Problems in Bone Marrow Pathology

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Introduction

The role of the Bone Marrow Biopsy

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Optimal Bone Marrow (BM) examination requires both Aspirate (BMA) and Core Biopsy (BMB).

BMA allows refined cytological examination (such as dysplastic changes, Auer rods, ring sideroblasts) as well as cytochemistry, flow cytometry, cytogenetics and BM cultures.

BMB definitively has some advantages over aspirate : examination of a greater volume of tissue with preserved architecture, assessment of cellularity and modifications of stroma and/or osseous trabeculae, detection of compact and/or fibrotic lesions, identification of granulomas and opportunistic micro-organisms and application of immunohistochemistry (IHC). BMB is mandatory when aspirate is not obtained ("dry aspirate" or "dry tap"). Molecular biology can be performed on both aspirate and biopsy.

The management of BMB requires refined processing so as to allow optimal preservation of morphological details. Plastic embedding - although expensive - is still advocated by some groups but most laboratories are now using paraffin-embedding. Both IHC and molecular biology can be performed on paraffin-embedded material. Gentle decalcification and thin sections (2-3 mu) are required.

The indications for the combined cyto-histological examination (BM aspirate and biopsy) can be summarized as follows :

- ✓ Lymphomas
- ✓ Multiple Myeloma (MM) and other gammopathies
- ✓ Chronic Myeloproliferative Diseases (MPD)
- ✓ Myelodysplastic Syndromes (MDS)
- ✓ Acute leukaemia (AL)
- ✓ Search for metastases
- ✓ Search for infectious processes (granulomas)
- ✓ Unexplained cytopenia

Guidelines for BMB examination are presented on table 1.

BM examination is an important procedure in the diagnosis and management of patients with **lymphoma**. The role of BM biopsy in lymphomas can be viewed as follows : assessment of initial diagnosis, staging of an already identified tumour, evaluation of residual haemopoiesis, patient follow-up and detection of residual disease after treatment. Different patterns of BM infiltration are described in lymphoma: nodular paratrabecular, nodular random, interstitial, diffuse, and intrasinusoidal.

Nodular involvement is the most common pattern. Interstitial infiltration is usually associated with generalized BM involvement, even though normal haematopoietic tissue and fat are not greatly compromised. Intrasinusoidal infiltration (marginal zone lymphoma, gamma-delta T cell lymphoma) may be difficult to appreciate on routinely stained sections without the aid of immunohistochemical stains. Lymphoma infiltration may be fibrotic and compact, so that aspirate is often not representative. Bilateral biopsy is recommended by some authors.
A panel of antibodies (Ab) reactive on paraffin sections is available. The most commonly used Ab are presented on Table 2. Immunophenotyping by flow cytometry or Fluorescent Activated Cell Sorter (FACS) is an important advantage of BM aspirate over biopsy, if enough cells of interest are present in the BMA material. It is particularly useful for the characterization and classification of small B-cell lymphomas according to the REAL/WHO scheme (Table 3).

The usefulness of molecular biology for diagnosis and follow-up of lymphomas has been reported by several authors.

For proper management of the patient, an integrative approach of all available data is necessary - i.e. peripheral blood and BM morphology, immunohistochemistry (IHC), flow cytometry, molecular biology and clinical data.

The frequency of BM involvement in lymphoma depends on the histological types and immunophenotypes.

Among *B-cell lymphoma*s, 3 groups can be distinguished.
1) B-cell lymphoma with constant BM involvement:
Chronic Lymphocyte Leukaemia (B-CLL), Prolymphocytic Leukaemia (B-PLL), Hairy Cell Disease (HCD).

2) B-cell lymphoma with a high frequency of BM involvement:
Immunocytoma (IC), Follicular (FL), Mantle Cell Lymphoma (MCL), lymphoblastic lymphoma

3) B-cell lymphoma with a low frequency of BM involvement
Diffuse large B-cell lymphoma of the REAL/WHO classification (high-grade category in the Kiel classification) and Burkitt's lymphoma.

In category 3), disseminated disease with or without BM involvement represents a poor prognostic factor. The marrow is infiltrated in 15-30 % of cases in large B-cell lymphomas. In cases presenting with interstitial and/or minor marrow involvement, tumour cells may be overlooked if IHC is not applied.
T-cell and/or histiocyte-rich B-cell lymphomas are characterized by the presence of reactive cells, sometimes in considerable number, and may be confused with a reactive process, Hodgkin's disease or T-cell lymphoma, if IHC is not performed

Most patients with *Peripheral T-cell lymphomas*, unspecified and angioimmunoblastic T-cell lymphoma present with generalized disease. BM involvement represents a poor prognostic factor. The diagnosis of a T-cell neoplasm on BM biopsy may be a challenge for the haematopathologist. A biopsy from another site (lymph node) is often necessary to establish the diagnosis.

In contrast to B-cell lymphomas, non specific reactive changes are common features in T-cell lymphomas. They are probably induced by a variety of cytokines produced by neoplastic T-cells. These changes may be very prominent and obscure the neoplastic process. Tumour cells constitute only a minor part of an infiltrate including lymphocytes, plasma cells, histiocytes (occasionally granulomas), macrophages, neutrophils, eosinophils, increased vascularity, fibrosis, and necrosis.

According to our experience and results, TCR-γ PCR represents an additional helpful tool for the detection of monoclonality and diagnosis of T-cell lymphomas in BM biopsies.

BM involvement has been reported in 7-30 % of cases *Anaplastic Large Cell Lymphoma (ALCL)*. BM infiltration is indicative of worse prognosis. In some cases, the pattern of infiltration is interstitial with isolated lymphoma cells or very small clusters of tumour cells. The lymphoma infiltration may be overlooked on HE or Giemsa sections and the detection of tumour cells is significantly higher when IHC with CD30 Ab is employed.

In *Hodgkin's disease*, BMA is not representative and BMB is mandatory since the tumour infiltration is usually heterogeneous and classically associated with severe fibrosis and inflammation. Blocks should be cut at multiple levels.

The most frequent **immunoproliferative diseases** are Multiple Myeloma (MM), Monoclonal Gammopathy of Undetermined Significance (MGUS), lymphoplasmocytic lymphoma (Immunocytoma).
In *Multiple Myeloma (MM)* and other gammopathies, tumour load, pattern of infiltration are best appreciated on histological preparations. Immunophenotyping on sections is necessary in cases of non secreting MM (about 1 % of the cases) and in follow-up of MM patients (distinction between residual disease and reactive plasma cell population). According to Bain et al.(1996), non-diagnostic biopsies are obtained in 5-10 % of the cases (early disease, heterogeneous infiltration) so that aspirate and biopsy should be regarded as complementary.

The monoclonality of the plasma cell population can be evaluated according to the " Light-chain Ratio " (LR) (Peterson et al. 1986).

$$LR = \frac{\text{N of plasma cells reacting for the predominant light-chain}}{\text{N of plasma cells reacting for the minority light-chain}}$$

According to Peterson et al. (1986), a LR of 4 or more corresponds to monoclonality.
In our institution, BMB is also part of the work-up of patients with *Monoclonal Gammopathy of Undetermined Significance (MGUS)* at diagnosis and during follow-up (progression to MM). Search for *amyloid deposits* (Congo red staining) should be applied on every BMB performed for gammopathy.

BMB is of diagnostic and prognostic value in the assessment of **Chronic Myeloproliferative Diseases (MPD)** at presentation and during follow-up. MPD correspond to a group of clonal diseases including Chronic Myeloid Leukaemia (CML), Polycythaemia Vera, (PV) Myelofibrosis with myeloid metaplasia (MMM) (syn.: primary or idiopathic myelofibrosis, agnogenic myeloid metaplasia) and Essential Thombocythaemia (ET). CML is the only MPD characterized by a specific chromosomal abnormality (Philadelphia (Phi) Chromosome). Overlapping cases exist and MMM can supervene during the course of another MPD. BM hyperplasia and fibrosis are common features in MPD. Aspirate is frequently not obtained. "Dry tap" is typical for MMM. BM cellularity, distribution of haemopoietic cell lines, quantification of BM fibrosis and blasts are best appreciated on biopsy.

The diagnosis of MPD is based on a combination of data: clinical status, assessment of PB and BM smears and BM biopsy, cytogenetics (Phi chromosome in CML) and erythroid cultures (particularly important in PV).

