The bone marrow is one of the more common organs to be involved by tumours that metastasize via the bloodstream. In adults the tumours most often seen are carcinomas of the prostate gland, breast and lung, although any tumour that gives rise to blood-borne metastases may infiltrate the marrow [1,2]. In children neuroblastoma, rhabdomyosarcoma, Ewing’s sarcoma, other primitive neuro-ectodermal tumours (PNET) and retinoblastoma account for the majority of metastases [3,4]. Bone marrow metastases from squamous cell carcinoma, other than that of the lung, and from soft tissue tumours of adults are uncommon [1]. Intracranial tumours rarely metastasize outside the cranial vault. Of those cases reported with bone marrow involvement, glioblastoma multiforme has been the most frequent [5]; examples of metastatic medulloblastoma [6] and oligodendroglioma [7] have also been recorded.

Infiltration of the marrow may be suspected on the basis of: (i) bone pain; (ii) pathological fractures, lytic lesions or sclerotic lesions demonstrated radiologically; (iii) unexplained ‘hot spots’ on isotopic bone scans; (iv) hypercalcaemia or elevated serum alkaline phosphatase activity; or (v) unexplained haematological abnormalities. The haematological abnormality most suggestive of marrow infiltration, though not specific for it, is leuco-erythroblastic anaemia (see below). Metastases are also demonstrated occasionally when bone marrow examination is carried out for staging purposes in the absence of any features suggestive of bone marrow infiltration. Overall, the presence of leuco-erythroblastic anaemia is a relatively insensitive indication of infiltration since it is observed in less than half of patients in whom bone marrow metastases can be demonstrated by biopsy [8–10]. Aspirates and trephine biopsies are occasionally positive even when skeletal radiology and isotopic bone scans [8,11] are normal.

Considering the small volume of tissue sampled, both bone marrow aspiration and trephine biopsy are relatively sensitive techniques for detecting bone marrow infiltration by metastatic tumours. In two autopsy studies which simulated biopsy procedures, it was estimated that, when osseous metastases were present, a bone marrow aspirate would give positive results in 28% of cases [12] and a single trephine biopsy in 35–45% [13]. Trephine biopsy is more sensitive than bone marrow aspiration and sensitivity is increased by performing bilateral biopsies or by obtaining a single large biopsy. The sensitivity of aspiration is increased if large numbers of films are examined and if a clot section is also examined. It is common for tumour cells to be detectable in trephine biopsy sections when none are demonstrable in films of an aspirate [8,14]. Overall, about three quarters of metastases detected by a trephine biopsy are also detected by simultaneous bone marrow aspirate. Discrepancy between biopsy and aspirate findings usually results from a desmoplastic stromal reaction to the tumour which renders neoplastic cells more difficult to aspirate than residual haemopoietic cells. It is also, to some degree, a consequence of the different volumes of tissue sampled. Because of its greater sensitivity, trephine biopsy should always be performed when metastatic malignancy is suspected. However, tumour cells are seen occasionally in aspirate films when trephine biopsy sections appear normal [2,8,14] and the two procedures should therefore be regarded as complementary.

Increasingly, bone marrow aspiration and trephine biopsy are being performed as staging procedures at the time of diagnosis in a number of solid tumours, principally neuroblastoma in children and...
carcinomas of the breast and lung in adults. Such investigations are indicated when there is a significant probability of bone marrow metastases and when knowledge of their presence would affect the choice of primary treatment. Biopsy may be indicated, for example, when radical surgery or radiotherapy with curative intent is to be undertaken or when intensive chemotherapy with autologous bone marrow transplantation is being considered.

It can be important to suggest the likely primary site of metastatic lesions detected in the bone marrow. This is particularly so in the case of adenocarcinoma since, although many such tumours are relatively resistant to therapy, those originating in the breast and prostate gland may respond to hormonal therapy. Identification of metastatic thyroid carcinoma is likewise important although, in practice, this tumour is rarely found unexpectedly in bone marrow biopsy samples since malignant thyroid tumours generally manifest themselves clearly at their primary site. Investigation for an unknown primary tumour is therefore rarely necessary in bone marrow biopsy samples since malignant thyroid tumours generally manifest themselves clearly at their primary site. Investigation for an unknown primary tumour is therefore rarely necessary in this context and radio-isotope imaging is the preferred staging technique for known thyroid malignancies.

