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REVIEW ARTICLE

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Immunohistochemistry in bone marrow pathology: a useful adjunct for morphologic diagnosis

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Abstract Pathomorphological examination of trephine biopsies of the bone marrow (BM) represents a standard method for the diagnosis and staging of hematologic neoplasms and other disorders involving the BM. The increasing knowledge about the genetic basis and biology of hematologic neoplasms, as well as the recently proposed WHO classification system, provide the framework for an accurate diagnosis. Although conventional morphology remains the gold standard for paraffin-embedded BM trephines, immunohistochemical stainings have become an integral part of the diagnostic workup. Antibodies suitable for paraffin sections are generally applicable to BM trephines, but modifications of staining protocols may be necessary due to the alternative fixatives and decalcification procedures used for BM biopsies. The indications for immunostainings range from confirmation and classification of lymphoma involvement, subclassification of acute leukemias, and estimating blast counts in myelodysplastic and myeloproliferative syndromes to characterization of BM involvement in nonhematologic neoplasms. Although subtyping of NHL in the BM is more difficult from the point of morphology, classification of the entities that frequently involve the BM, especially the small B-cell lymphomas, can easily be achieved with the help of immunohistochemistry. In this review, we try to summarize the current state of the art in BM immunohistochemistry for the diagnosis of hematologic disorders. Moreover, diagnostic algorithms and useful antibody panels are proposed for a rational and cost-effective approach.

Keywords Trephine biopsies · Hematologic disorders · Immunohistochemistry · Diagnostic algorithms

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Introduction

Morphological examination of trephine biopsies of the bone marrow (BM) is a standard method for diagnosis and staging of patients with malignant lymphoma (ML), plasma cell dyscrasias, chronic myeloproliferative syndrome (MPS) and myelodysplastic syndrome (MDS), acute leukemia, and metastatic tumors. Occasionally, the diagnosis of nonneoplastic conditions such as infectious diseases or, to a lesser extent, anemias also require examination of a BM trephine [10]. In addition, determination of BM cellularity following BM transplantation or administration of chemotherapeutic agents is most reliably established by trephine biopsies. Ideally, trephine biopsy, aspirate smears, and flow cytometry should be viewed as integral parts of a combined diagnostic approach. In some instances, e.g., myelofibrosis, the trephine biopsy will be the only specimen available for examination. Due to both technical and historical reasons, the use of immunohistochemistry for BM diagnostics has lagged behind other applications. On one hand, the availability of antibodies suitable for paraffin was limited for a long time, and flow cytometry was (and for many indications it still is) the primary technique for immunophenotyping of hematologic neoplasms. On the other hand, the aim of alternative fixation, decalcification, and embedding techniques at optimizing morphology frequently proved detrimental for the preservation of antigens. However, recent years have seen an exponential increase in antibodies suitable for paraffin-embedded tissues, plus improvements in antigen retrieval, leading to a rapid integration of immunohistochemistry into BM diagnostics. Of note, the necessity for immunohistochemical studies on trephine biopsies is greater in countries like

Germany, where the biopsy is signed out by the pathologist, whereas flow cytometry and, frequently, also cytology is seen by the hematologist, making an integrated diagnostic approach more difficult.

Since diagnosis and staging of ML are among the most frequent clinical indications for trephine biopsies, this review provides a detailed description of the morphological and immunophenotypical criteria for making the diagnosis of ML in the BM.

The role of immunohistochemistry in the classification of myeloproliferative disorders, MDSs, acute leukemias, and nonneoplastic BM changes comprises the second part of this review.

Technical issues of BM immunohistochemistry

A variety of fixatives and decalcification procedures are routinely used for BM trephines in different parts of the world. Many, but not all of them, are well suited for immunohistochemical stains. In our opinion, fixation in buffered formalin, decalcification in EDTA, and embedding in paraffin is a good compromise, because it not only provides good morphology and excellent conditions for immunohistochemistry but also allows DNA- and RNA-based molecular studies. Acceleration of the decalcifying process can be achieved by using ultrasound energization, which reduces the decalcification time to approximately 6–12 h. (Medite, Burgdorf, Germany) [106, 119]. In addition, formalin fixation allows routine processing of BM sections on automated immunostainers, which improves reproducibility and reliability of immunostaining [21, 32, 42, 161]. However, certain recommendations should be considered for obtaining optimal staining results. The fixation time should not exceed 24 h, since staining intensity decreases with prolonged fixation time. In our experience, staining results are not significantly affected by the duration of EDTA decalcification. Nevertheless, staining protocols and especially antigen retrieval used for other paraffin tissues such as lymph nodes may have to be adjusted for BM trephines to avoid inadequate staining or tissue damage due to overly strong antigen retrieval.

Schaefer's fixative (0.4% glutaraldehyde/10% formalin), a fixative solution widely used in Germany, and methacrylate embedding are two techniques that offer some advantages over conventional formalin fixation, such as excellent morphological detail and the ability to perform numerous histochemical reactions, but both techniques severely limit immunohistochemical studies [18, 60, 133]. Fixation with B5 or Zenker fixative for up to 2.5 h, frequently used in the USA, renders good staining results, but prolonged fixation of the specimen may abolish immunoreactivity. An important drawback of these fixatives, despite the good morphology achieved, is that molecular analysis, in general, cannot be performed.

Currently, a wide array of antibodies is available for BM diagnostics. Table 1 shows a list of primary antibodies used in our institution. Obviously, this selection represents the personal choice of the authors and is by no means complete. Of note, some standard antibodies for lymphoma subtyping such as CD15, CD43, or CD68 (KP1) show broad reactivity with myeloid cells and, therefore, are less suited for BM trephines than for lymph nodes. The choice of antibodies and the amount of stains necessary depend on the type of the disease and the clinical question. For instance, the primary classification of neoplasms in the BM usually requires a larger panel of antibodies, whereas confirmation of BM infiltration by lymphoma diagnosed at another site, assessment of treatment response, or detection of minimal residual disease or relapse is usually possible with a few selected stains.

Malignant lymphoma

Peripheral B-cell lymphomas

Non-Hodgkin lymphomas (NHL) composed of small B lymphocytes comprise the majority of lymphoma infiltrates in the BM. Bone marrow involvement is seen in virtually 100% of B-cell chronic lymphocytic leukemia (B-CLL)/small lymphocytic lymphoma (SLL), in almost all cases of lymphoplasmacytic lymphoma (LPL) and hairy cell leukemia (HCL), in 55–93% of mantle cell lymphoma (MCL), in 50–60% of follicular lymphoma (FL), and invariably, in splenic marginal zone B-cell lymphoma (SMZL). The only notable exception within these groups is nodal and extranodal marginal zone B-cell lymphoma, which involves the marrow in approximately 10–30% of cases [34, 37, 51, 57, 82, 83, 95, 103, 120, 121, 135, 149, 164]. Distinction of the most common types of B-NHL in the trephine biopsy can be difficult on morphological grounds alone due to overlapping cytological features and distribution patterns, and therefore, requires integration of immunophenotypic and occasionally molecular data for primary diagnosis. Nevertheless, the morphology of an infiltrate gives important clues for subclassification and should guide the selection of antibodies. The pattern of BM involvement is one of the most important hints for diagnosis. Five main patterns of BM infiltration by NHLs are recognized, namely, paratrabecular, randomly focal intertrabecular, interstitial, diffuse, and sinusoidal/intravascular [37, 72, 82, 135]. In some cases, multiple or mixed patterns are present in the same specimen. Although most infiltrates are easily recognizable in standard stains, sparse interstitial and sinusoidal involvement can be practically undetectable by conventional morphology and requires immunohistochemistry to highlight the neoplastic population.