The **Myelodysplastic Syndromes (MDS)** represent a group of clonal diseases characterized by ineffective haemopoiesis. MDS usually present with PB cytopenia of one or several cell lines with a hyper- or normocellular BM in most cases. The diagnosis of MDS relies on a combination of data including clinical status, morphologic evaluation of the peripheral blood (PB) smear, BM aspirate and biopsy as well as cytogenetic analysis. It is often a diagnosis of exclusion and BMB is necessary to exclude other conditions associated with cytopenia, such as Aplastic Anaemia (AA) or a metastatic process. About 12 % of MDS patients present with the hypocellular variant of MDS, thus raising a differential diagnosis with AA. The use of the FAB classification (1982) on sections has allowed a better correlation between cytology and biopsy. Over the last fifteen years, the introduction of BM biopsies in MDS has led to consideration of histological prognostic parameters such as cellularity, fibrosis, Abnormal Localization of Immature Precursors (ALIP) and CD34 positive blasts. The negative prognostic impact of histological parameters such as quantity of blasts, quantity of CD34+ immature cells, marrow fibrosis and ALIP has been demonstrated.
The differential diagnosis between MDS and MPD may be difficult and overlapping cases exist, such as Chronic Myelomonocytic Leukaemia (CMML).

In **Acute Leukaemia (AL)**, BMB has to be performed in cases of unsatisfactory aspirates due to packed and/or fibrotic BM. One of the main roles of the biopsy is to rule out other malignant tumours involving the BM. In most cases a rough characterization of the blast cells can be established by IHC, but most often a precise application of the FAB classification is not possible. Flow cytometry is the most reliable technique to identify the phenotype of blast cells in AL but a panel of IHC markers can be usefully applied on BM sections, including TdT, MPO, CD34, CD68, CD79a, FVW.

Precursor lymphoid cells are positive for TdT (terminal deoxynucleotidyl transferase), whereas peripheral B- and T- cells are negative for that marker. The histological and cytological features and immunophenotypes are identical in lymphoblastic lymphomas and Acute Lymphoblastic Leukaemias (ALL). The distinction is usually based on the percentage of lymphoblasts in the marrow at time of diagnosis (25 % of BM blasts correspond to ALL).

Acute non lymphoid (myeloblastic or myelomonocytic) leukaemias (ANLL) are positive for myeloperoxydase (MPO) and/or for the myelomonocytic marker CD68, whereas lymphoblasts are regularly negative for MPO.

Aberrant expression of lymphoid markers may be observed in ANLL (e.g. aberrant positivity for B-cell markers). CD34 and CD45 can be expressed by both lymphoblasts and myeloblasts.

In daily practice, **unexplained cytopenia** represents one of the major indications for BM examination. Precise assessment of BM features such as hypocellularity or aplasia (e.g. aplastic anaemia), hyper-/or normocellularity (e.g. peripheral destruction, hypersplenism, inefficient haemopoiesis, HIV-positive patients, Myelodysplastic Syndromes), expanding process in BM (e.g. granulomas, tumour cells, MPD) has to be done on histological sections.

BMB is part of the routine clinical work-up of haematological and oncological patients. Optimal interpretation of BMB requires an integrative approach of all available data.

TABLE 1

Analyse of the BM biopsy. Guidelines.

- ✓ Size / quality of the biopsy
- ✓ Bone trabeculae (thickness, modifications)
- ✓ Cellularity according to age (see below)
- ✓ Haemopoietic cell lines (representation, distribution, maturation)
- ✓ Reactive cells: lymphocytes, plasma cells, histiocytes and mast cells (quantity, distribution, morphology)
- ✓ Quantification of BM reticulin and collagen according to Bauermeister (see below)
- ✓ Iron stores
- ✓ Abnormal cells: blasts or lymphoma or myeloma or metastatic cells
- ✓ Specific lesions: granulomas, necrosis, amyloidosis, serous atrophy
- ✓ Immunohistochemistry results if available
- ✓ Diagnosis, interpretation and comments

Assessment of cellularity in daily practice. In adults (30 - 60 yr-old), a 50 % cellularity may be viewed as normal; cellularity of less than 25 % indicates hypoplasia and more than 75 % hyperplasia. The BM in neonates is extremely cellular and cellularity declines steadily with age. The cellularity is expressed as percentage of the marrow cavity.
Caution! Adequate size necessary! A small biopsy containing only a small amount of subcortical marrow does not allow assessment of cellularity (this area can be of low cellularity in normal subjects)

Assessment of BM reticulin ("marrow fibrosis") according to Bauermeister (1971).

0 No reticulin fibres demonstrable
1 Occasional fine individual fibres and foci of a fine fibre network
2 Fine fibre network throughout most of the section, no coarse fibres
3 Diffuse fibre network with scattered thick coarse fibres but no mature collagen
4 Diffuse often coarse fibre network with areas of collagenization

0 and 1 can be viewed as normal.
The term "myelofibrosis" should be restricted to the myeloproliferative disease. Although a significant percentage of patients with BM fibrosis have MPDs, lymphoproliferative disorders and metastatic tumours together account for a greater number. Inflammatory diseases may also result in marrow fibrosis (e.g. HIV infection). The term *marrow fibrosis* should be used for the non - specific reaction associated with a variety of disorders.

TABLE 2

BONE MARROW BIOPSY
IMMUNOHISTOCHEMISTRY ON PARAFFIN SECTIONS

(Table not exhaustive !)

CD45	Leukocyte Common Antigen (LCA)
CD138/syndecan-1	Plasma cells
Immunoglobulins	A, G, M, Kappa, Lambda
CD20 (L26)	Pan B
CD45RA (4KB5)	Pan B
CD79a (JCB117)	Pan B
DBB42	Pan B, a few T-cells
CD72/DBA44	Hairy cells, a few B-cells
CD3	Pan T
CD45R0 (UCHL1)	Pan T, a few granulocytic, monocytic and B-cells
CD8 (C8/144B)	T8
CD1a (NA1/34)	Langerhans histiocytosis cells, immature T cells
CD57 (Leu7)	T cells, NK cell subsets
CD56 (Leu19)	NK cells, cytotoxic T cell subsets, neuroectodermal cells
Glycophorine A	Erythroid cell line
Myeloperoxydase	Granulocytic cell line
Elastase (NP57)	Granulocytic cell line
CD68 (KP1)	Myelomonocytic cells
CD68 (PGM1)	Histiocytes and monocytes
AA1 (Tryptase)	Mast cells
Factor VIII (VW)	Megacaryocytes, endothelium
CD61 (Y2/51)	"
UEA (Ulex)	"
CD30 (BerH2)	Activated lymphoid cells, Hodgkin's cells, ALCL cells
CD15 (LeuM1)	Granulocytes, Hodgkin's cells
CD34 (QBEND10)	Immature lymphoid and myeloid cells or stem cells, endothelium
TdT	Immature lymphoid cells, lymphoblastic ML, ALL, rarely undifferentiated AML
EMA	Epithelial Membrane Antigen, plasma cells, ALCL cells
Cytokeratin (MNF116)	Epithelial cells

Abbr.,: NK, Natural Killer, ML, Malignant Lymphoma. ALCL, Anaplastic Large Cell Lymphoma.
TdT, Terminal Desoxynucleotidyl Transferase.
ALL, Acute Lymphoblastic Leukaemia. AML, Acute Myeloid Leukaemia.

TABLE 3

PERIPHERAL B-CELL LYMPHOMAS
FLOW CYTOMETRY : IMMUNOLOGICAL FEATURES

B-CLL/SLL	sIg+, cIg-/+, CD5+, CD10-, CD23+, CD43+
Lymphoplasmocytic	sIg+, cIg+, CD5-, CD10-, CD23-, CD43-/+
Mantle cell	sIg+, cIg-, CD5+, CD10+/-, CD23-, CD43+
Follicular	sIg+, cIg-, CD5-, CD10 +/-, CD23 -, CD43-
Marginal zone	sIg+, cIg-/+, CD5-, CD10-, CD23-, CD43-/+
Hairy Cell Disease	sIg+, cIg-, CD5-, CD10-, CD23-, CD43-, CD11c+, CD25+, CD103+

TABLE 4

BM BIOPSY: LYMPHOID NODULES
MALIGNANCY VERSUS REACTIVE PROCESS

	Nodules in FCL*	Reactive nodules
Size	usually large	usually small
Number	multiple	unique or multiple
Localization	paratrabecular	intertrabecular
Borders	not sharp	sharp
Germinal centers	usually absent	possible, unusual
IHC	usually B > T	usually B < T
FDC**	often present	unusual

*FCL = Follicular Center Lymphoma, **FDC = Follicular Dendritic Cell.

No absolute criteria !

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Workshop

Case 1

Clinical and haematological data.
69-yr-old lady. A diagnosis of lymphoma, diffuse large B-cell (REAL/WHO) was made in a cervical LN. No other adenopathy, no hepatosplenomegaly.
PB count and smear within normal range.
BM aspirate: overall normal BM cellularity. One/12 smears characterized by a lymphocytic infiltrate, exhibiting small lymphocytes with mature chromatin.