The main areas of difficulty in the diagnosis of metastatic tumour in bone marrow are:

1. distinguishing metastatic tumour cells from tumours of haemopoietic cells—for example, marrow involvement by high grade non-Hodgkin’s lymphoma (NHL) or M7 AML (WHO categories of acute megakaryocytic leukaemia and acute panmyelosis with myelofibrosis);
2. determining the site of origin of metastatic tumour when the primary is unknown;
3. detecting small foci of metastatic tumour in biopsies performed as part of tumour staging; and
4. identifying scanty metastatic malignant cells in severely sclerotic deposits.

Immunohistochemistry is very useful in the identification of metastatic tumours in the bone marrow (Table 10.1).

**Peripheral blood**

Normocytic normochromic anaemia is commonly present when there is infiltration of the bone marrow by malignant cells; other cytopenias are less common. In a third to a half of patients with bone marrow infiltration there are nucleated red cells and neutrophil precursors in the blood—designated leuco-erythroblastic anaemia when the patient is also anaemic. The presence of a leuco-erythroblastic anaemia correlates with the degree of reactive bone marrow fibrosis rather than with the extent of malignant infiltration [9]: it is most commonly seen in association with carcinoma of the breast, stomach, prostate gland and lung. Sometimes bone marrow infiltration is identified in the absence of anaemia or any other abnormality in the peripheral blood.

Significant numbers of circulating malignant cells are rare but may occur in the small cell tumours of childhood, particularly neuroblastoma, rhabdomyosarcoma and medulloblastoma. Circulating neoplastic cells may also be seen in adult patients with carcinoma but this is a very rare occurrence.

Patients with metastatic malignant cells in the bone marrow may show peripheral blood abnormalities which are caused by the underlying malignant disease but are not directly due to bone marrow infiltration. Such abnormalities can include: (i) iron deficiency anaemia; (ii) the anaemia of chronic disease; (iii) micro-angiopathic haemolytic anaemia; (iv) neutrophilia; (v) eosinophilia; (vi) thrombocytopenia; (vii) thrombocytosis; and (viii) increased rouleaux formation.