Immunohistochemical classification of small B-cell lymphomas in the BM

A practical approach to the primary diagnosis of small B-cell lymphomas involving the BM is based on (1) infiltration pattern and cytology and (2) immunohistochemistry with a limited panel of antibodies based on morphological findings. This strategy, which is directed at reducing

Table 1 Primary antibodies employed for BM diagnostics in our institution

Antigen	Clone	Company	Main cellular expression
CD1a	MTB-1	Novocastra	T cell (thymic)
CD2	AB75	Novocastra	T cell (thymic, NK)
CD3	F7.2.38	DAKO	T cell (mature)
CD4	IF6	Ventana	T cell (helper)
CD5	4C7	Novocastra	T cell (mature)
CD7	272	Novocastra	T cell (thymic)
CD8	C8/144B	DAKO	T cell (cytotoxic)
CD10	56C6	Novocastra	LPC, GC B cell
CD15	MMA	BD	Granulocytes, monocytes
CD20	L26	DAKO	Pan B cell (except PC)
CD21	1F8	DAKO	B cell (subset), FDC
CD23	IB12	Novocastra	B cell (subset), FDC, LC
CD30	BerH2	DAKO	Activated B/T cell, HC
CD31	JC/70A	DAKO	EC, Platelets
CD34	QB-END/10	BMA	Hemopoietic SC, EC
CD43	MT1	Novocastra	T cell, myeloid cells
CD45 LCA	X16/99	Novocastra	Panleukocyte
CD56	IB6	Novocastra	T cell (NK, MM)
CD61	Y2/51	DAKO	Platelets, Megakaryocytes, EC
CD68	PGM-1	DAKO	Monocytes, LC, Macrophages
CD68	KP1	DAKO	Monocytes, Macrophages, myeloid cells
CD79a	HM57	DAKO	B cell, PC
CD117	c-kit	DAKO	SC, mast cells, Melanoc
CD138	MI15(4)	DAKO	Plasma cells
ALK 1	ALK1(2)	DAKO	Anaplastic lymphoma kinase (ALCL)
BCL-2	124(3)	DAKO	Lymphocytes (subset)
BCL-6	P1F6	Novocastra	GC, activated B cells
Cyclin D1	SP4	DC Systems	Cell cycle (MCL, MM)
DBA.44	DBA.44	DAKO	Hairy cells, B cell
Granzyme B	GrB-7	DAKO	T cell (cytotoxic)
Glycophorin C	Ret40f	DAKO	Erythroblasts, erythrocytes
Lysozyme	Polyclonal	DAKO	Monocytes
Mast cell tryptase	AA1	DAKO	Mast cells
MUM-1	MUM-1p	DAKO	PC, post-GC B cells
Myeloperoxidase	MPO-7	DAKO	Myeloid cells
PAX5	Polyclonal	BD	HC, B-cell transcription factor
p27	F8	Santa Cruz	Cell cycle
TDT	polyclonal	Zytomed	Immature B/T cells
TIA-1	2G9	Immunotech	T cell (cytotoxic)
VS38c	VS38c	DAKO	Plasma cells
kappa	R10-21-F3	DAKO	Light chain
lambda	N10/2	DAKO	Light chain
IgG	7701	BioGenex	Immunoglobulin class G
IgA	NIF2	BioGenex	Immunoglobulin class A
IgM	R1/69	DAKO	Immunoglobulin class M
IgD	Polyclonal	DAKO	Immunoglobulin class D

The most frequently used antibodies are highlighted
Abbreviations: *NK* Natural killer, *LPC* lymphoid progenitor cells, *GC* germinal center cells, *PC* plasma cells, *FDC* follicular dendritic cells, *LC* Langerhans cells, *HC* Hodgkin cells, *EC* endothelial cells, *SC* stem cells, *MM* multiple myeloma, *ALCL* anaplastic large-cell lymphoma, *MCL* mantle cell lymphoma

unnecessary stains and misinterpretations, is outlined in a flow chart (Fig. 1).

In cases with nodular or interstitial centrally located lymphoid infiltrates composed of small cells, the differential diagnoses mainly encompass B-CLL/SLL, MCL, and

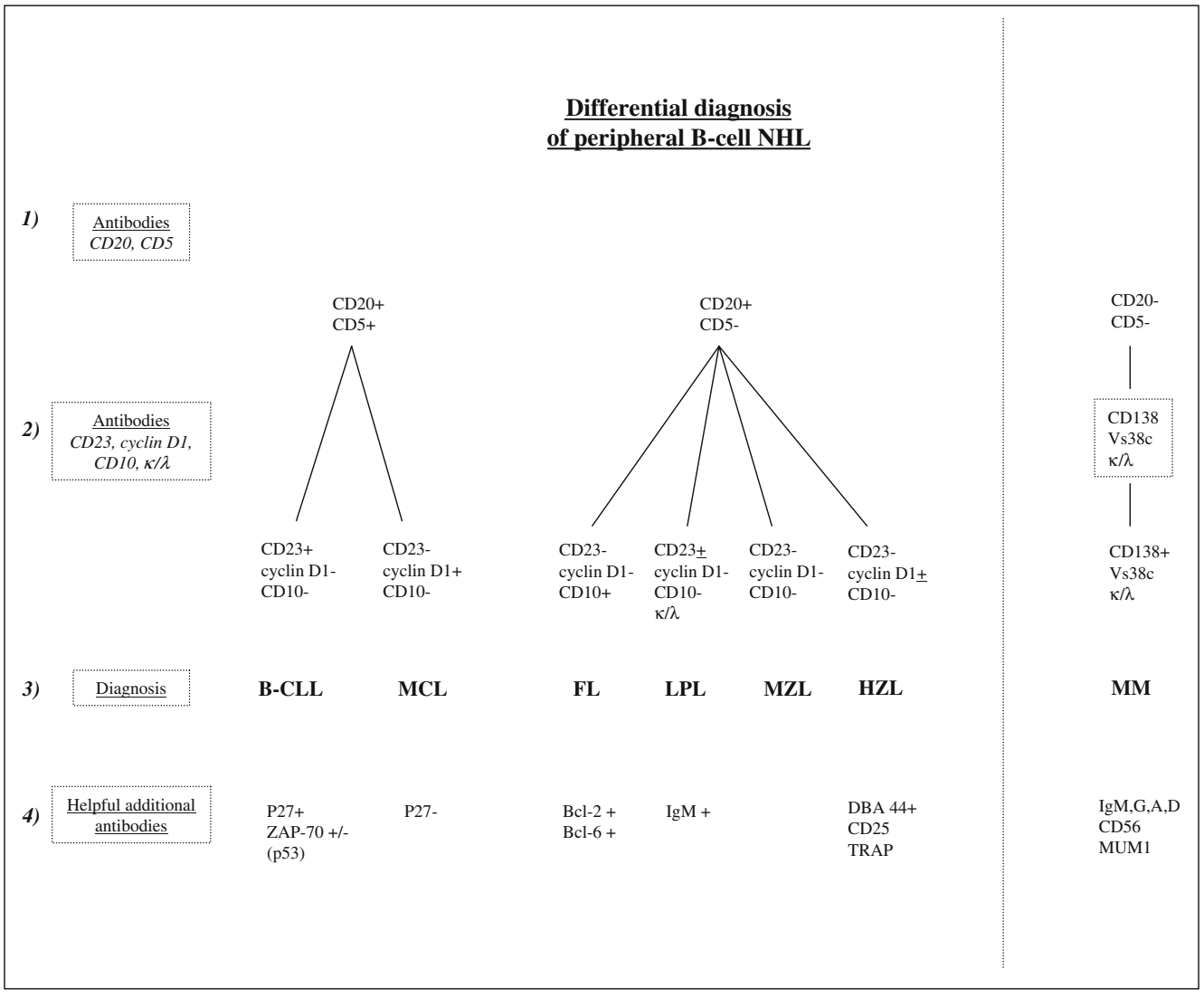

Fig. 1 Flow chart for the differential diagnosis of mature B-cell non-Hodgkin's lymphoma. If a low-grade B-cell NHL or an MM is suspected, we recommend a "first-line" staining with two basic antibodies, CD20 and CD5 (*1*). In cases of CD20-positive infiltrates, an array of antibodies consisting of CD23, cyclin D1, CD10, and possibly light chains, kappa, and lambda is required for further analysis (*2*). In most cases, these stains will lead to identification of the most frequent B-cell NHL, affecting the BM (*3*). Additional stainings for each entity, including optional prognostic markers such as ZAP-70 or p53 as shown at the baseline (*4*), conclude the immunohistochemical analysis of mature B-cell NHL. If MM is suspected, or the "first-line" immunostaining results lead to a CD20- and CD5-negative cell population; CD138, Vs38c, and light chains should be added in a second step for the diagnosis of MM. Further stains with antibodies against IgM, G, A, D, CD56, and MUM1 may help in ambiguous cases. Note the exception of cyclin D1- and CD20-positive lymhoplasmacytic MM as shown in Fig. 3D,E

MZL. In these cases, CD20 or CD5 are the most informative markers. The simultaneous expression of CD5 and CD20 helps to narrow down the differential diagnosis to mainly B-CLL and MCL, since both lymphomas express CD5 in more than 90% of cases [22, 45, 145, 157]. The choice of further antibodies depends on the main differential diagnoses and the need for additional prognostic information. Of practical importance in patients with B-NHL is the downregulation of CD20 antigen expression occasionally observed after treatment with anti CD20 antibodies (rituximab) [81]. In doubtful cases with nodular lymphoid aggregates after therapy, other B-cell antigens such as CD79a can serve to discriminate residual B-cell infiltrates from CD3+ reactive T-cell infiltrates.