Bilateral BM biopsy performed.
Both biopsies show an overall cellularity within normal range, although somewhat heterogeneous. Three cell lines present with maturation. Several lymphoid aggregates or "nodules", are present. They are not paratrabecular in position, well circumscribed, and composed of small-sized lymphocytes exhibiting a very slight pleomorphism.
At IHC examination, mixed B- and T- cell population, both in the aggregates and interstitial.
A few polyclonal plasma cells.

Diagnosis and interpretation. BM lymphocytosis, interstitial and focal, most consistent with a reactive process; absence of involvement by a large B-cell lymphoma.

Comments
The interpretation of lymphoid nodules is often a difficult task, although some guidelines exist (see Table 4). No absolute criteria! The application of flow-cytometry and/ or molecular biology on BM and/or PB is certainly of help in difficult situations.
The lymphoid aggregates of our patient present many features in favour of a reactive process: "non-paratrabecular" localisation, no frank cytological atypia and mixture of B – and T-cells at IHC examination. Note that neoplastic nodules of B-cell lymphomas are most commonly composed of an homogeneous B-cell population but exceptions do exist.
Benign lymphoid aggregates are relatively common findings in BM biopsy. The incidence appears to increase with age and is higher in females than males. The aggregates may occur in patients with a wide range of disorders. Usually large aggregates may occur in the BM of patients with diseases related to the immune system such as autoimmune disease, HIV infection, thymoma, old age... In the case presented here, an important point is to exclude BM involvement by a low-grade component of the large B-cell lymphoma diagnosed in LN. According to Brunning and Mc Kenna (1994), histological discordance between the LN and BM is observed in 15 to 40 % of the cases.

In our patient, flow cytometry applied on the BM lymphocytes was in favour of a reactive process: predominantly small T-cell population and absence of B-cell monoclonality.

Case 2

Clinical and haematological data.
53 yr-old women. Presented with polyadenopathy and hepatosplenomegaly.
Hb 109 g/l, L 4.9 G/l, T 117 G/l
A few "atypical" lymphocytes observed on the PB smear.
BM aspirate: overall BM cellularity normal; lymphocytic infiltration (40%); lymphoma suspected.

A bilateral BM biopsy is performed.
Both biopsies are characterized by focal and paratrabecular large lymphoid infiltrates; some of them coalescing and becoming diffuse, exhibiting predominantly small to medium sized lymphocytes - many of them with "cleaved" nuclei" – associated with a few blasts (isolated cells or small clusters). Interstitial spillage of the lymphoid cells in the residual surrounding haemopoietic tissue. Reticulin fibres significantly increased..
IHC: Lymphoid infiltration positive for B-cell markers (CD20/L26 and DBB42); minority of CD3 +T-cells.

Interpretation and diagnosis.
Focal and diffuse infiltration of the BM biopsy by a predominantly small B - cell lymphoma with paratrabecular involvement consistent with a Follicular Lymphoma (FL) (REAL/WHO).

Comments
Pattern and morphology typical of FL.
The differential diagnosis usually encompasses other types of small B-cell lymphomas, mainly mantle cell lymphoma. The lymphoid population in MCL is usually more uniform. In this particular case, a cervical LN biopsy revealed a FL grade 3.

Patients with Follicular Centre Cell lymphoma (FCCL) usually present with *advanced disease*. BM involvement is frequently detected, even when the PB is normal. BM involvement is more common in the predominantly small cell variant : 50-85 % of the cases, according to different series. Leukaemic cells may be recognized in PB smears in a minority of cases. Lymphocytosis > 5 G/l, which could be confused with B-CLL is recognized as part of FCCL by the presence of notched nuclear cells. BM aspirate is often not representative due to patchy infiltration and increase in reticulin fibres in the neoplastic areas.
On histological sections, BM involvement is usually patchy with a characteristic *paratrabecular pattern*. A focal nodular ("non-paratrabecular") pattern may be observed in some cases and raises a difficult differential diagnosis with reactive or benign lymphoid nodules (see Table 4). The *reticulin* is significantly increased in direct relationship to the degree of cellular infiltrate. The infiltrates are composed of *small - to medium size cells with numerous "cleaved" forms* and a minority of blast cells, which are strongly stained by B-cell markers. When histology is inconclusive, the precise immunophenotype has to be defined by flow cytometry. Remember that the follicular architecture of the lymphoma is not identified on BM sections. A LN biopsy is recommended. Cellular composition may vary from one site to another and discrepancy between BM and lymph nodes may be observed. The more aggressive pattern is usually seen on the lymph node.

Case 3

Clinical and haematological data.
57-yr-old man. No hepatosplenomegaly and no adenopathy.
Hb 137 g/l, L 19 G/l, T 208 G/l.
80 % lymphocytes without significant atypia on PB smear.

At low power, the BM biopsy shows a global cellularity within normal range, although somewhat heterogeneous. Three cell lines present with maturation. Closer inspection reveals an interstitial infiltration by small lymphocytes with occasional nodular condensation and no significant atypia. In some areas, the haemopoietic cells are interspersed with lymphocytes.
IHC. The majority of lymphoid cells are positive for B-cell markers, DBB42 and CD45RA; only weak positivity for CD20. A minority of lymphocytes are CD3+ T - cells.

Interpretation and diagnosis.
B-cell infiltration of BM, predominantly interstitial, consistent with Chronic Lymphocytic Leukaemia (CLL)

Comments
This case illustrates the interstitial or early pattern of BM infiltration by CLL. Careful morphological and IHC examination are required for a correct interpretation.
Diagnosis of B-CLL is based on laboratory studies which include a full blood count with careful attention to the morphology of the lymphocytes, a BM aspirate and trephine biopsy and a minimum of cell markers (flow cytometry and/or BM biopsy). Persistent lymphocytosis over 10 G/l without apparent cause seems necessary for the diagnosis. Blood film exhibits a predominance of small lymphocytes with scanty cytoplasm, round nucleus, clumped nuclear chromatin and absence of azurophil granules.
Four histological patterns of BM infiltration have been described 1) interstitial, 2) focal / nodular, 3) mixed interstitial and focal / nodular and 4) diffuse.
The *diffuse pattern* represents the most advanced degree of infiltration with complete replacement of BM spaces. The pattern – diffuse versus non diffuse – has been shown by some authors to represent a significant prognostic parameter. *Small lymphocytes* are usually predominant in the BM infiltrates. A minority of prolymphocytes (PL) and/or immunoblast-like cells are usually observed. Clusters of prolymphocytes or so called "proliferative centres" resembling those observed on lymph node biopsy- may be seen in some cases. There is little if any increase in reticulin. Two types of *transformation* have been recognized in B-CLL and can occasionally be detected on BM biopsy: prolymphocytoid and large B-cell lymphoma (so-called "Richter's Syndrome").
The differential diagnosis includes:
- Reactive lymphocytosis**.** Usually characterized by a mixture of B- and T-cells, but reactive B-lymphocytosis may occur (mainly in autoimmune diseases or viral infection). Flow cytometry may be necessary to assess clonality.
- Other B-cell lymphoproliferative disorders, small cell type, such as Hairy Cell Disease (positivity for DBA.44), Immunocytoma (monotypic plasma cells), Mantle Cell Lymphoma (should be positive for PRAD1/Cyclin D1), Splenic Marginal Zone Lymphoma. In difficult situations, flow cytometry required (Table 3).

In summary, BM trephine is useful in diagnosis and follow-up of CLL patients
1) to confirm the diagnosis and exclude other lymphoproliferative diseases
2) to define the pattern of BM infiltration
3) to assess the BM haemopoietic capacity in relation to the peripheral blood findings
4) to detect morphological changes of B-CLL.

Case 4

Clinical and haematological data.
73-yr-old man. Suffers from fatigue. Presented with splenomegaly and pancytopenia. No adenopathy, no hepatomegaly.
Hb 72 g/l, L 1,6 G/l, T 109 G/l
No atypical cells described on PB smear.
BM examination
Dry tap at BM aspiration
BM trephine reveals an interstitial and loose infiltration by small to medium sized cells with bean-shaped or indented nuclei, surrounded by a clear zone. Decreased haemopoiesis. Moderate and diffuse increase in reticulin.
IHC: the atypical cells are positive for B-cell markers with a strong positivity for DBA44. Tumour cells negative for CD 3, CD 34 and MPO.

Interpretation and diagnosis: Morphology and IHC results typical of Hairy Cell Disease (HCD) with "hypoplastic" BM.