**Bone marrow cytology**

When bone marrow infiltration has led to reactive myelofibrosis, attempts at aspiration may result in a ‘dry tap’ or a ‘blood tap’, or a small amount of marrow containing haemopoietic cells, tumour cells or both may be aspirated with difficulty. When there is an associated increase in bone turnover, the aspirate may contain a mixture of tumour cells, osteoblasts and osteoclasts (Fig. 10.1). Sometimes the aspirate is wholly or partly necrotic and this observation should lead to the suspicion of malignant infiltration. When a satisfactory aspirate is obtained, it may contain large numbers of tumour cells mixed with a variable number of residual haemopoietic cells, or tumour cells may be scanty and found only after a prolonged search. Examination of the tail and edges of the film and examination of many films are important if scanty tumour cells are to be detected. The detection of scattered neoplastic cells in films of bone marrow aspirates is enhanced by the use of
Table 10.1  Antigens expressed by non-haemopoietic cells, useful for demonstration of metastatic tumours by immunohistochemistry in fixed, decalcified bone marrow trephine biopsy specimens.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Antibody</th>
<th>Specificity</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low molecular weight cytokeratins</td>
<td>CAM5.2, AE1, MNF116</td>
<td>Epithelial cells (cytoplasmic expression)</td>
<td>Cells in occasional cases of anaplastic large cell lymphoma are also positive</td>
</tr>
<tr>
<td>High molecular weight cytokeratins</td>
<td>AE3</td>
<td>Epithelial cells (cytoplasmic expression)</td>
<td>Cells in occasional cases of anaplastic large cell lymphoma are also positive</td>
</tr>
<tr>
<td>Epithelial membrane antigen</td>
<td>E29</td>
<td>Epithelial cells (membrane expression)</td>
<td>Anaplastic large cell lymphomas and non-neoplastic plasma cells are also positive</td>
</tr>
<tr>
<td>Carcino-embryonic antigen (epitopes include CD66)</td>
<td>Polyclonal antisera and 85A12</td>
<td>Epithelial cells (membrane expression, often apical or periluminal distribution)</td>
<td>Metamyelocytes and mature neutrophils are also positive</td>
</tr>
<tr>
<td>Prostate-specific antigen</td>
<td>ER-PR8, PSA 28/A4</td>
<td>Prostatic epithelial cells (cytoplasmic expression)</td>
<td></td>
</tr>
<tr>
<td>Prostatic acid phosphatase</td>
<td>PASE/4LJ</td>
<td>Prostatic epithelial cells (preferential apical/periluminal distribution)</td>
<td></td>
</tr>
<tr>
<td>Thyroglobulin</td>
<td>DAK-Tg6 1D4</td>
<td>Thyroid epithelial cells (cytoplasmic expression)</td>
<td></td>
</tr>
<tr>
<td>Calcitonin</td>
<td>Polyclonal antisera and CAL-3-F5</td>
<td>Thyroid medullary C-cells (cytoplasmic expression)</td>
<td>Of limited diagnostic value in metastases from an unknown primary source but may assist in prognostication in breast carcinoma</td>
</tr>
<tr>
<td>Oestrogen receptor</td>
<td>1D5</td>
<td>Oestrogen-sensitive cells, including those of breast, ovary and endometrium (nuclear expression)</td>
<td>Of limited diagnostic value in metastases from an unknown primary source but may assist in prognostication in breast carcinoma</td>
</tr>
<tr>
<td>Progesterone receptor</td>
<td>1A6</td>
<td>Oestrogen-sensitive cells, including those of breast, ovary and endometrium (nuclear expression)</td>
<td>Of limited diagnostic value in metastases from an unknown primary source but may assist in prognostication in breast carcinoma</td>
</tr>
<tr>
<td>PS3</td>
<td>D07</td>
<td>Tumours of diverse origins with excessive wild-type PS3 or mutant PS3 (nuclear expression)</td>
<td>Perineurial cells, a subset of macrophages, cells of Langerhans cell histiocytosis and 20% of breast cancers are positive Varying proportions of cells are negative in many tumours</td>
</tr>
<tr>
<td>S100 protein</td>
<td>Polyclonal antisera</td>
<td>Malignant melanoma (nuclear and cytoplasmic expression)</td>
<td></td>
</tr>
<tr>
<td>Melanosome matrix protein gp100-cl</td>
<td>HMB-45</td>
<td>Malignant melanoma (cytoplasmic expression)</td>
<td></td>
</tr>
<tr>
<td>Melanoma-associated MART-1 gene product</td>
<td>Melan A</td>
<td>Malignant melanoma (cytoplasmic expression)</td>
<td></td>
</tr>
<tr>
<td>Protein gene product 9.5 (PGP9.5)—ubiquitin related</td>
<td>Polyclonal antisera</td>
<td>Neuro-ectodermal tumours (especially neuroblastoma) and small cell carcinomas from lung and other sources (nuclear and cytoplasmic staining)</td>
<td>Newly produced reticulin, chondrocytes and late granulocyte precursors may be positive Extensive background nuclear staining occurs with some fixation protocols Endothelial cells and some adult malignant epithelial tumours may also be positive</td>
</tr>
<tr>
<td>Uncharacterized</td>
<td>NB84</td>
<td>Primitive neuro-ectodermal tumours, especially neuroblastoma (cytoplasmic expression)</td>
<td></td>
</tr>
<tr>
<td>Uncharacterized</td>
<td>NeuN</td>
<td>Primitive neuro-ectodermal tumours, especially neuroblastoma (cytoplasmic expression)</td>
<td></td>
</tr>
</tbody>
</table>
### Table 10.1 (cont'd)