B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma

B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma is one of the most frequently encountered B-cell neoplasms in BM trephines. The phenotype of B-CLL is characterized by coexpression of CD5, CD20, and CD23, and these three markers are usually sufficient for diagnosis (Fig. 2A,B). Of note, the staining intensity of CD20 is almost

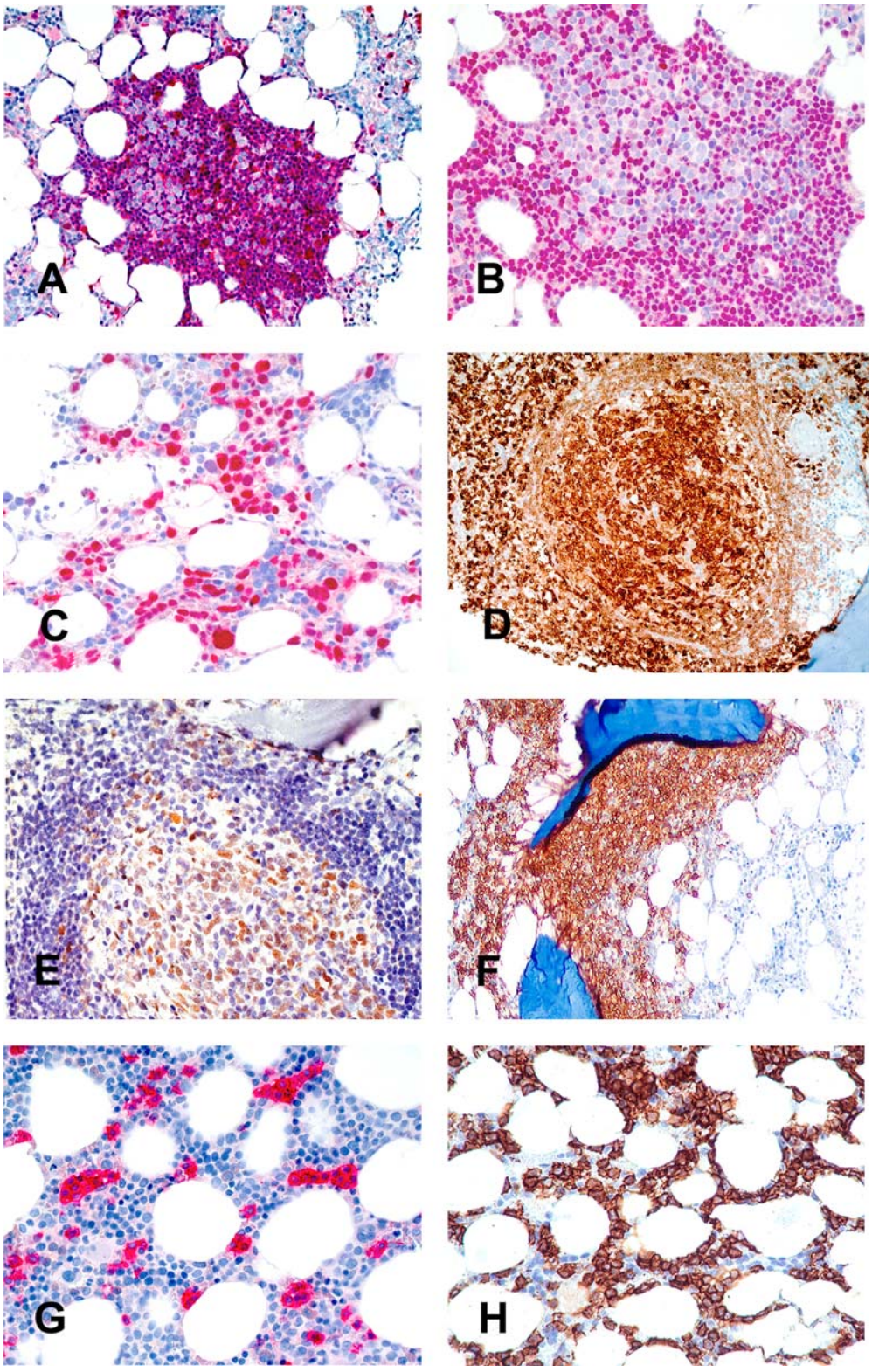

Fig. 2 Immunostainings of small B-cell NHL in the BM. **A** CD5 staining of a characteristic nodular B-CLL infiltrate. The neoplastic cells exhibit a relatively faint staining, whereas the "reactive" intermingled T cells show a strong immunostaining with CD5. Of note is the sharp border between unaffected BM cells and the neoplastic infiltrate (×200 ABC-AP). **B** $P27^{Kip1}$ staining of the same B-CLL case, with a strong immunoreactivity of the small lymphocytes. The intermingled "paraimmunoblasts" are negative for p27 (×300, ABC-AP). **C** Cyclin D1 reactivity of a MCL, pleomorphic variant. Note the interstitial infiltration pattern in this case (×400 ABC-AP). **D**, **E** These microphotographs represent an exceptional case of an FL with true neoplastic germinal centers. The tumor cells are positive for Bcl-2 (**D**) and Bcl-6 (**E**) (×200 ABC-POX, ×300 ABC-AP). Microphotograph (**F**) shows the more common peritrabecular infiltration pattern of an FL (CD20+) (×300, ABC-POX). **G** SMZL with characteristic intrasinusoidal infiltration stained for CD20. This pattern of infiltration is easily overlooked in routine stains (×200, ABC-AP). **H** HCL with characteristically strong membranous CD20 staining. (×400 ABC-POX)

always weaker than in other B-NHL [117]. The expression of CD23 in most cases is helpful, especially to rule out the diagnosis of MCL [90]. The coexpression of CD20 and CD5 is a universal finding in B-CLL. Still, there are cases of small infiltrates with large numbers of reactive T cells where comparison of CD3 and CD5 staining may be necessary to determine the presence or absence of CD5 coexpression by the B-cell population. A feature that can be very helpful is the double intensity of positivity of CD5. Reactive T cells are strongly positive for CD5, whereas the neoplastic B lymphocytes show a weaker reactivity (Fig. 2A).

In the last few years, the systematic analysis of gene expression profiling have led to the discovery of new diagnostic and prognostic markers. One of these markers is the zeta-chain-associated protein 70 (ZAP-70), which is normally expressed in T cells and was found to be highly expressed in a subgroup of B-CLL cases [165]. This finding was confirmed by flow cytometry, where the expression of ZAP-70 was found in almost 100% of patients with nonmutated immunoglobulin heavy chain (IgH) genes and only in 10% of patients with mutated IgH genes. The mutational status of the IgH gene is a strong predictor of survival in B-CLL [136]. Of interest, ZAP-70 is not only a surrogate marker for the mutational status but also seems to have independent predictive value and may provide a cheaper and easier way to obtain prognostic information [27, 136, 165]. ZAP-70 can also be detected by paraffin section immunohistochemistry, thus potentially making it an excellent prognostic marker for routine diagnostics. P53 protein and CD38 represent two other prognostic markers which can be assessed by immunostaining [102, 136]. Whether immunohistochemical demonstration of ZAP-70 and CD38 in BM biopsies renders equal prognostic information as their determination by flow cytometry remains to be shown. Worth mentioning in this context is the so-called secretory B-CLL with a detectable monoclonal paraprotein in the serum and cytoplasmic light chain expression, which is found in approximately 5% of otherwise typical cases. These cases also express CD5 and CD23, distinguishing them from LPL (see below).

