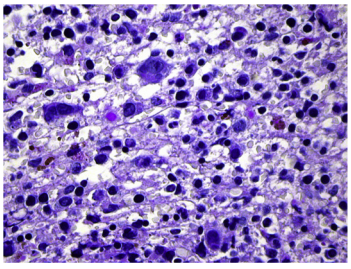

Fig. 19. Human immunodeficiency virus myelopathy: increased, small to pyknotic megakaryocytes with hypolobulated, bare nuclei, stromal edema, dyserythropoiesis and siderosis (PAS).

lineage shows both presence of mature and immature forms (left shifting) and occasionally easily recognizable pyknotic megakaryocytes, i.e. smaller forms with increased nucleo-cytoplasmic ratio and roundish, deeply basophilic and hyperchromatic nuclei. No significant clustering or overt dysplasia – including micromegakaryocytes, i.e. megakaryocytes that are as large as promyelocytes and only recognizable by means of specific (e.g. CD42b or CD61) staining - should be present. This histological appearance is also seen in other conditions associated with increased peripheral platelet consumption such as mechanic destruction, (auto)inflammatory (e.g. collagenoses), infectious (e.g. HIV), septic or microangiopathic conditions.

Several medications are notorious to induce thrombocytopenia, yet heparin is by far the most feared as it induces one the most severe forms. Heparin-induced thrombocytopenia (HIT) is an immune-mediated disorder, in which HIT-antibodies form ultra-large immune complexes with platelet-factor 4 (PF4), a cytoplasmic protein physiological present in the cytoplasm of platelets [43]. The formation of these ultra-large immune complexes is enhanced by the presence of heparin. These immune complexes induce a prothrombotic state, probably by secretion of thrombin and activation of the coagulation cascade, resulting in potential life-threatening venous and arterial thrombotic events. Besides a temporal/clinical/anamnestic concurrence of heparin use and thrombocytopenia with or without thrombotic events, these anti-PF4 antibodies can also be demonstrated by immunoassays.

Although many infective agents can cause isolated thrombocytopenia, HIV deserves to be mentioned separately as it exhibits some particular histologic features. Apart from immune mediated peripheral destruction that is morphologically reflected by megakaryocytic thrombocytopenia, the direct infection of megakaryocytes by the virus impairs the production of platelets [44,45]. The most striking histologic feature are small, pyknotic megakaryocytes with dark nuclei and almost no cytoplasm (so-called naked nuclei) (Fig. 19). They probably represent megakaryocytes, undergoing apoptosis after viral infection. The overall BM background may show features of HIV-myelopathy with increase of histiocytes, dyserythropoiesis, plasmacytosis, and gelatinous transformation [46].

Despite the hematopathologists perception of a common need to perform a BMB in clinically suspected ITP, this procedure is in fact rarely indicated and usually performed in refractory states to rule out specific underlying disease such as MDS, lymphoma or, in infants, constitutional disorders, and is thus performed in < 10% of all ITP patients. An important pitfall to consider along these thoughts is the use of thrombopoietin-analogues and –receptor agonists for ITP, especially in refractory instances, which may not be communicated to the hematopathologist. In such cases, the BMB may exhibit MPN-like features, such as clustering of hyperlobated, hyperchromatic megakaryocytes and even myelofibrosis [47,48].

2.6. Monocytopenia

Monocytopenia deserves special attention since it very commonly accompanies hairy cell leukemia, the infiltrates of which might be subtle and occasionally missed on morphologic evaluation [49]. Ancillary studies for the expression of e.g. CD20, $BRAF^{V600E}$, CD103 and/or cyclin D1 can be of decisive help in such occasions. Furthermore, monocytopenia is one of the early presentation in patients with *GATA2* mutation-associated disorders, explaining – along with the reduced B- and NK- cells - the immunodeficient state and the high incidence of mycobacterial and viral infections of the affected individuals [50]. Finally, monocytopenia can also be linked to chronic use of corticosteroids.