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Antibody</th>
<th>Specificity</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD99 (MIC2)</td>
<td>12E7, HO36-1.1</td>
<td>Primitive neuro-ectodermal tumours, especially Ewing’s sarcoma (membrane expression)</td>
<td>Some normal T lymphocytes and cells in many cases of ALL are also positive</td>
</tr>
<tr>
<td>Desmin</td>
<td>D33</td>
<td>Rhabdomyosarcoma (cytoplasmic expression)</td>
<td>Cytoplasmic staining is found in most neuroblastomas and occasional cases of Ewing’s sarcoma/PNET. Consistent good performance with the currently available McAb is difficult to achieve in fixed tissue</td>
</tr>
<tr>
<td>MyoD1</td>
<td>5.8A</td>
<td>Rhabdomyosarcoma (nuclear expression)</td>
<td></td>
</tr>
<tr>
<td>Myogenin</td>
<td>F5D</td>
<td>Rhabdomyosarcoma (nuclear expression)</td>
<td>Megakaryocytes are also positive</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>MG-1 and polyclonal antisera</td>
<td>Rhabdomyosarcoma (cytoplasmic expression)</td>
<td>Endothelium and tumours of endothelial origin (cytoplasmic expression)</td>
</tr>
<tr>
<td>von Willebrand factor</td>
<td>F8/86 and polyclonal antisera</td>
<td>Rhabdomyosarcoma (cytoplasmic expression)</td>
<td>Megakaryocytes are also positive</td>
</tr>
<tr>
<td>CD34</td>
<td>Q8End10</td>
<td>Endothelium and tumours of endothelial origin (cytoplasmic expression)</td>
<td>Some non-endothelial spindle cell tumours, normal and neoplastic early haemopoietic cells are also positive</td>
</tr>
<tr>
<td>CD31</td>
<td>JC70a</td>
<td>Endothelium and tumours of endothelial origin (cytoplasmic expression)</td>
<td>Macrophages, monocytes, megakaryocytes and plasma cells are also positive</td>
</tr>
<tr>
<td>a-1-fucosyl residues,</td>
<td>Ulex europaeus</td>
<td>Endothelium and tumours of endothelial origin (cytoplasmic expression)</td>
<td>Megakaryocytes, erythroid cells and some epithelial cell types are also positive</td>
</tr>
<tr>
<td>including those expressed</td>
<td>agglutinin-1 (the lectin is used</td>
<td>Endothelium and tumours of endothelial origin (cytoplasmic expression)</td>
<td></td>
</tr>
<tr>
<td>on blood group H core</td>
<td>as an antibody equivalent)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>glycoprotein</td>
<td></td>
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**Fig. 10.1** BM aspirate, carcinoma of prostate, showing carcinoma cells and an osteoclast. MGG ×377.
appropriate monoclonal antibodies such as those reactive with cytokeratins, carcino-embryonic antigen, human milk-fat globulin and epithelial membrane antigen (EMA) \[15,16\]. Positive reactions with such antibodies allow single neoplastic cells to be identified with more confidence.

Malignant cells are usually considerably larger than any haemopoietic cells other than megakaryocytes. However, in the small cell tumours of childhood, malignant cells may be similar in size to blast cells and acute leukaemia then enters into the differential diagnosis. Malignant cells are commonly cohesive and therefore occur as tight clumps with or without dispersed cells. Sometimes only irregularly distributed, dispersed cells are present. Neoplastic cells are usually pleomorphic with regard to size, shape and nuclear characteristics. Cell outlines may be indistinct or cells may appear smudged. Some cells may be multinucleated. The nuclei are often hyperchromatic and may contain nucleoli. Mitotic figures may be numerous. Carcinoma cells usually have moderately abundant cytoplasm which shows a variable degree of basophilia and may contain vacuoles; they are sometimes phagocytic. In the small cell tumours of childhood, cytoplasm may be scanty, thus increasing the resemblance to leukaemic blast cells, and sometimes, because of their marked fragility, the cells are represented only by single or clustered bare nuclei. It should also be noted that neuroblastoma cells are positive for \(\alpha\)-naphthyl acetate esterase activity \[17\]. However, they do not resemble cells of the monocyte lineage cytologically and they lack \(\alpha\)-naphthyl butyrate esterase activity.

It is not usually possible to predict the tissue of origin from the cytological features of neoplastic cells in films of bone marrow aspirates. In view of this, it is important also to examine histological sections of marrow particles, particularly if a trephine biopsy has not been performed. Sections may show features, such as gland formation, which are helpful in suggesting the tissue of origin. In a small percentage of cases, cytological features in aspirate films may suggest the tissue of origin. Melanoma cells may be recognized by the presence of pigment (Fig. 10.2), the nature of which can be confirmed by specific stains (see below). Such stains may be positive even when no pigment is detected in routinely stained films but otherwise the cells of amelanotic melanoma cannot be distinguished from other neoplastic cells. Melanin may also be present in macrophages. Clear cell carcinomas are distinctive and suggest a renal primary; the cells have a relatively small nucleus and abundant, very weakly basophilic cytoplasm (Fig. 10.3). Cells of metastatic carcinoid tumour also have a relatively small nucleus and moderately abundant cytoplasm (Fig. 10.4). In children, neuroblastoma (Fig. 10.5) may sometimes be identified by the presence of extracellular blue–grey fibrillar material or by the presence of cells with irregular ‘tails’; rosettes of tumour cells are distinctive and are found in up to two thirds of

Fig. 10.2 BM aspirate, malignant melanoma, showing melanoma cells containing melanin. MGG \(\times 940\). (By courtesy of Dr J Luckit, London.)
Fig. 10.3  BM aspirate, carcinoma of kidney, showing ‘clear cells’ with voluminous pale cytoplasm. MGG ×377. (By courtesy of Dr D Gill, Brisbane.)