B-prolymphocytic leukemia

B-prolymphocytic leukemia (B-PLL) is a rare disease defined mainly by clinical and laboratory features and shows high lymphocyte counts in the peripheral blood with more than 55% prolymphocytes, splenomegaly, and BM infiltration. The neoplastic cells are larger than lymphocytes of classical B-CLL. They typically lack CD23 and show CD5 only in a minority of cases. Cases of B-PLL carrying a t(11;14)(q13;q32) translocation as described in the earlier literature will nowadays be regarded as leukemic MCL by most investigators [128]. Further investigations will have to demonstrate whether the remaining cases of B-PLL represent a true entity or just variants of other leukemic B-NHLs.

Mantle cell lymphoma

The main differential diagnosis of CLL/SLL in the BM is MCL. MCL is characterized by the t(11;14)(q13;q32) translocation, resulting in aberrant expression of cyclin D1, which is the hallmark of the diagnosis [19, 25, 115]. MCL have a very characteristic phenotype, cyclin D1+, CD20+, CD5+, CD10−, and CD23−. Nevertheless, in the past, the inconsistency of the cyclin D1 staining in BM biopsies often precluded an accurate diagnosis. This problem seems to have been overcome with the introduction of the new monoclonal rabbit antibody against cyclin D1 (clone SP4, DC System, Germany), which gives a reliable and strong staining. Another useful marker for the diagnosis of MCL is the anti-$p27^{Kip1}$ antibody [147]. The lack of $p27^{Kip1}$ protein expression is a characteristic feature of MCL, which is not found in other small B-cell NHL. The lack of p27 staining in a small B-cell infiltrate in the BM is highly suspicious of the diagnosis of MCL [86, 123, 132]. A word of caution, since T cells are strongly positive for p27, the parallel assessment of the number of reactive T cells within the neoplastic infiltrate is important to avoid misinterpretation of staining results. As mentioned above, CD23 is very helpful in distinguishing MCL from B-CLL, although one has to keep in mind that rare cases of bona fide MCL have been reported to express CD23. Another potential problem in the differential diagnosis of MCL is the more aggressive blastic and pleomorphic variants. Aggressive MCLs show a spectrum of cytological appearances ranging from lymphoblast-like cells to large pleomorphic cells mimicking large-cell lymphomas (Fig. 2C). Similar to the classic variant of MCL, the blastic variant shows focal infiltration in the BM, a useful feature in the differential diagnosis of lymphoblastic leukemia/lymphoma that tends to infiltrate in a diffuse pattern. Immunohistochemically, blastic variants of MCL can be separated from lymphoblastic leukemia/lymphoma by their strong staining for CD20 and cyclin D1 and the lack of terminal desoxyribonucleotidyl transferase (TdT) and/or CD3. P53 overexpression and high proliferation rate in MCL are associated with the cytologically more aggressive blastic and pleomorphic variants of MCL and a worse outcome [25, 64, 66, 71, 73, 114, 123, 146].

Follicular lymphoma

Follicular lymphoma shows a characteristic infiltration pattern in the BM with peritrabecular rather than centrally located infiltrates with significant lesional fibrosis. This allows a definite diagnosis of FL by morphology in many instances. Immunostainings are useful in the presence of extensive diffuse infiltrates and in minimal paratrabecular lesions, which are hard to spot in routine stains. The phenotype of FL in the BM is virtually identical to its nodal counterpart. The tumor cells express antigens of germinal center cells, including CD10, Bcl-6, and Bcl-2. Rare cases express CD43, and even less commonly, CD5 [44]. CD10 also marks BM stromal cells and immature B cells and may be difficult to interpret in small infiltrates. A meshwork of follicular dendritic cells can be demonstrated in larger infiltrates with stains against CD21 and CD23. Rare cases of FL show centrally located neoplastic follicles with mantle zones, imitating reactive follicles [151]. In these cases, demonstration of bcl-2 positivity in the follicle centers is necessary to confirm malignancy even in patients with known FL, since most true BM follicles are of a reactive nature (Fig. 2D–F). Similar to FL in lymph nodes, BM infiltrates of FL frequently contain high numbers of intermingled “reactive” T cells within the neoplastic in-

filtrate, which should not discourage from a diagnosis of lymphoma, if the other diagnostic criteria are met.

In contrast to nodal FL, grading of FL in the BM has not been studied systematically to date. However, many cases of FL grade 3a/3b and even diffuse large-cell lymphoma show a predominance of small lymphoid cells in the BM, frequently with typical paratrabecular infiltration, also referred to as discordant BM infiltration. This phenomenon seems to be associated with a better clinical outcome than concordant infiltration by large-cell lymphoma. Discordant BM infiltration should not be confused with those cases where the large B-cell component is obscured by small reactive T cells. In these cases, immunostaining highlights the presence of malignant large B cells immersed in a sea of reactive T lymphocytes [88]. Approximately one to two thirds of patients with FL transform into large B-cell lymphoma during the course of their illness [38, 104, 105, 168].

Lymphoplasmacytic lymphoma

Lymphoplasmacytic lymphoma is a rare disease, infiltrating the BM in approximately 80% of patients at presentation and virtually all patients during the clinical course, usually with an interstitial and diffuse pattern [4, 69]. LPL in the WHO classification is a diagnosis of exclusion and is, in most cases, associated with the clinical syndrome of Waldenstrom's macroglobulinemia. Immunohistochemically, CD20 and CD79a are strongly positive, with an occasionally observed coexpression of CD43 in 20% of the cases [3, 92]. Immunoglobulin heavy (typically IgM, sometimes IgG, and rarely IgA) and light chains are virtually always demonstrable as cytoplasmic staining in paraffin sections [22, 45, 69, 70, 145, 157]. By definition, CD5 is negative. Most cases of the so-called lymphoplasmacytoid variant of immunocytoma recognized in the Kiel classification, which frequently expressed CD5, are regarded as variants of B-CLL/SLL with cytoplasmic immunoglobulin in the new WHO classification. The plasmacytoid differentiation of LPL can be easily confused with other ML showing lymphoplasmacytic or plasmacytic differentiation such as MZL, B-CLL, and multiple myeloma (MM) with lymphoplasmacytic morphology. The lymphoplasmacytic or small-cell variant of MM is frequently CD20+, adding to the potential for misdiagnosis (see below).

Nodal and extranodal (MALT-type) marginal zone B-cell lymphoma

Marginal zone B-cell lymphoma has to be considered in the differential diagnosis of CD20+ and CD5− nodular BM infiltrates [26, 51, 70]. The incidence of BM involvement in extranodal MZL is lower (~20%) than in other low-grade B-NHL, albeit recent studies described BM infiltration in up to 44% of cases [51, 83, 94, 120, 124, 149]. For nodal MZL, the available data are sparse and contradictory, with the incidence of BM infiltration ranging from 35 to 77% [11, 17, 83]. The tumor cells of both nodal and extranodal MZL express CD20, CD79a, and, in a subset of cases, CD43 [24, 92]. They are usually CD5- and CD23-negative, although rare CD5-positive MZL have been reported, potentially complicating the differential diagnosis with MCL and CLL/SLL [11, 12, 51].

Splenic marginal zone lymphoma

In contrast to nodal and extranodal MZL, BM involvement in SMZL is frequent and often shows a characteristic distribution pattern which is of great help in establishing the diagnosis. A significant percentage of SMZL show circulating B cells with small cytoplasmic projections, reflected in their previous designation as splenic lymphoma with villous lymphocytes [8, 57]. Although different patterns of infiltration can occur, the presence of intrasinusoidal tumor cells is highly characteristic for this entity. Intrasinusoidal spread may be found in the absence of obvious lymphoid nodules and can be so subtle that it is frequently overlooked in conventional stains [56, 57, 91]. Therefore, immunostainings are mandatory if SMZL is suspected (Fig. 2G). The neoplastic cells show positivity for CD20, CD79a, PAX5, Bcl-2, and express surface IgM and IgD. They are consistently negative for CD23, CD10, Bcl-6, cyclin D1, and CD43 [41, 56, 57, 108, 155]. The clinical course of SMZL is relatively indolent. A recent study documented clinical progression in 13% of cases, histologically accompanied by an increased number of blasts in the BM. Transformation usually correlates with a higher proliferation index and p53 overexpression [24, 41, 98].