3. Conclusion

Evaluating BMB for cytopenias can sometimes feel like a hopeless task, yet it is also one of the most challenging ones in the field of hematopathology. Interdisciplinary consultation and input are of paramount importance in this setting and each one – treating physician, laboratory hematologist and hematopathologist – should not hesitate to actively ask for additional information. A combined histopathological pattern-based and peripheral blood cell count-based approach as the one described above may help hematopathologists to tackle such conditions and to establish a diagnosis or, at least, an as specific as possible differential diagnosis.

Conflict of interest

The authors declare to have no competing interests.

References

[1] F. Fend, A. Tzankov, K. Bink, S. Seidl, L. Quintanilla-Martinez, M. Kremer, S. Dirnhofer, Modern techniques for the diagnostic evaluation of the trephine bone marrow biopsy: methodological aspects and applications, Prog. Histochem. Cytochem. 42 (4) (2008) 203–252.
[2] A. Tzankov, S. Dirnhofer, C. Beham-Schmid, Normal bone marrow and common reactive alterations, Pathologe 33 (6) (2012) 496–507.
[3] E.E. Torlakovic, R.K. Brynes, E. Hyjek, S.H. Lee, H. Kreipe, M. Kremer, R. McKenna, Y. Sadahira, A. Tzankov, M. Reis, A. Porwit, H. International Council for Standardization in, ICSH guidelines for the standardization of bone marrow immunohistochemistry, Int. J. Lab. Hematol. 37 (4) (2015) 431–449.