Comments
HCD is a disease of the adult age group (mean age 50 yrs; range 24-80 yrs) with a M:F ratio of 5:1. Splenomegaly is present in 90 % and hepatomegaly in 50 % of the cases. Lymphadenoapthy is uncommon.
The PB counts always reveal variable degrees of cytopenia, 75 % of the patients presenting with pancytopenia. Monocytopenia is frequent and has been implicated in the pathogenesis of the infections which characteristically accompany the disease. The proportion of hairy cells on the PB varies from case to case and may be rare and difficult to find (only 25 % of the patients have more than 50 % hairy cells in the differential count).
A diagnosis of HCD, suspected on the basis of clinical and haematological features needs a confirmation by either cytochemical*, histological or immunological methods.
BM is always involved. BM aspirate is usually unsuccessful due to increase in reticulin fibres. BM histology represents a reliable diagnostic tool and the histopathologist can make the diagnosis with confidence. BM trephine and IHC are necessary for the differential diagnosis with other lymphoproliferative disorders. BM biopsy shows a diffuse infiltration by hairy cells in most cases. Fibrosis is typical. Hairy cells infiltrate the BM in a very characteristic loose fashion with a well-defined rim of cytoplasm which leaves a clear zone around the cells. These appearances facilitate the differential diagnosis from other lymphoproliferative disorders. In a minority of cases, patchy infiltration may be seen, more frequently in early stages of the disease. Rare cases of HCD are characterized by an interstitial infiltration together with a hypoplastic BM. Such cases should not be confused with an aplastic anaemia. Classically, hairy cells are small to medium sized, with oval or "bean"-shaped or indented nuclei. Nucleoli and mitotic activity are inconspicuous. The "hairy" features of cytoplasms are not visible on tissue sections
IHC. Tumour cells are positive for B – cell markers (L26/CD20, DBB42). The positivity for DBA44 is highly characteristic of HCD, although not entirely specific. According to the literature and our experience, IHC - especially DBA44 immunostaining - is mandatory for the detection of subtle interstitial infiltration or residual disease after treatment (example shown: slide 4 b)

*Hairy cells also have tartrate resistant acid phosphatase (TRAP) activity. This cannot be applied routinely on tissue sections but can be performed on imprints from the biopsy.

Case 5 and 6 (To be discussed together)

Case 5

Clinical and haematological data.
54-yr-old woman. Rheumatoid arthritis (RA) since 12 yrs, treated by chloroquin.
Presented with splenomegaly, anaemia, neutropenia and thrombocytopenia.
No peripheral adenopathy, but mediastinal and retroperitoneal adenopathy at CT-scan.
Hepatomegaly. Lymphoma suspected.
BM aspirate: lymphocytic infiltrates on 8 / 10 smears, without significant atypia.
BM biopsy: Overall increased cellularity. Granulocytic cell line predominant. Lymphoid foci - intertrabecular and occasionally paratrabecular- with atypical small to medium-sized lymphocytes with irregular nuclear contours and condensed chromatin. Granulomatous reaction associated with the lymphoid foci. Increased number of eosinophils. No microorganisms detected by special stains. Increase of the reticulin.
On IHC preparation, positivity of the atypical lymphoid cells for CD3. Rare CD20 positive B-cells. No CD30 positive cells. Numerous histiocytes coloured by CD 68/PGM1.
Molecular biology: no monoclonal rearrangement could be found by PCR.

Interpretation and diagnosis: picture raising a differential diagnosis between a T-cell lymphoma, a T-cell rich B-cell lymphoma and Hodgkin's disease. Reactive process less probable because of the cell atypia.
Because of the IHC results, BM histology most consistent with a peripheral T-cell lymphoma. Non specific granulomatous reaction.

PB smears reviewed later on: Moderate lymphocytosis. Presence of Large Granular Lymphocyte (LGL) with azurophilic granules. Flow cytometry: 95 % CD3+ lymphocytes, with 83 % lymphocytes presenting a coexpression CD3/ CD57

Final diagnosis : LGL leukaemia.

Case 6

Clinical and haematological data.
53-yr-old woman. Presented with pulmonary abscess and pseudomonas aeruginosa septicaemia. Splenomegaly. No adenopathy. Neutropenia.
Hb 88 g/l, L 7 G/ l, T 209 G/l
Neutrophils 4.6 %, Lymphocytes 86.9 %, Monocytes 7.6 %

BM biopsy was performed because of suspicion of lymphoma or of Myelodysplastic Syndrome.
BM trephine shows an overall increased cellularity. Erythrocyte cell line present with minor dysplastic changes. Shift to the left of the granulocytic cell line. Moderate increase of the number of megakaryocytes without any significant atypia. On HE section, a few clusters of lymphocytes are to be seen. Some lymphocytes exhibit an irregular or indented nucleus. Few plasma cells. Moderate increase of the reticulin network.
Lymphocytosis much more apparent on IHC preparations. Clear-cut predominance of CD3 and CD8 T-cells.
Molecular biology; a monoclonal TRC-γ rearrangement was demonstrated by PCR.

Interpretation and diagnosis: on HE section, the picture could be suggestive of a reactive process - due to infection and/or hypersplenism - with hyperplastic BM and lymphoplasmacytosis. However, because of the IHC and molecular results, a diagnosis of peripheral T-cell lymphoma is favoured.

PB smears reviewed (2nd look): about 50 % lymphocytes of the Large Granular Lymphocyte (LGL) type (azurophilic granules).
At flow cytometry, 98 % lymphocytes CD3+, with 88% of them co-expressing CD8 and 57 % CD57.

Final diagnosis : LGL leukaemia

Comments
LGL leukaemia does not have distinctive features on BM biopsy and can be confused with a reactive process or various small cell lymphoproliferative disorders. The BM lymphocytic infiltration may be subtle and difficult to detect if IHC is not applied. Simultaneous examination of the PB smears and complementary investigations such as flow cytometry are necessary to recognize the disease.
Clonal diseases of LGL disorders can arise from a CD3+ T-cell lineage or from a CD3 – NK-cell lineage. According to Lamy and Loughran (1999), CD3+ LGL leukaemia is the most frequent form of LGL leukaemia. It is a disease of elderly people. Approximately 60 % of patients are symptomatic: recurrent infections secondary to chronic neutropenia, anaemia, and RA. In the recent paper by Lamy and Loughran it is suggested that "dysregulated apoptosis could be an underlying mechanism for both malignancy and autoimmune disease".

Case 7

Clinical and haematological data
67-yr-old man. Presents now with marked splenomegaly and anemia.
A diagnosis of Polycythemia Vera was made 12 years ago.
No hepatomegaly, no adenopathy. Hb 100g/l, L 10 G/l, T 160 G/l
On PB smears: erythroblasts 3%, myelocyte 0.5%. RBC aniso-poikilocytosis with tear-drop shaped RBCs.
BM aspirate: dry tap.

BM biopsy. Histological examination disclose an hypercellular marrow with strong hyperplasia of the megakaryocytic cell line. The megakaryocytes form large clusters and present variable morphological features. Most of them exhibit large and hyperlobulated nuclei but some small forms can also been observed characterized by dark and hyperchromatic nuclei and poor cytoplasm. The erythroid cell line is present with rare dysplastic changes. The granulocytic cell line predominates with good maturation and no shift to the left. No excess of blast cells. Diffuse increase in reticulin (Bauermeister grade 2). The marrow sinusoid are distended and contain foci of haemopoietic cells. No excess of CD34+ cells at IHC.

Interpretation and diagnosis (taking in account the clinical history and the haematological data) :
Postpolycythemia Myeloid Metaplasia (PPMM) or Myelofibrosis with myeloid metaplasia supervening during the course of PV.

Comments
The BM picture is virtually indistinguishable from that of Chronic Idiopathic or Primary Myelofibrosis in cellular phase. This case underlines the importance of careful correlation of clinical, laboratory, and histological findings in reaching the best diagnosis.
Approximately 15 % of patients with PV develop Myelofibrosis or PPMM also termed "spent phase of PV". This phase appears after an average interval of 10 years from diagnosis. The incidence among patients with PV who survive for 15 years or more is reported to approach 50%. For some time it has been thought that the use of radioactive phosphorus (^{32}P) in the treatment of PV was associated with an increase incidence of PPMM. However, this was not confirmed by the results of the PVSG (Polycythemia Vera Study Group). Patients with PPMM are characterized by splenomegaly, leukoerythroblastosis, teardrop RBC poikilocytosis, normalization or decrease of the RBC mass, BM failure and extensive marrow fibrosis. The onset of PPMM is associated with a shorter survival than for patients with primary or idiopathic myelofibrosis. Most patients die in less than 3 years from the diagnosis of PPMM and transformation to acute leukaemia is a frequent cause of death. Approximately 1% to 2% of PV patients treated with phlebotomy alone develop acute leukaemia. However, the frequency is higher (10% to 15%) in patients treated with myelosuppressive agents and in nearly 50% of these patients, the blastic transformation is preceded by a myelodysplastic period. Thus, the finding of myelodysplastic features in the follow-up biopsies of PV patients should be viewed with concern.
The differential diagnosis of myelofibrosis includes: leukoerythroblastosis due to a metastatic process involving BM, myelodysplastic syndromes (MDS) with fibrosis (possibly therapy-related MDS). According to the literature, patients with MDS and fibrosis are characterized by minimal organomegaly and prominent dysplasia in megakaryopoiesis. They also have a higher frequency of cytogenetic aberrations.