An important diagnostic pitfall is persistent polyclonal B lymphocytosis with binucleated lymphocytes, a benign lymphoproliferation almost exclusively observed in female smokers, which shows an increase in intravascular B cells in the BM closely resembling the highly characteristic intrasinusoidal infiltration pattern by SMZL. Correlation with clinicopathologic findings, careful cytologic examination, and even molecular analysis may be required to avoid overdiagnosis of ML in these cases [39, 52, 154].

Hairy cell leukemia

Hairy cell leukemia involves the BM in almost all patients at the time of diagnosis. The morphology is highly characteristic, showing a patchy infiltration of widely spaced cells with oval nuclei and ample, clear cytoplasm, with progressive replacement of normal hematopoetic cells and increased reticulin fibers [137, 150]. HCL shows very strong staining for CD20, and staining of the cytoplasmic processes of the neoplastic cells may give them a "hairy" appearance (Fig. 2H). In contrast to other small-cell NHL, the combined positivity of DBA44 and tartrate-resistant acid phosphatase (TRAP) is highly suggestive for HCL. Another useful HCL marker is CD25, the interleukin (IL)-2 receptor. Very recently, Falini and coworkers described a promising new HCL-specific antibody against annexin 1

(ANXA1) suitable for paraffin [50, 163]. SMZL represents the main differential diagnosis for HCL. SMZL may also express DBA44 and TRAP, albeit the staining intensity in SMZL is generally weak [68, 78, 127, 131]. In contrast to SMZL, a significant subset of HCL shows staining for cyclin D1 in variable intensity accompanied by a reduction in p27 staining, which should not pose any problem with the differential diagnosis of MCL [86]. An important role of immunohistochemistry in HCL is the detection and quantification of residual disease. With the availability of highly efficient drugs such as 2-CDA, complete remission is frequently obtained, and histological and immunohistochemical criteria for response evaluation in the BM trephine have been defined [67].

Immunosecretory disorders: monoclonal gammopathy of undetermined significance and multiple myeloma

Immunosecretory disorders such as monoclonal gammopathy of undetermined significance (MGUS), MM, and Waldenstrom's macroglobulinemia represent a frequent indication for BM trephine examination. MM is the second most frequent B-cell tumor in Western countries and accounts for approximately 10% of all hematologic neoplasms [74, 139]. In most cases with significant infiltration, MM is a straightforward diagnosis. BM infiltration in MM is highly variable and can range from randomly distributed sheets or clusters of abnormal plasma cells to large nodules or complete obliteration of the marrow space. Immunohistochemistry is of importance for the diagnostic workup of MM in several settings, namely, (1) discrimination from MGUS, (2) identification of small numbers of tumor cells after therapy, (3) distinction from other neoplasms in morphologically unusual cases, and (4) for the identification of prognostic markers. Given the close biological relatedness of MGUS and MM, a distinction of these two disorders is somehow arbitrary in the presence of a non-tumor-forming plasmacytosis without cytologic atypia, but the presence of a clear-cut light chain restriction with a kappa/lambda ratio of more than 16:1 or the reverse and more than 10% plasma cells are suspicious for a diagnosis of MM (Fig. 3A–C) [54]. Since well-differentiated plasmacytomas can be cytologically indistinguishable from normal plasma cells, the finding of plasma cells in sheets or clusters without association with vascular structures is another important criterion for the diagnosis of MM. Immunophenotypically, neoplastic plasma cells lack in most cases the pan-B-cell antigen CD 20, whereas CD38, CD79a, the collagen-1 binding proteoglycan, syndecan (CD138), and VS38c are usually strongly expressed. The latter two markers are well suited for plasma cell enumeration in trephine biopsies. The neural adhesion molecule CD56 is abnormally expressed in approximately 75% of cases of MM and is frequently associated with lytic bone lesions. In contrast, CD56 is absent both from polyclonal plasma cells and other mature B-NHL with plasmacytic differentiation [46, 65, 87]. Together with CD138, CD56 is thought to be important for plasma-cell anchoring in the BM. Immunoglobulin heavy chains are frequently expressed, with IgG more common than IgA. IgM expression is rare in MM and usually indicates LPL. Occasionally, MM expresses aberrant antigens such as CD10 or even myeloid markers, but their importance in terms of prognosis remains unclear [47, 107]. Although currently not specified in the WHO classification, the lymphoplasmacytic or small-cell variant of MM mentioned above has some specific features such as more frequent secretion of rare Ig isotypes (IgE and IgM), common CD20 positivity, and a high frequency of the t(11;14) translocation associated with strong nuclear cyclin D1 protein expression (Fig. 3D,E). These cases represent a potential diagnostic pitfall because MCL may be suspected based on morphological and phenotypical findings. Prognostically, this MM variant seems to have a better prognosis, possibly due to a paradoxically low proliferation rate [9, 53, 122, 126, 142, 166]. Another subset of MM shows weaker, heterogeneous cyclin D1 expression frequently associated with trisomy 11.

High-grade B-cell lymphomas

Diffuse large B-cell lymphoma

Diffuse large B-cell lymphoma has a relatively low incidence of BM involvement at time of diagnosis (20%) [2, 35, 82, 109, 111, 116, 156]. Apart from discordant involvement (see above), large-cell BM infiltrates in DLBCL are highly variable, ranging from minimal small foci rarely observed intrasinusoidal involvement to subtotal replacement of the BM [111]. In cases of a minimal infiltration, immunostainings facilitate the search for tumor cells, which express CD20 and CD79a in most cases, and in up to 50% CD10, Bcl-2, and Bcl-6. Primary DLBCL expressing CD5 have to be distinguished from the blastic variant of MCL as well as Richter's transformation of B-CLL/SLL [167]. Whereas cyclin D1 positivity helps to distinguish blastic MCL, separation of secondary de novo DLBCL from clonally related Richter's transformation in patients with B-CLL may be impossible by immunohistochemistry. AML M6 is another, albeit rather infrequent differential diagnosis of DLBCL. Stainings for gylocphorin A or hemoglobin A are helpful in these cases, but immature erythroblasts can fail to show immunoreactivity for glycophorin A, whereas myeloid markers such as CD117 and MPO are variably expressed [59, 96].

Bone marrow involvement by the T-cell/histiocyte-rich variant of DLBCL requires special attention, since it resembles BM involvement by Hodgkin's lymphoma (HL) morphologically, due to its high number of reactive T cells and histiocytes and the resemblance of the rare neoplastic cells to L&H or Reed–Sternberg (RS) cells [30]. The tumor cells are usually CD20- and epithelial membrane antigen (EMA)-positive but lack CD30 and CD15. Nodular lymphocyte-predominant HL (nodular paragranuloma)