[4] S. Adam, D. Melguizo Sanchis, G. El-Kamah, S. Samarasinghe, S. Alharthi, L. Armstrong, M. Lako, Concise review: getting to the core of inherited bone marrow failures, Stem Cells 35 (2) (2017) 284–298.
[5] H. Iwafuchi, The histopathology of bone marrow failure in children, J. Clin. Exp. Hematop. 58 (2) (2018) 68–86.
[6] P.P. Khincha, S.A. Savage, Genomic characterization of the inherited bone marrow failure syndromes, Semin. Hematol. 50 (4) (2013) 333–347.
[7] R.J. Leguit, J.G. van den Tweel, The pathology of bone marrow failure, Histopathology 57 (5) (2010) 655–670.
[8] D.P. Steensma, New challenges in evaluating anemia in older persons in the era of molecular testing, Hematol. Am. Soc. Hematol. Educ. Program 2016 (1) (2016) 67–73.
[9] P. Valent, ICUS, IDUS, CHIP and CCUS: diagnostic criteria, separation from MDS and clinical implications, Pathobiology 86 (1) (2019) 30–38.
[10] J.K. Davies, E.C. Guinan, An update on the management of severe idiopathic aplastic anaemia in children, Br. J. Haematol. 136 (4) (2007) 549–564.
[11] S.H. Swerdlow, E. Campo, N.L. Harris, E.S. Jaffe, S.A. Pileri, H. Stein, J. Thiele, D.A. Arber, R.P. Hasserjian, M.M. le Beau, A. Orazi, R. Siebert, WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (Revised 4th Edition), IARC, Lyon, France, 2017.
[12] N.S. Young, Aplastic Anemia, N. Engl. J. Med. 379 (17) (2018) 1643–1656.
[13] C.J. Parker, Update on the diagnosis and management of paroxysmal nocturnal hemoglobinuria, Hematol. Am. Soc. Hematol. Educ. Program 2016 (1) (2016) 208–216.
[14] R.A. Brodsky, Paroxysmal nocturnal hemoglobinuria, Blood 124 (18) (2014) 2804–2811.
[15] R. Agarwal, P. Chapple, M. Brown, J. Szer, S. Juneja, Analysis of abnormal clones by the fluorescent aerolysin method in paroxysmal nocturnal haemoglobinuria and other marrow disorders, Int. J. Lab. Hematol. 37 (1) (2015) 14–21.
[16] T. Menter, A. Tzankov, Aplastic anemia, in: J. van Krieken (Ed.), Encyclopedia of Pathology, Springer, Cham, 2018.
[17] S. Ogawa, Clonal hematopoiesis in acquired aplastic anemia, Blood 128 (3) (2016) 337–347.
[18] J.P. Maciejewski, A. Risitano, E.M. Sloand, O. Nunez, N.S. Young, Distinct clinical outcomes for cytogenetic abnormalities evolving from aplastic anemia, Blood 99 (9) (2002) 3129–3135.
[19] F.G. Rosado, A.S. Kim, Hemophagocytic lymphohistiocytosis: an update on diagnosis and pathogenesis, Am. J. Clin. Pathol. 139 (6) (2013) 713–727.
[20] M.B. Jordan, C.E. Allen, S. Weitzman, A.H. Filipovich, K.L. McClain, How I treat hemophagocytic lymphohistiocytosis, Blood 118 (15) (2011) 4041–4052.
[21] S. Nikiforow, N. Berliner, The unique aspects of presentation and diagnosis of hemophagocytic lymphohistiocytosis in adults, Hematol. Am. Soc. Hematol. Educ. Program 2015 (2015) 183–189.
[22] J. Diebold, T. Molina, S. Camilleri-Broet, A. Le Tourneau, J. Audouin, Bone marrow manifestations of infections and systemic diseases observed in bone marrow trephine biopsy review, Histopathology 37 (3) (2000) 199–211.
[23] A. Tzankov, M. Kremer, R. Leguit, A. Orazi, J. van der Walt, U. Gianelli, K.M. Hebeda, Histiocytic cell neoplasms involving the bone marrow: summary of the workshop cases submitted to the 18th Meeting of the European Association for Haematopathology (EAHP) organized by the European Bone Marrow Working Group, Basel 2016, Ann. Hematol. 97 (11) (2018) 2117–2128.
[24] K. Foucar, K. Reichard, D. Czuchlewski, Bone Marrow Pathology, ASCP Press, Chicago, USA, 2010.
[25] T.I. George, D.A. Arber, Atlas of Bone Marrow Pathology, Springer Science +Business Media, New York, 2018.
[26] J.E. Sadler, Pathophysiology of thrombotic thrombocytopenic purpura, Blood 130 (10) (2017) 1181–1188.
[27] Z.W. Myint, T.H. Oo, K.Z. Thein, A.M. Tun, H. Saeed, Copper deficiency anemia: review article, Ann. Hematol. 97 (9) (2018) 1527–1534.
[28] M.D. Cappellini, I. Motta, Anemia in clinical practice-definition and classification: does hemoglobin change with aging? Semin. Hematol. 52 (4) (2015) 261–269.
[29] R.T. Means Jr., Pure red cell aplasia, Blood 128 (21) (2016) 2504–2509.