Fig. 10.4  BM aspirate, carcinoid tumour, showing cells with relatively small nuclei and a variable amount of cytoplasm. MGG ×377.

Fig. 10.5  BM aspirate, neuroblastoma, showing neoplastic cells which are relatively small and have a high nucleocytoplasmic ratio and a diffuse chromatin pattern. Neurofibrillary bundles are apparent. MGG ×376.
patients [18]. Rosettes are uncommon in other small cell tumours of childhood but small numbers may be seen in Ewing’s sarcoma [18] and other PNET. In metastatic rhabdomyosarcoma there may be multinucleated giant cells or spindle-shaped binucleated rhabdomyoblasts [4]. The cytoplasm is often vacuolated and large vacuoles may coalesce to form lakes [19] (Fig. 10.6). Such cells are periodic acid–Schiff (PAS)-positive. In some cases, some of the tumour cells are phagocytic. Less specific changes, such as foamy or vacuolated cytoplasm or displacement of nuclei by cytoplasmic mucin, may be noted in metastatic adenocarcinoma originating from various primary sites (Fig. 10.7). In squamous cell carcinoma, metastatic tumour cells have sometimes been noted, with Romanowsky stains, to have a reddish cytoplasmic margin; the cytoplasm adjacent to the nucleus is more basophilic [20]. In small cell carcinoma of the lung, the neoplastic cells are usually smaller than those of most carcinomas but are nevertheless still larger than haemopoietic blasts. They have scanty, weakly basophilic cytoplasm and nuclei with coarse chromatin and inconspicuous nucleoli. The nuclei may appear to be bare.
and ‘moulded’ by the nuclei of adjacent tumour cells (Fig. 10.8).

Non-haemopoietic neoplastic cells in a bone marrow aspirate must be distinguished from lymphoma cells, blast cells of acute leukaemia and the neoplastic cells of Langerhans cell histiocytosis or systemic mastocytosis. Other cells which are sometimes confused with malignant cells include: (i) osteoblasts; (ii) osteoclasts; (iii) stromal fibroblasts; (iv) endothelial cells; (v) atypical megakaryocytes; and (vi) crushed erythroblasts.

When the bone marrow is infiltrated by malignant cells there may be associated reactive changes including increased plasma cells or mast cells, granulocytic or megakaryocytic hyperplasia, increased macrophages and increased storage iron. Gelatinous degeneration is rare but may be seen in severely cachectic patients.

**Immunocytochemistry**

Immunocytochemistry can be useful both to confirm the presence of carcinoma cells in a bone marrow aspirate and to detect infrequent cells (Fig. 10.9). A number of studies have been undertaken to assess the value and reliability of immunocytochemistry for epithelial antigens as a means of assessing the extent of bone marrow involvement by metastatic carcinoma (see page 455).

**Cytogenetics and molecular genetics**

Cytogenetic analysis may be useful in suggesting the non-haemopoietic nature of malignant cells infiltrating bone marrow and in confirming the specific diagnosis in small cell tumours of childhood. For example, t(2;13)(q35;q14) can be demonstrated in many cases of rhabdomyosarcoma [21] and the variant translocation, t(1;13)(p36;q14) [22], in a minority. In neuroblastoma, +7 and 17q+ are typical, the latter often resulting from an unbalanced translocation with chromosome 1 in which 1p is lost [23]. Ewing’s sarcoma and other PNET may be associated with t(11;22)(q24;q12); examples of Ewing’s sarcoma have also been found with t(7;22)(p22;q12) and t(21;22)(q22;q12). Recurrent cytogenetic abnormalities have also been reported in several types of adult sarcomas but we have not yet seen examples of these involving bone marrow.