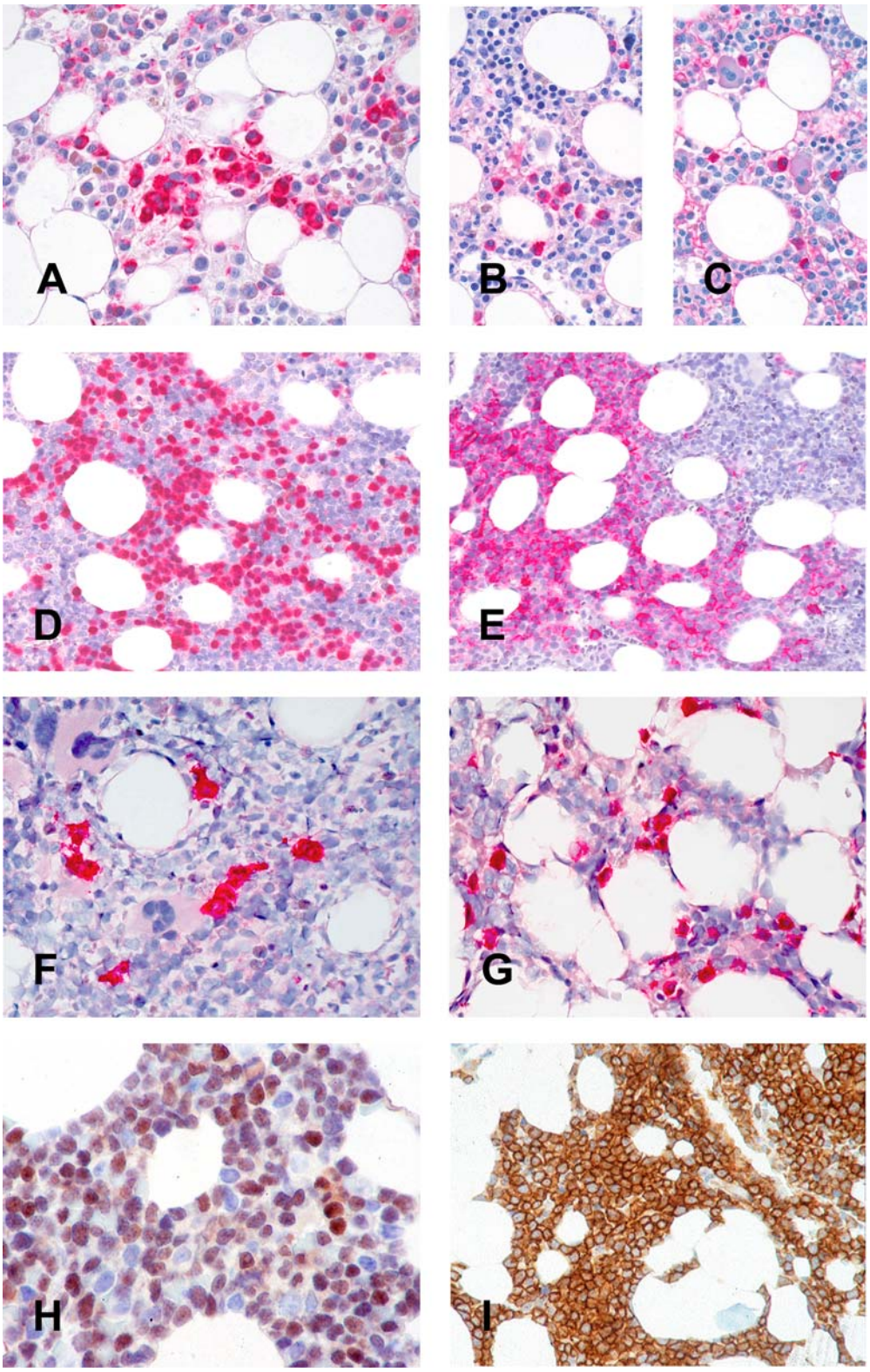

Fig. 3 Immunostainings of immunosecretory disorders, T-cell NHL, and acute lymphoblastic leukemia/lymphoblastic lymphoma. **A**–**C** Microphotographs showing a case with marked reactive BM plasmacytosis without evidence of light chain restriction. Staining for Vs38c (**A**) demonstrate the mainly perivascularly located clusters of plasma cells. There is a polyclonal distribution of immunoglobulin light-chains kappa (**B**) and lambda (**C**) (×300, ×200 ABC-AP). **D**, **E** t(11;14) positive MM with characteristic lymphoplasmacytic morphology. Note the strong cyclin D1 staining intensity with the recently introduced antibody (clone SP4, DC System, Germany) in **D**. The tumor cells in this MM subtype are regularly CD20-positive (**E**) (×300 ABC-AP). **F** BM involvement by ALCL with scattered strongly CD30-positive tumor cells. (×300 ABC-AP). **G** T-LGL with a morphologically inconspicuous interstitial infiltrate of CD8-positive T lymphocytes (×300 ABC-AP). **H**, **I** T-ALL/T-LBL. TdT shows strong homogeneous nuclear reactivity (**H**; ×400 ABC-POX). The tumor cells express the pan-T-cell marker CD7 (**I**) (×400 ABC-POX)

shows an immunophenotype similar to T-cell/histiocyte-rich DLBCL, but BM involvement, especially at primary presentation, is rare [84].

Burkitt's lymphoma

Burkitt's lymphoma (BL) involves the BM in up to 60% of cases. The pattern is nearly always diffuse or interstitial, and the extent of involvement varies from occasional

scattered lymphoma cells to complete replacement of the marrow [23, 144]. Areas of necrosis are observed frequently. Immunohistochemically, BL cells have a germinal center cell phenotype with expression of CD20, CD79a, CD10, Bcl-6, and CD43 but with constant absence of bcl-2. The proliferative activity, assessed with MIB1, should be virtually 100%. In contrast to lymphoblastic lymphoma (LBL)/acute lymphoblastic leukemia (ALL), BL lacks TdT [16, 20, 33].

Peripheral T-cell non-Hodgkin lymphoma

General considerations

The histopathology of BM involvement by T-cell non-Hodgkin lymphoma (T-NHL) has recently been reviewed comprehensively by Dogan and Morice [43]. T-NHL are rare diseases accounting for approximately 15% of all lymphoproliferative disorders in the Western world, with peripheral T-cell lymphoma (PTCL) unspecified, angioimmunoblastic T-cell lymphoma (AITL), cutaneous T-cell lymphomas, and anaplastic large-cell lymphoma (ALCL) representing the most common entities [129]. The frequency and the histology of BM involvement vary greatly between the different entities. Diagnosis of BM infiltration is hampered by frequent secondary changes in the BM microenvironment induced directly or indirectly by the lymphoma cells [49]. Some PTCL such as AITL show a vaguely nodular or interstitial infiltration pattern, whereas others, like large granular lymphocyte leukemia and hepatosplenic T-cell lymphoma (HSTCL), are characterized by a predominantly intrasinusoidal involvement [29, 31, 58, 61, 63, 99]. Immunophenotyping reveals an aberrant T-cell phenotype with considerable variations, mostly with the absence of one or more pan T-cell antigens. In T-cell NHL, in general, immunostainings are helpful in identifying the frequently subtle infiltrates and should be performed whenever possible.

Anaplastic large-cell lymphoma

Bone marrow involvement in ALCL is seen in about one quarter of the systemic cases but, as a rule, is absent in the cutaneous form. Most systemic cases have translocations involving the ALCL kinase-1 (ALK-1) gene, resulting in nuclear and/or cytoplasmic staining for ALK-1 protein [93, 143]. BM infiltration is usually interstitial and often too subtle to be recognized in routinely stained sections. This is particularly the case for the small-cell variant of ALCL, in which the neoplastic cells can easily be confused with immature megakaryocytes [14, 55]. For this reason, immunostainings for CD30 and/or ALK-1 are mandatory to identify a minimal infiltration in BM biopsies (Fig. 3F). The great majority of ALCL express one or more T-cell antigens; however, some cases may have an apparent "null cell" phenotype but, nevertheless, show evidence for T-cell lineage at the genetic level [55, 143]. Classic HL is the main differential diagnosis of ALCL, although this issue will rarely be raised in a BM biopsy.

Angioimmunoblastic T-cell lymphoma

Bone marrow involvement is seen in the majority of the cases (50–80%) and often mimics the features seen in peripheral lymph nodes [61, 62]. The infiltration pattern may be focal (paratrabecular) or diffuse and is characterized by a patchy polymorphic infiltrate often accompanied by a proliferation of arborizing vessels and fibrosis. Immunophenotypically, the phenotype of the neoplastic cells is very difficult to define, similar to the situation in involved lymph nodes. AITL cells express CD3 and CD4, whereas cytotoxic markers are negative. Interestingly, the recently described CD10 reactivity of the neoplastic cells observable in peripheral lymph nodes is often absent in the tumor cells infiltrating the BM [6, 7]. The frequent presence of large, Epstein–Barr virus (EBV)-positive B cells in AITL, which occasionally can show the morphology of Hodgkin and RS cells, may lead to confusion with either large B-cell lymphoma or HL. Another important differential diagnosis of BM involvement by AITL are reactive infiltrates with granulomas.

T-cell granular lymphocytic leukemia and hepatosplenic T-cell lymphoma

T-cell granular lymphocytic leukemia (T-LGL) is an indolent lymphoproliferative disorder, characterized by a distinct clonal cytotoxic T-cell population in the peripheral blood, which is easily identified by flow cytometric immunophenotyping. However, in BM trephines, the neoplastic cells of T-LGL cannot be reliably identified by morphological assessment alone. Immunostainings with antibodies against CD3, CD8, TIA-1, and granzyme B reveal the neoplastic infiltrate present as small interstitial infiltrates or typically in marrow sinusoids (Fig. 3G).

An important differential diagnosis is a reactive increase in cytotoxic T cells, which usually lacks the relatively distinct intrasinusoidal and intravascular pattern observed in T-LGL [110]. Like T-LGL, HSTCL shows an intrasinusoidal and subtle interstitial infiltration pattern, which is difficult to recognize by morphology alone. HSTCL is usually positive for CD3 and TIA-1 and frequently expresses CD56, whereas CD4, CD8, granzyme B, and perforin are negative [15, 36, 162].