[30] P. Valent, G. Busche, I. Theurl, I.Z. Uras, U. Germing, R. Stauder, K. Sotlar, W. Fureder, P. Bettelheim, M. Pfeilstocker, R. Oberbauer, W.R. Sperr, K. Geissler, J. Schwaller, R. Moriggl, M.C. Bene, U. Jager, H.P. Horny, O. Hermine, Normal and pathological erythropoiesis in adults: from gene regulation to targeted treatment concepts, Haematologica 103 (10) (2018) 1593–1603.
[31] R. Green, Vitamin B12 deficiency from the perspective of a practicing hematologist, Blood 129 (19) (2017) 2603–2611.
[32] B.B. Ganguly, D. Banerjee, M.B. Agarwal, Impact of chromosome alterations, genetic mutations and clonal hematopoiesis of indeterminate potential (CHIP) on the classification and risk stratification of MDS, Blood Cells Mol. Dis. 69 (2018) 90–100.
[33] P.A. Blaker, M. Arenas-Hernandez, M.A. Smith, E.A. Shobowale-Bakre, L. Fairbanks, P.M. Irving, J.D. Sanderson, A.M. Marinaki, Mechanism of allopurinol induced TPMT inhibition, Biochem. Pharmacol. 86 (4) (2013) 539–547.
[34] P. Katara, H. Kuntal, TPMT polymorphism: when shield becomes weakness, Interdiscip. Sci. 8 (2) (2016) 150–155.
[35] S. Berentsen, Cold agglutinin disease, Hematol. Am. Soc. Hematol. Educ. Program 2016 (1) (2016) 226–231.
[36] G. Garratty, Immune hemolytic anemia caused by drugs, Expert Opin. Drug Saf. 11 (4) (2012) 635–642.
[37] P.G. Fraenkel, Understanding anemia of chronic disease, Hematol. Am. Soc. Hematol. Educ. Program 2015 (2015) 14–18.
[38] T. Lamy, A. Moignet, T.P. Loughran Jr., LGL leukemia: from pathogenesis to treatment, Blood 129 (9) (2017) 1082–1094.
[39] M. Shi, R. He, A.L. Feldman, D.S. Viswanatha, D. Jevremovic, D. Chen, W.G. Morice, STAT3 mutation and its clinical and histopathologic correlation in T-cell large granular lymphocytic leukemia, Hum. Pathol. 73 (2018) 74–81.
[40] A. Johnston, J. Uetrecht, Current understanding of the mechanisms of idiosyncratic drug-induced agranulocytosis, Expert Opin. Drug Metab. Toxicol. 11 (2) (2015) 243–257.
[41] P.E. Newburger, D.C. Dale, Evaluation and management of patients with isolated neutropenia, Semin. Hematol. 50 (3) (2013) 198–206.
[42] M. Kuwana, Y. Okazaki, M. Kajihara, J. Kaburaki, H. Miyazaki, Y. Kawakami, Y. Ikeda, Autoantibody to c-Mpl (thrombopoietin receptor) in systemic lupus erythematosus: relationship to thrombocytopenia with megakaryocytic hypoplasia, Arthritis Rheum. 46 (8) (2002) 2148–2159.
[43] L. Rauova, G. Arepally, M. Poncz, D.B. Cines, Molecular and cellular pathogenesis of heparin-induced thrombocytopenia (HIT), Autoimmun. Rev. 17 (10) (2018) 1046–1052.
[44] A. Scaradavou, HIV-related thrombocytopenia, Blood Rev. 16 (1) (2002) 73–76.
[45] P. Vishnu, D.M. Aboulafia, Haematological manifestations of human immune deficiency virus infection, Br. J. Haematol. 171 (5) (2015) 695–709.
[46] D. Ricci, M. Ponzoni, M.C. Zoldan, L. Germagnoli, A. Faravarelli, Bone marrow biopsy in 50 AIDS patients: a diagnostic approach, Pathologica 87 (6) (1995) 640–645.
[47] V.K. Douglas, M.S. Tallman, L.D. Cripe, L.C. Peterson, Thrombopoietin administered during induction chemotherapy to patients with acute myeloid leukemia induces transient morphologic changes that may resemble chronic myeloproliferative disorders, Am. J. Clin. Pathol. 117 (6) (2002) 844–850.
[48] D.J. Kuter, G.J. Mufti, B.J. Bain, R.P. Hasserjian, W. Davis, M. Rutstein, Evaluation of bone marrow reticulin formation in chronic immune thrombocytopenia patients treated with romiplostim, Blood 114 (18) (2009) 3748–3756.
[49] E. Kraut, Infectious complications in hairy cell leukemia, Leuk. Lymphoma 52 (Suppl. 2) (2011) 50–52.
[50] A.P. Hsu, E.P. Sampaio, J. Khan, K.R. Calvo, J.E. Lemieux, S.Y. Patel, D.M. Frucht, D.C. Vinh, R.D. Auth, A.F. Freeman, K.N. Olivier, G. Uzel, C.S. Zerbe, C. Spalding, S. Pittaluga, M. Raffeld, D.B. Kuhns, L. Ding, M.L. Paulson, B.E. Marciano, J.C. Gea-Banacloche, J.S. Orange, J. Cuellar-Rodriguez, D.D. Hickstein, S.M. Holland, Mutations in GATA2 are associated with the autosomal dominant and sporadic monocytopenia and mycobacterial infection (MonoMAC) syndrome, Blood 118 (10) (2011) 2653–2655.