The case presented here illustrates the diagnostic and prognostic value of BM biopsy in the assessment of MPD at presentation and during follow-up.

Case 8

Clinical and haematological data
77-yr-old woman, suffering from chronic macrocytic anaemia
Hb 55g/l, MCV 109 fl, L 3.1, T 115 G/l
No hepato-splenomegaly.
Serum iron within normal range.
No vitamin deficiency
No inflammatory process.
PB blood smear
RBC: anisopoïkilocytosis, macrocytes, dacryocytes. Granulocytopenia. Platelet anisocytosis.

BM aspirate: Hypercellularity. Myelo/erythroid ratio = 1. Increased number of megakaryocytes. Myelodysplasia of the 3 cell lines. No excess of blasts. No ring sideroblasts.

BM histology: Hypercellularity. Erythroid cell line: dyserythropoiesis, macrocytosis. Granulocytic cell line present with shift to the left. Increased number of megakaryocytes with abnormal small forms showing non-lobulated rounded nuclei.
No excess of blasts. No significant fibrosis.
IHC: small excess of CD34+ cells (5-10%) .

Interpretation and diagnosis: hyperplastic BM with dysplastic features (dysmegakaryopoiesis) strongly suggestive of MDS. The chronic macrocytic anaemia together with the morphology of the megakaryocyte raise a suspicion of 5 q – syndrome. Cytogenetics recommended.

Final diagnosis, after cytogenetic analysis : MDS of the 5q- syndrome type

Comments
The 5q- Syndrome is a neoplastic condition belonging to the group of MDS (stem cell clonal disease). Age > 65 yrs, female > male. Refractory anaemia; thrombocytosis +/-. Splenomegaly rare (16 % of the cases). BM exhibiting an increased number of megakaryocytes characterized by non - lobulated nuclei but relatively abundant cytoplasm. Favourable prognosis. Supportive therapy.
BM biopsy is an important part of the clinical work-up of patients with chronic PB cytopenia and suspicion of MDS and has to rule out tumour infiltration or aplastic anaemia as alternative cause of cytopenia.
Remember that myelodysplastic features can be seen in benign and malignant conditions so that the diagnosis of MDS is made by exclusion and the differential diagnosis includes

- nutritional disorders (vit. B12 or folate deficiency);
- dysplastic changes in AIDS or other infection (tuberculosis),
- Dysplastic marrow changes resulting from exposure to toxins and drugs can mimic a MDS (anticonvulsivants, alcohol abuse, antipurines, antipyrimidines, hydroxyurea, cyclophosphamide, procarbazine, acyclovir)

In cases of 5q- syndrome with thrombocytosis, a chronic myeloproliferative syndrome has also to be taken in consideration. Myelodysplastic features may be present in MPD at presentation but are usually less obvious than in MDS. In this situation, cytogenetics is mandatory.
In summary, a combination of data must be taken in account for the correct interpretation of myelodysplastic features in BMB

- Clinical status
- Morphological evaluation of the PB smear, BM aspirate and biopsy specimen
- Cytogenetic analysis.

Case 9

Clinical and haematological data
33-yr-old woman, HIV+. Presented with fever and jaundice.
History of gastro-enteritis 2 weeks ago.
Hepatosplenomegaly. No peripheral adenopathy. Retroperitoneal adenopathy on CT scan.
Therapy: Ciproxine, Fenoxypen
Lymphoma and/or opportunistic infection suspected.
Hb 82g/l, L 1.4 G/l, T 148.
PB smear: macrocytic anaemia, neutropenia, lymphopenia.
BM aspirate: normocellular BM with dysmyelopoiesis of the 3 cell lines, strong plasmacytosis, 5% lymphocytes, absence of opportunistic microorganisms, absence of lymphoma.
BM biopsy (unilateral): increased cellularity, three cell lines present, dyserythropoiesis, shift to left of the granulocytic cell line, increased number of megakaryocytes, dysmegakaryopoiesis (small forms, hypolobulated nuclei, apoptotic nuclei), increased number of plasma cells, a few epithelioid and histiocytic cells, very rare atypical large nucleolated cells observed on some levels of section, special staining for microorganisms negative.
IHC: polyclonal plasmacytosis, slight lymphocytosis with a mixture of B- and T- cells; no atypical cell positive for CD30.

Interpretation and diagnosis
Non specific reactive BM in the context of an HIV infection.
Rare atypical - but non-diagnostic - large nucleolated cells (Hodgkin's cells?)

Because of the suspicion of lymphoma, liver and retroperitoneal adenopathy biopsies were performed. Both biopsies disclosed Hodgkin's disease, mixed cell type with granulomatous reaction.

Comments
Peripheral blood (PB) cytopenia is one of the major indications for BM examination in HIV-positive patients and this case illustrates the complexity of the BM analyse in the context of a HIV infection. The BM trephine presented here exhibits a constellation of findings, which are characteristic - although not specific - of HIV infection: hypercellularity with increased number of megakaryocytes, plasmacytosis, fibrosis and dysmyelopoiesis (especially dysmegacaryopoeisis). Gelatinous transformation of the stroma, opportunistic infections and lymphoma may also be observed in advanced disease
Our case is an example of dysmyelopoiesis or myelodysplastic features associated with a reactive process. Hyperplasia of the megakaryocytic cell line together with dysmegakaryopoiesis represent the histological hallmark of the ineffective thrombocytopoiesis in HIV infection. In this context, the term "dysmyelopoiesis" is used in a descriptive sense, referring to "maturation abnormalities" of the haematopoietic cell lines and does not imply a "premalignant state". The pathogenesis of the haematological abnormalities in HIV infection appears to be multifactorial: coexisting infections, neoplasm, drugs, antibodies against PB elements, circulating immune complexes, hypersplenism and HIV itself are potential candidates. Both in vitro and in vivo studies have shown that megakaryocytes are susceptible to HIV infection.
The diagnostic of Hodgkin's disease (HD) in BM trephine may be a challenge for the histopathologist. Because of the heterogeneity of the infiltration, diagnostic foci may be overlooked. HD in HIV-infected patients has been reported mainly in Europe. An unusually aggressive tumour behaviour has been reported, including higher frequency of unfavourable histologic subtypes, advanced stages, extranodal involvement and poorer therapeutic outcome, as compared with the behaviour of HD outside of the HIV setting.

Case 10

Clinical and haematological data
31-yr-old female. Suffers from fatigue since 1 month. Presented with pancytopenia. No adenopathy, no hepatosplenomegaly. Hb 115 g/l, L 2 G/l, T 39 G/l
PB smear: rare blast cells.

BM histological examination
Increased cellularity, diffuse and monotonous cellular infiltration. The cells are medium-sized with somewhat eccentric rounded or lobulated nuclei, fine chromatin and dense non-granular cytoplasm.
Some residual islands of erythropoiesis and a few megakaryocytes are present. Focally, slight increase of the reticulin fibre network.
IHC: tumour cells positive for MPO and CD68/KP1 and negative for CD34,CD68/PGM1 Tdt, lymphoid markers and CD138.

Histologic interpretation and diagnosis
The differential diagnosis includes acute lymphoblastic leukaemia, multiple myeloma (some "plasma cell-like features"), Hairy Cell Disease and metastatic tumour. Positivity of the tumour cells for MPO allows a diagnosis of Acute Myeloid Leukaemia (AML), not further classified.

BM smears: blast cell population with granular cytoplasms and Auer rods and positive for Black Sudan. At flow cytometry: the blast cells co- express CD13 and CD33 and are positive for CD15 and CD65. Negativity for TdT and CD34.

Final diagnosis: AML M3 (FAB) according to the FAB system.

Comments
Histopathologists have to be familiarized with the morphological and IHC features of AL. BMB may viewed as complementary to the PB and BM smears for the diagnosis and follow-up of Acute Leukaemia (AL) (assessment of BM cellularity and residual haemopoiesis, quantity and rough characterization of the blasts). The trephine biopsy is necessary in cases showing dry tap at bone marrow aspiration or for classification of cases which lack circulating blasts.

Case 11

Clinical and haematological data
This 60-yr-old man was admitted to hospital because of thoracic pain and presented with a right-sided thoracic mass involving soft tissues and one rib. No hepato-splenomegaly, no adenopathy. No other skeletal lesion. Hb 111 g / l, L 4.9 G / l, T 29 G /l. No paraprotein, no Bence Jones protein.