Hodgkin's lymphoma

The incidence of BM involvement in HL ranged between 5 and 30% according to older studies, but was found in only 5.2% of patients in a recent large series of HL [77]. The lowest incidence was reported in lymphocyte-predominant HL (<5–10%), and the highest incidence was reported in HL with lymphocyte depletion (50–75%). Of note, BM

infiltration is frequent in HIV-associated HL and may be the site of primary diagnosis [80]. The infiltration pattern may be diffuse or focal, occasionally occupying the entire trephine volume. The infiltrate consists of a polymorphous inflammatory background with histiocytes, eosinophils, lymphocytes, and plasma cells with an accompanying dense collagen fibrosis. Diagnostic RS cells and variants are rare and difficult to identify even if immunohistochemistry for CD15 and CD30 is employed. However, the presence of a polymorphous lymphohistiocytic infiltrate with fibrosis, without diagnostic cells, can be regarded as sufficient evidence for BM infiltration if a diagnosis of classical HL has been established at a nodal site. ALCL, PTCL, T-cell-rich B-cell lymphoma, mast cell diseases, granulomatous or histiocyte disorders, or even metastatic carcinoma are among the differential diagnoses. If encountered as primary diagnosis in the BM, an extended panel of antibodies, including CD20, PAX5, OCT2, BOB1, CD3, granzyme B, EMA, ALK-1, and pancytokeratin may be necessary. In contrast, the neoplastic cells in nodular lymphocyte-predominant HL are characteristically positive for CD20, raising the differential diagnosis of T-cell/histiocyte-rich DLBCL.

Lymphoblastic lymphoma/acute lymphoblastic leukemia

In BM biopsies, lymphoblasts are relatively uniform in appearance with round to oval nuclei. Nucleoli are generally inconspicuous, and the chromatin is typically finely dispersed. The infiltration pattern is almost always interstitial or diffuse. Characterization of LBL/ALL is best performed using flow cytometry and cytogenetics, but a number of markers suitable for trephines have become available recently. The most constant and reliable marker for LBL/ALL of both lineages is TdT, which is expressed in almost all cases of ALL (Fig. 3H). TdT usually shows strong nuclear staining of virtually all cells. TdT expression also occurs in a significant subset of acute myeloid leukemia (AML) but is usually less intense and not as uniform as in ALL/LBL. In most cases of ALL/LBL, assignment to either B- or T-cell lineage is possible. The most reliable marker for B-LBL/ALL is the B-cell transcription factor PAX5 followed by CD10 and CD79a, whereas CD20 is variably expressed [152]. Of note, CD79a is not absolutely lineage-specific in this context and may be present both in rare cases of T-ALL and in AML [5]. T-LBL/ALL can usually be confirmed by staining for a variety of T-cell antigens, including CD1a, CD3, CD4, CD5, CD7, and CD8, with CD7 being the earliest T-cell marker to appear (Fig. 3I). Coexpression of CD4 and CD8 can be found frequently and is diagnostic for T-LBL/ALL. The number and sequence of T-cell antigens present may be used for stratification according to maturation stage. Both B- and T-ALL/LBL can show an expression of CD34 and, less frequently, CD117. In rare biphenotypic acute leukemias, an aberrant coexpression of myeloid markers is observed [13, 89, 118, 153]. The differential diagnosis of ALL/LBL includes such diverse entities as AML, BL, blastoid MCL, reactive BM with increased CD10+ hematogones, and even nonhematopoietic tumors such as Ewing sarcoma or rhabdomyosarcoma. Of practical importance is the immunoreactivity of LBL for CD99 (MIC-2), which may lead to confusion with Ewing sarcoma [100, 169].

Myeloid disorders

Myelodysplastic syndromes/acute myeloid leukemias

The diagnosis of MDS rests largely on clinicopathologic and morphological (cytological) findings complemented by cytogenetics. However, immunohistochemistry provides a useful tool for accurate classification of MDS in BM trephines. The identification of abnormally located precursors for a diagnosis of refractory anemia with excess of blasts (RAEB) type 1 or 2 is easily achieved with antibodies against the hematopoietic stem cell antigen CD34 (Fig. 4A) [76, 112]. Several studies investigated CD117 (c-kit) as an additional marker for the characterization of blasts in MDS. However, in our experience, the application of CD117 is problematic, since this marker is also expressed on immature erythroid cells [79, 101, 112]. For the differentiation between hypoplastic MDS and aplastic anemia, CD34 staining can be helpful to identify small numbers of immature precursors, which are a strong indicator for hypoplastic MDS [113].

In AML, a panel of antibodies, including CD34, TdT, myeloperoxidase, CD68 (KP-1 and PG-M1), glycophorin A, CD61, CD20, CD79a, CD3, and CD1a, is a useful adjunct for the differentiation between AML and ALL and for a tentative subgrouping of AML according to the French–American–British Group (FAB) criteria as illustrated by Pileri and coworkers [118]. CD34, TdT, and lymphoid markers are necessary for discrimination of AML M0 (Fig. 4B,C) and ALL (B/T). AML M2 and M4 can be separated by the use of two CD68 clones: PG-M1 is restricted to AML with monocytic/monoblastic differentiation (M4 and M5), whereas KP-1 is additionally positive in myeloblasts (M2). The sometimes difficult distinction between promyelocytic leukemia (AML M3) and a reactive increase in promyelocytes can be resolved by staining for CD117, which is aberrantly expressed in malignant promyelocytes of AML M3 [28, 125]. CD61 and glycophorin A are helpful markers for the identification of acute megakaryoblastic leukemia (M7) or acute erythroid leukemia (M6) (Fig. 4D,E).

The very recently described mutations in the nucleophosmin (NPM) gene, leading to abnormal cytoplasmatic protein expression in karyotypically normal AML, can be demonstrated with anti-NPM antibodies. Nevertheless, the importance of this observation in terms of prognosis has to be elucidated [1, 48].

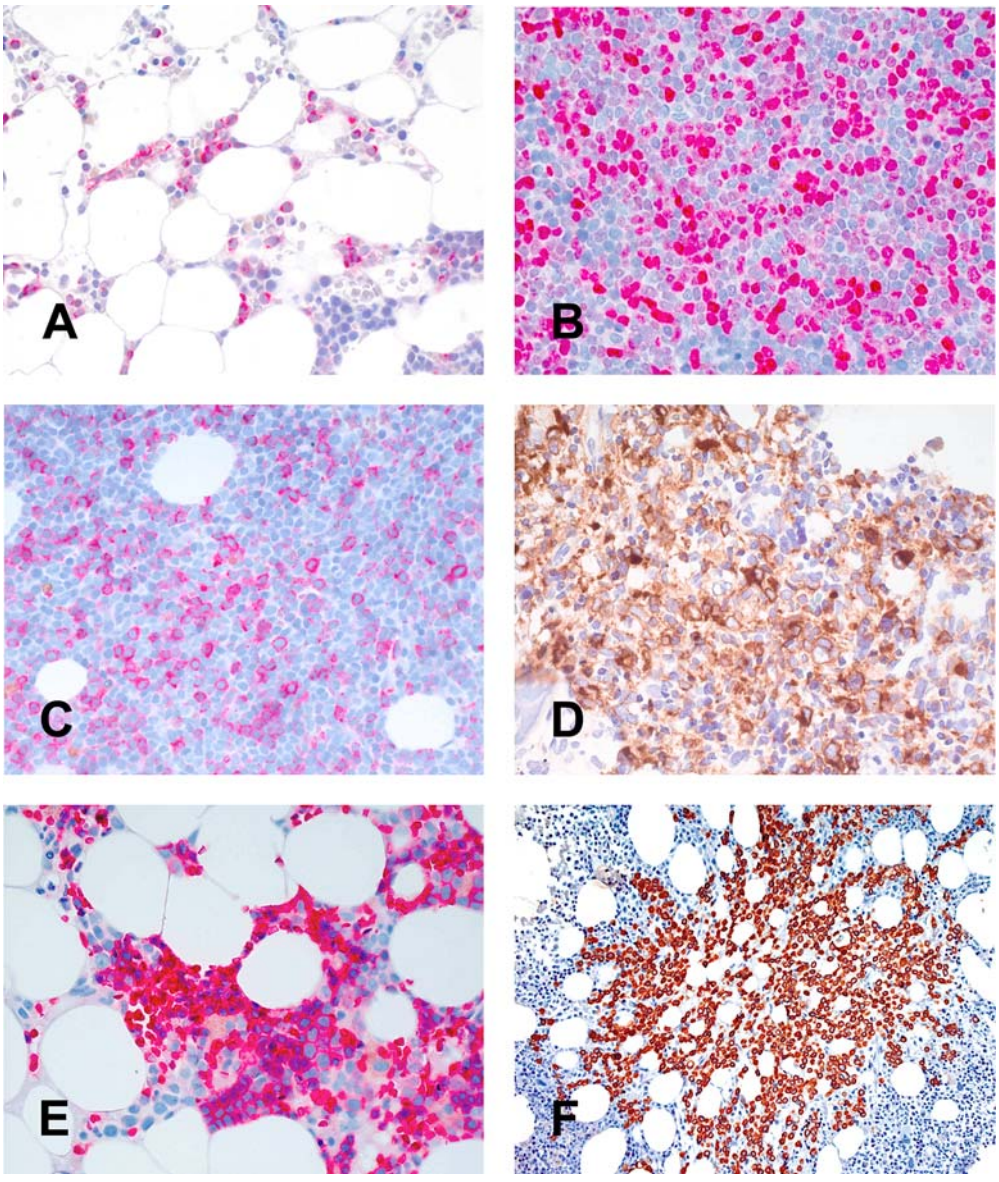

Fig. 4 Immunostainings of acute myeloid leukemias, myelodysplastic syndromes, and metastatic bone marrow disease. **A** Hypocellular MDS (RAEB-1 WHO). CD34 highlights the small interstitial aggregates of myeloblasts. The corresponding aspirate contained 9% blasts (×300 ABC-AP). **B**, **C** AML M0 (FAB) TdT (**A**) and CD34 staining (**B**) of a "packed marrow." Note the heterogeneous expression of TdT in the AML blasts in contrast to ALL (×300 ABC-AP). **D** AML M7 (acute megakaryoblastic leukemia) CD61 shows a strong cytoplasmic and membrane staining of cells of the megakaryocytic lineage. Note the mixture of small mononuclear megakaryoblasts and more differentiated cells (×300 ABC-POX). **E** Marked erythroid hyperplasia in a patient with a secondary erythrocytosis stained for glycophorin C. Maturation from erythroblasts to normoblasts is easily recognized (×300 ABC-AP). **F** BM infiltration by a lobular breast carcinoma. The infiltration by neoplastic epithelial cells is highlighted by pancytokeratin staining (×200 ABC-POX)

Chronic myeloproliferative syndromes

The diagnosis of chronic myeloproliferative syndromes such as chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), and idiopathic myelofibrosis (IMF) is generally based on a synopsis of clinical, morphological, and molecular data. However, immunostainings, including glycophorin A or C, hemoglobin, MPO, or CD61, are undoubtedly helpful for assessing the distribution and quantities of the different cell types and to identify morphologically abnormal forms such as micromegakaryocytes. In addition, CD34 is useful for determining the number of blasts present in the BM if an accelerated phase or acute transformation is suspected. Interestingly, mature megakaryocytes of MPS frequently show an aberrant cytoplasmatic CD34 expression, which can aid in the distinction from reactive processes.

Systemic mastocytosis

The BM is one of the major sites of involvement of systemic mastocytosis. The most common form of systemic mast cell disease is indolent systemic mastocytosis usually associated with cutaneous lesions. Due to the generally pronounced concomitant fibrosis in mast cell infiltrates, a BM trephine should always be performed. Although mast cells usually are easily demonstrated with 3-hydroxy-4′-nitro-2-naphthanilide chloroacetate (NASD) esterase or Giemsa stains, neoplastic mast cells may be poorly granulated, and occasionally, immunostains are required for their identification. They stain strongly for CD117 and mast cell tryptase [159, 160]. In addition, mast cells in systemic mastocytosis have been reported to aberrantly express CD2 and CD25 [141].

Metastatic disease

Bone marrow trephines generally exhibit the highest yield for detecting metastatic disease because neoplastic cells are frequently inaspirable due to concomitant fibrosis. Although metastatic solid tumors are rarely confused with hematopoetic neoplasms, immunostainings may be required for identification of the putative primary site. A diagnostic challenge, especially if minor involvement is suspected, are infiltrates of small blue round cell tumors, e.g., Ewing's sarcoma, or small-cell carcinoma. The primary antibody panel for these cases should at least contain anticytokeratin, CD99, CD56, and TdT.

The role of immunohistochemistry in reactive BM changes

In healthy adults, the mature lymphocyte compartment in the BM comprises less than 10% of nucleated cells and consist mainly of T lymphocytes (CD 3+ and CD5+) and, to a lesser extent, B lymphocytes (CD20+) [148]. However, lymphoid aggregates are commonly seen especially in the elderly or in patients with autoimmune disease. Distinction between benign nodular lymphoid aggregates and infiltrates of ML remains a difficult problem in BM histology and has been the subject of a number of studies [72, 85, 130, 134]. Benign nodules tend to have well-defined borders, are distributed at random in the marrow spaces, and have a more polymorphic cellular composition. True germinal centers can be seen occasionally. Although intralesional fibrosis, a typical feature of neoplastic infiltrates, is usually absent or mild in reactive lymphoid nodules, this feature lacks specificity. Immunohistochemically, benign lymphoid infiltrates show a polytypic staining pattern with a mixed population of B and T cells, with the latter predominating. An analysis of a light chain restriction is useful in cases with increased plasma cells. As outlined above, identification of an aberrant immunophenotype such as CD5 coexpression allows identification of early nodular involvement by B-CLL or MCL, but this feature is not present in other subtypes of B-NHL. Although most cases will be resolved by morphological and immunohistochemical studies, ambiguous cases may require molecular analysis [85]. Another important reactive condition, which may require immunostaining for its identification as mentioned above, are increased numbers of hematogones. Hematogones are immature B-cell precursors at various stages of maturation and may cause diagnostic problems when present in great numbers such as in regenerative marrow postchemotherapy or in children. In contrast to LBL/ALL, they show a range of maturation with variable expression of TdT, CD34, CD10, CD20, and/or CD79 and lack clonal antigen receptor gene rearrangements. Usually, mature forms already lacking TdT expression predominate [140].

Immunohistochemistry may also aid in the identification of infectious agents especially in immunosuppressed hosts. One of the agents worth mentioning in this context is human Parvovirus B19, which causes aplastic crisis and also the failure of nonerythroid lineages. Although B19 infections exhibit a typical morphology, with giant erythroblasts containing viral inclusions and arrest of erythroid maturation, immunostainings using a monoclonal antibody against the virus capsid protein (R92F6) can facilitate the detection [97].

Acute hemophagocytic syndrome is an important and distinctive manifestation of acute viral/bacterial infections (i.e., EBV, Parvovirus B19, hepatitis virus, and mycobacteria) especially in immunocompromised patients. The BM is characterized by an overall hematopoietic hypocellularity, variable reticulin fibrosis, and pronounced histiocytic hyperplasia, which can be highlighted by immunostainings with CD68. Highly characteristic are ingested mature and immature BM cells in the cytoplasm of histiocytes, giving them a "sack-like" appearance. The differential diagnosis includes acute monocytic leukemia and a paraneoplastic hemophagocytic syndrome in patients with peripheral T-cell NHL [75, 138, 158].

Bone marrow granulomas are encountered in both infectious and noninfectious conditions. They consist of cohesive clusters of histiocytes, lymphocytes, and occasionally, eosinophils. Immunohistochemistry can aid in the identification of microorganisms and in differentiating granulomas in the setting of ML, mastoocytosis, or reactive disorders [40].

Conclusion

Immunohistochemistry is a powerful auxiliary technique for the diagnosis of hematologic disorders in BM trephine biopsies. If standard fixation and decalcification procedures are employed, primary antibodies suitable for paraffin-embedded tissues can be applied to BM trephines without restrictions. An accurate characterization and primary classification of lymphoid neoplasms is possible in the majority of cases and expands the diagnostic scope of BM histology. Moreover, diagnostic algorithms provide a rational, fast, and cost-effective diagnostic approach.

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