BM biopsy : Shows a diffuse and massive infiltration by medium – to large sized cells presenting pleomorphic and often eccentric nuclei, fine chromatin, nucleoli, and moderately abundant cytoplasm. Increase in reticulin.
At IHC examination, the cells are negative for all tested markers (epithelial, lymphoid, melanoma, Ewing, Ig, CD30…), except for CD138 /Syndecan-1
A needle biopsy of the thoracic mass yielded the same morphological and IHC results

Interpretation and diagnosis : Multiple Myeloma, non - secretory, expressing CD138

BM aspirate contained some atypical cells, so that flow cytometry was performed and confirmed the diagnosis. The tumour cells coexpressed CD38 and CD138. No sIg could be detected. CIg were tested : IgA expression could be found; but no light chain was detected.

Comments
The role of BMB and IHC in the diagnosis of MM is well illustrated by this cases of non secretory MM, exhibiting very atypical morphology. According to the literature and our own experience, the monoclonal Ab CD138 is very helpful in characterising the plasma cells and selectively differentiating malignant plasma cells from other malignancies (immunoblastic lymphoma, poorly differentiated carcinoma). Note that in a proportion of MM cases expression of CD45 and/or B-cell associated antigens and, occasionally, aberrant expression of CD45RO (UCHL-1) or CD30 or non-haematopoietic antigens (EMA, vimentin) represent diagnostic pitfalls.

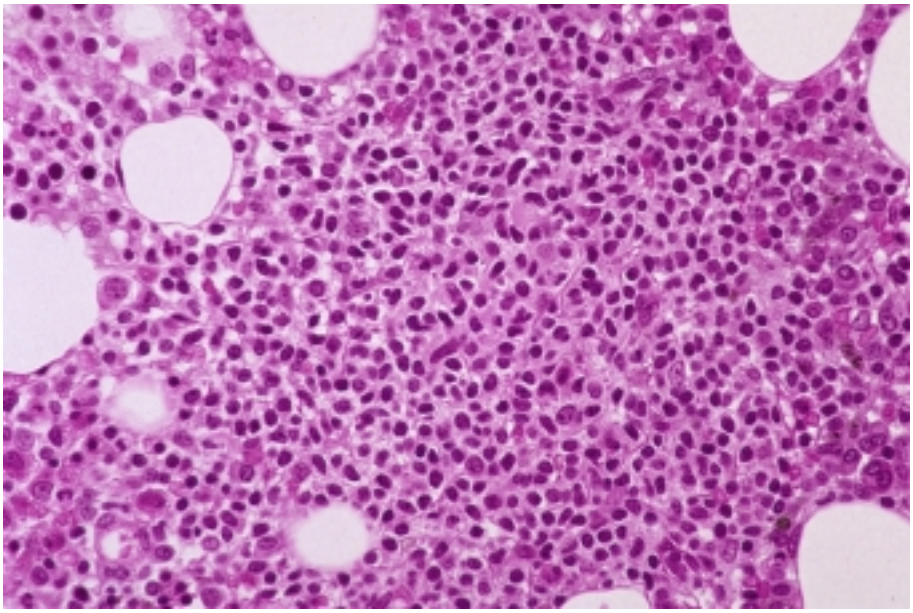
Syndecan-1 (CD138) is a transmembrane heparin sulphate proteoglycan expressed in different stages of differentiation of normal lymphoid cells, i.e. in pre-B-cells and Ig producing plasma cell. Its normal function or presence in lymphoid malignancies is largely unknown. According to Chilosi et al (1999), CD138/Syndecan -1 is a useful immunohistochemical marker of normal and neoplastic plasma cells. On routine trephine bone marrow biopsies (paraffin embedded and decalcified) virtually all normal and neoplastic plasma cells express clear-cut membrane CD138 immunostaining.

Case 12

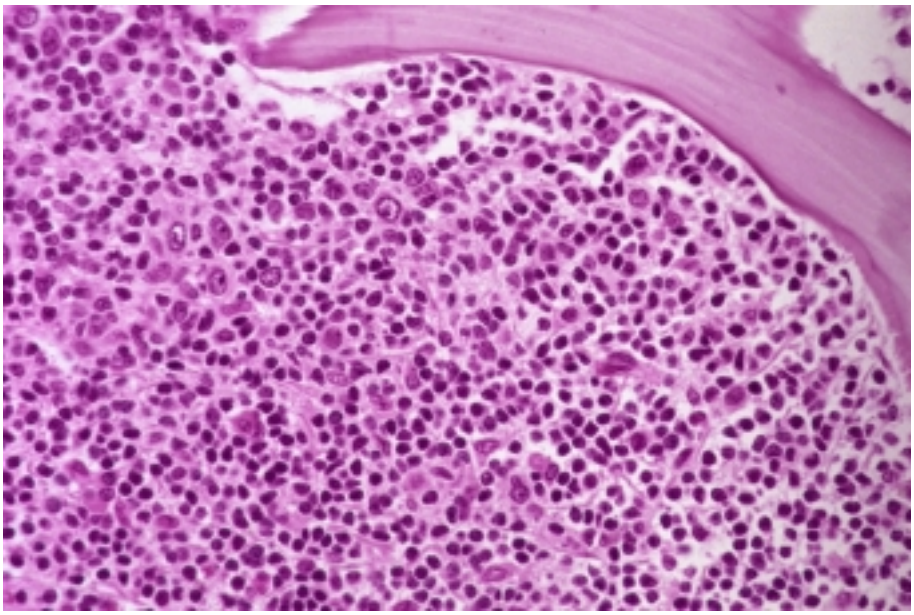
Clinical and haematological data.
30-yr-old lady (MA).
Presented with renal failure, hypercalcemia and multiple osteolytic lesions.
Adenopathy, splenomegaly, no hepatomegaly. Anaemia. No paraprotein in the serum.
Neutrophils precursors on the PB smear.
BM aspirate : dry tap.
BM biopsy : diffuse infiltration by large cells characterized by eccentric nuclei, prominent nucleoli, abundant and dense cytoplasms without granules.
IHC: tumour cells positive for Melan A +, S100+, negative for keratin C11, CD138, Ig, CD45, B (CD20) - and T (CD3) - cell markers.

Interpretation and diagnosis: melanoma, metastatic.

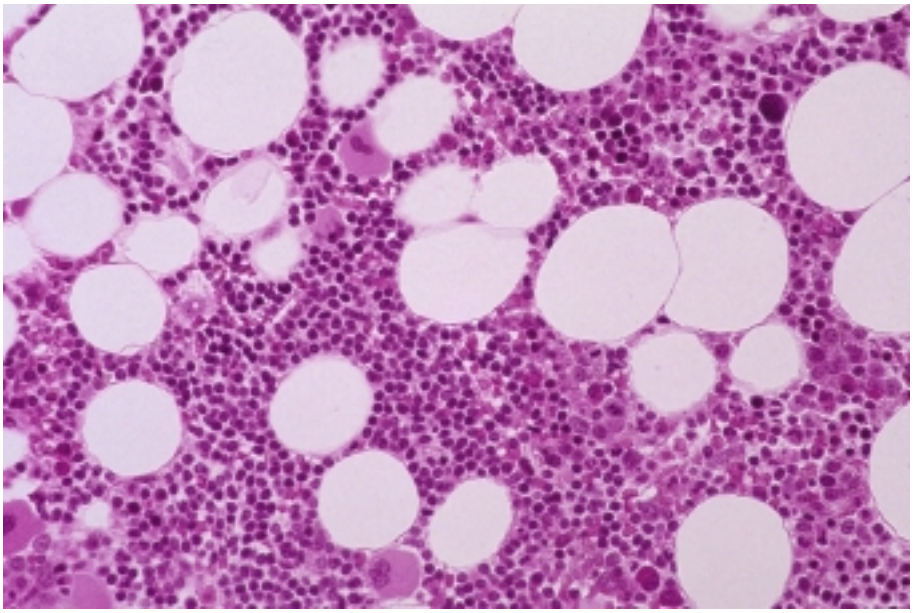
Comments
BMB is more often positive for metastatic tumour than is aspirate, mainly because metastatic tumours induce fibrosis and or necrosis or may be too compact. In adults, breast carcinoma (female) and prostate and lung carcinoma (males) are the most frequent metastatic tumours involving the BM. In children, neuroblastoma is the most common solid tumour and has the highest frequency of BM involvement. In such situations, the panel of antibodies applied to BM sections is identical to that used in surgical pathology.
When the marrow is infiltrated by malignant cells, non-specific changes may be observed, including increased number of plasma cells, granulocytic and/or megakaryocytic hyperplasia and increased storage iron. A leucoerythroblastic reaction with circulating erythroblasts and immature granulocytes is a relatively common finding in the PB.
Malignant melanoma is found in the BM in approx. 5 % of patients with disseminated disease. Malignant melanoma should be suspected if metastatic tumour is composed of polygonal or spindle cells with prominent nucleoli. If melanin is present within the tumour cells, the diagnostic is relatively easy. However, not infrequently, metastatic melanoma is amelanotic and IHC mandatory for the diagnostic.
In the case presented here, the tumour cells are amelanotic and their morphological features can suggest a plasmablastic plasmocytoma or immunoblastic lymphoma and the correct diagnosis cannot be made without IHC.



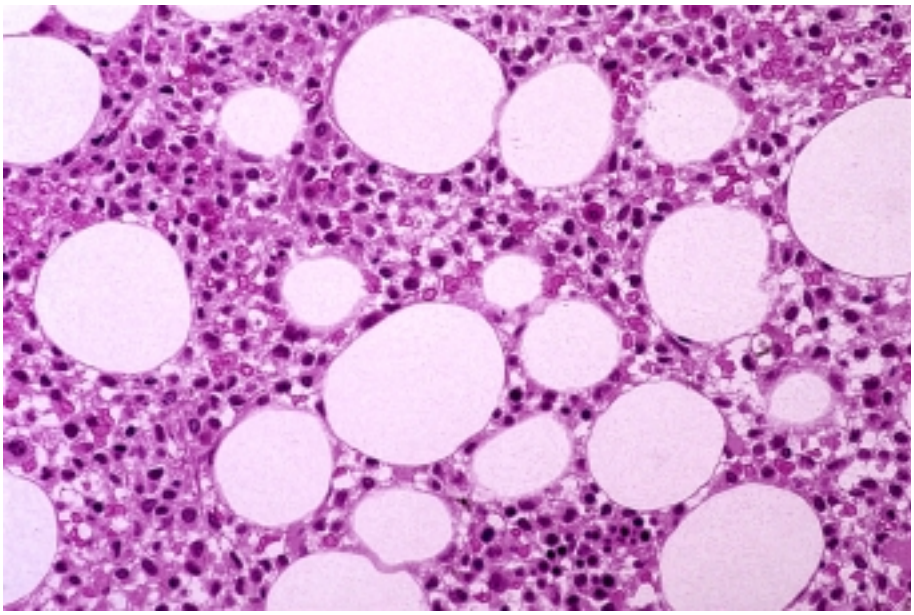
Case 1. BM lymphocytosis, reactive process.



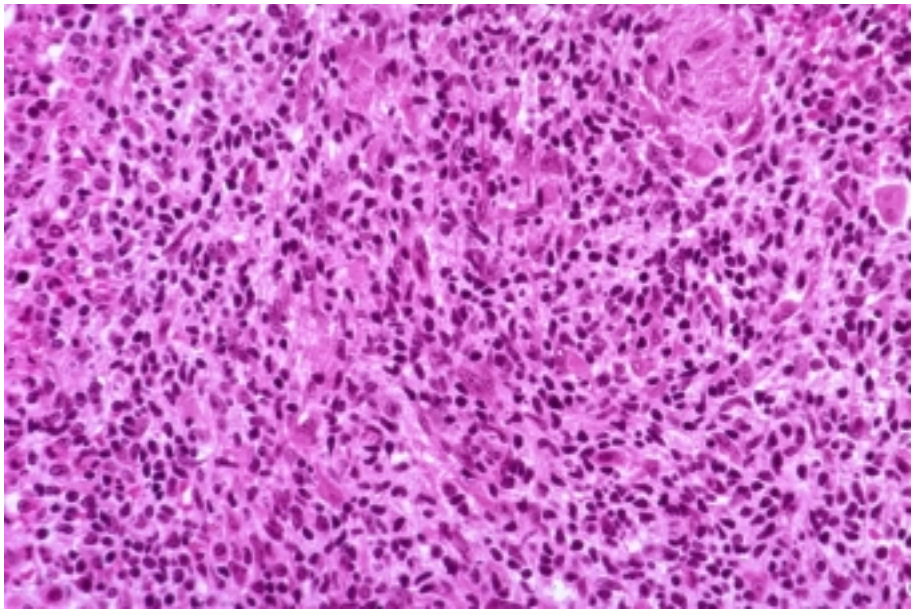
Case 2. Follicular Lymphoma.



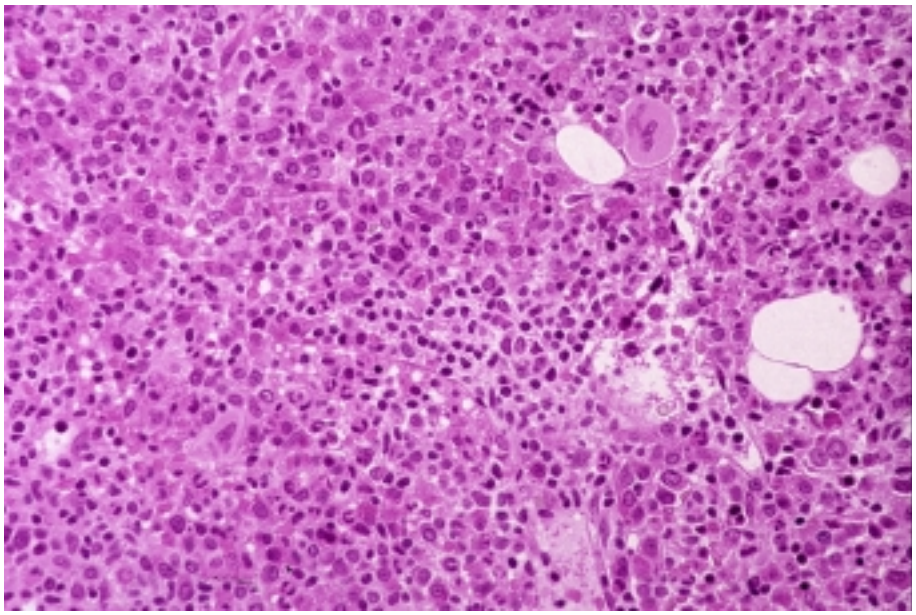
Case 3. Chronic Lymphocytic Leukaemia (CLL).



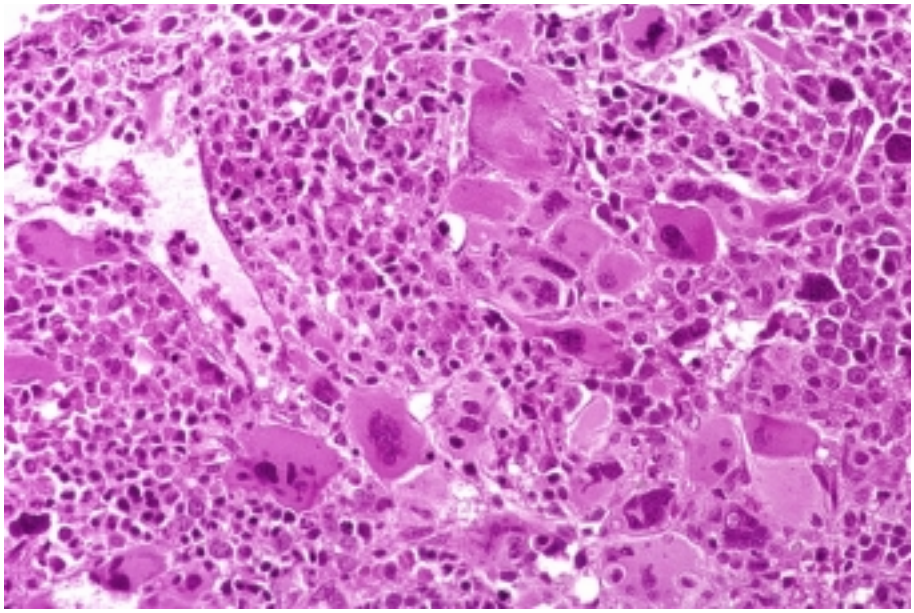
Case 4. Hairy Cell Disease.



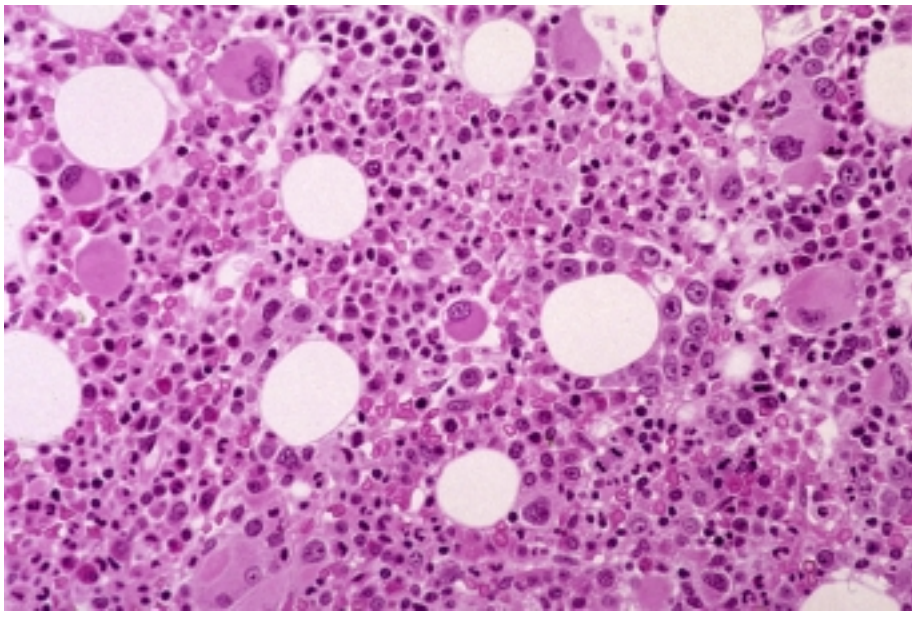
Case 5. Large Granular Lymphocyte Leukaemia.



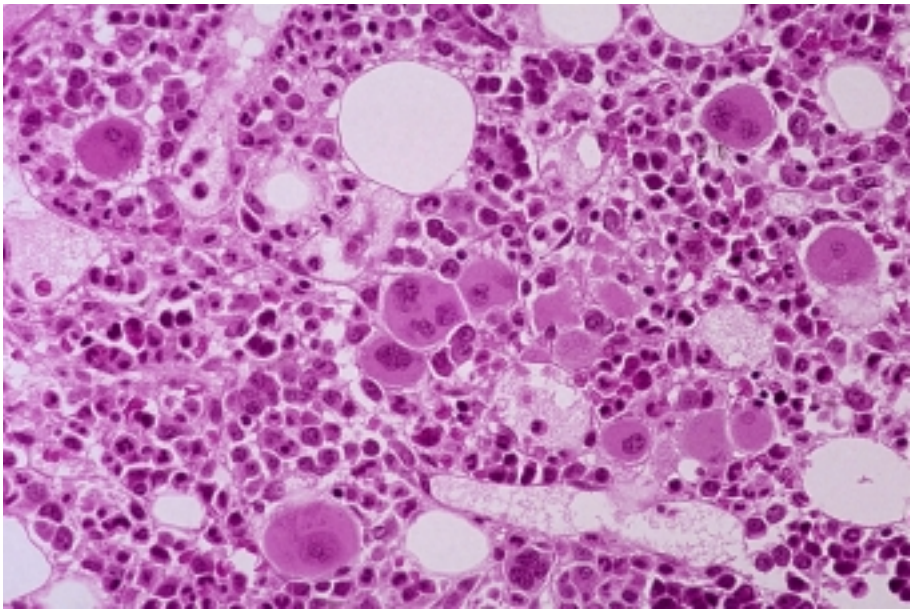
Case 6. Large Granular Lymphocyte Leukaemia.



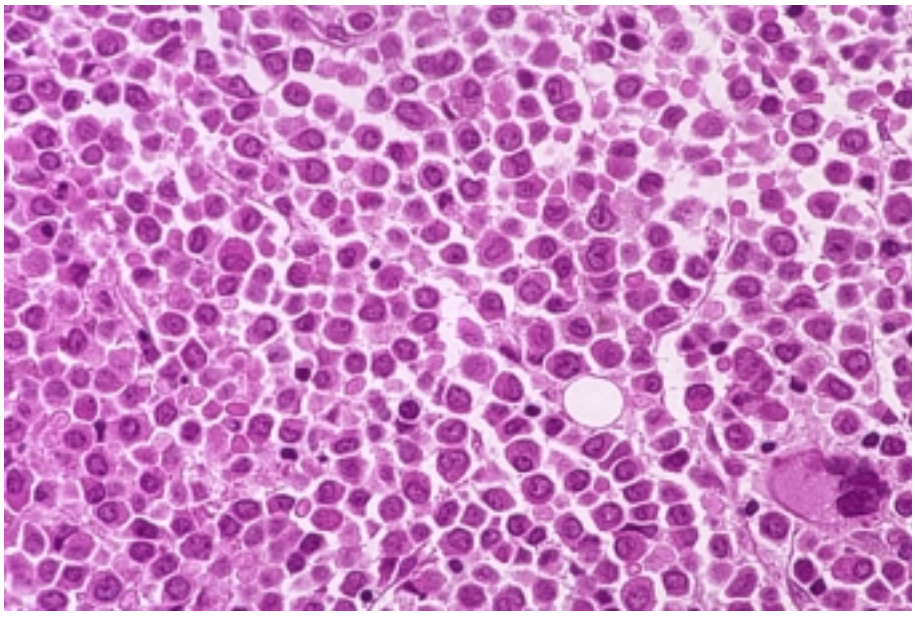
Case 7. Postpolycythemia Myeloid Metaplasia.



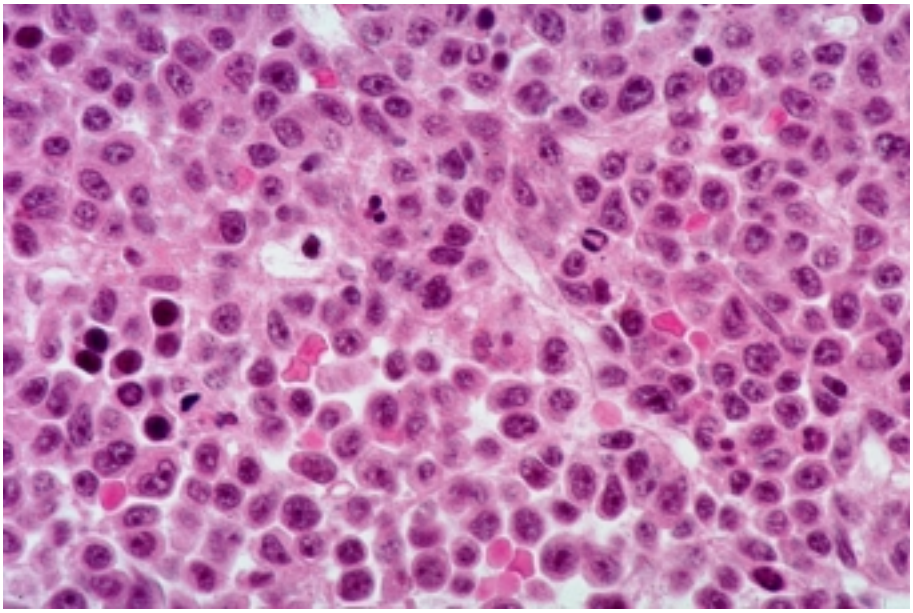
Case 8. MDS of the 5q- syndrome type.



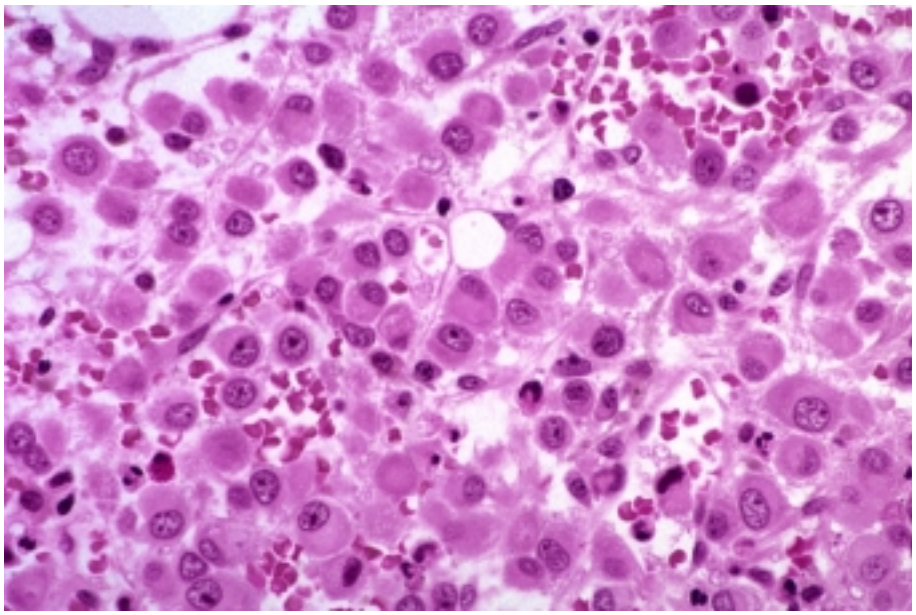
Case 9. Reactive BM in the context of an HIV infection.



Case 10. Acute Myeloid Leukaemia M3 (FAB)



Case 11. Multiple Myeloma.



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