Molecular genetic analysis in Ewing’s sarcoma shows rearrangement of the EWS gene at 22q12. In alveolar rhabdomyosarcoma, the PAX3 and PAX7 genes at 2q25 and 1p36, respectively, are rearranged resulting in formation of PAX3-FKHR and PAX7-FKHR fusion genes [22]. Neuroblastoma may be associated with N-MYC oncogene amplification; when present, such amplification is associated with an adverse prognosis.
Bone marrow histology

Marrow infiltration by metastatic tumour may be focal or diffuse. Reticulin and collagen fibrosis are commonly present. Marked fibrosis is most frequent in carcinomas of the breast and prostate gland but is also found relatively commonly in metastases from cancers of the stomach and lung [9,24,25]. The proportion of tumour cells to stroma is variable and, in cases with severe myelofibrosis, there may be associated osteosclerosis (Figs 10.10 and 10.11). Failure to recognize tumour cells within the fibrous stroma can result in a mistaken diagnosis of idiopathic myelofibrosis. The degree of differentiation of metastatic tumour is very variable and it is often impossible to be certain of the site of the primary tumour on purely morphological grounds. Frequently, metastases are undifferentiated and the differential diagnosis includes poorly differentiated carcinoma, high grade NHL and malignant melanoma (Figs 10.12–10.14); immunohistochemistry is invaluable (see below) [26]. In undifferentiated or poorly differentiated carcinomas it is not usually possible to determine the site of origin of the tumour. In tumours showing differentiation it may be possible to determine the type of carcinoma and suggest the likely site of origin—for example, in metastatic squamous carcinoma, lung is the most

Fig. 10.9 BM aspirate, metastatic carcinoma of breast. (a) MGG ×940. (b) Immunoperoxidase with anti-cytokeratin antibody ×940.
Fig. 10.10 BM trephine biopsy section, carcinoma of the breast, showing osteosclerosis and replacement of the marrow by dense fibrous tissue containing tumour cells. Paraffin-embedded, H&E ×39.

Fig. 10.11 BM trephine biopsy section, carcinoma of the breast (same case as in Fig. 10.10), showing a group of tumour cells with hyperchromatic nuclei and vacuolated cytoplasm. Paraffin-embedded, H&E ×390.

Fig. 10.12 BM trephine biopsy section, poorly differentiated prostatic carcinoma, showing osteosclerosis and replacement of the marrow by dense fibrous tissue containing tumour cells. Paraffin-embedded, H&E ×97.
likely primary site. Squamous differentiation is recognized by the formation of keratin and the presence of intercellular bridges. A mixed pattern of differentiation, with squamous cell carcinoma, small cell carcinoma and adenocarcinoma in any combination, is highly suggestive of origin from the lung.

Metastatic adenocarcinoma (Fig. 10.15) can be diagnosed on the basis of the formation of glands, the presence of signet ring cells and/or the presence of mucin (best detected using a combined diastase-treated PAS–alcan blue stain). A mucin stain facilitates the detection of small numbers of carcinoma cells which may be difficult to detect when they are present as an interstitial infiltrate (Fig. 10.16). Some adenocarcinomas produce large amounts of extracellular mucin, also detectable with a mucin stain (Fig. 10.17). It should be noted that, very rarely, signet ring cells occur in lymphomas [27]. Metastatic adenocarcinoma may arise from primary tumours in the gastro-intestinal tract, breast, prostate gland, ovary, endometrium, pancreas and many other sites. The two primary sites whose identification is most important because of their sensitivity to hormonal therapy are breast and

---

**Fig. 10.13** BM trephine biopsy section, poorly differentiated prostatic carcinoma, showing expression of cytokeratin by tumour cells (same case as in Fig. 10.12). Paraffin-embedded, peroxidase–antiperoxidase, anti-cytokeratin monoclonal antibody ×97.

**Fig. 10.14** BM trephine biopsy section (as for Fig. 10.13), ×390.
Fig. 10.15 BM trephine biopsy section, well-differentiated prostatic carcinoma, showing a tumour composed of small, well-defined glandular structures. Plastic-embedded, H&E ×97.

Fig. 10.16 BM trephine biopsy sections, adenocarcinoma, showing interstitial infiltrate. (a) H&E ×370. (b) Alcian blue stain ×370. (By courtesy of Dr S Wright, London.)
Fig. 10.17 BM trephine biopsy sections, adenocarcinoma, showing intracellular mucin and abundant extracellular mucin. (a) H&E ×370. (b) PAS stain ×370.

Fig. 10.18 BM trephine biopsy section, prostatic adenocarcinoma, showing microglandular pattern with extensive new bone formation. Paraffin-embedded, H&E ×188.
prostate gland. Prostatic origin is suggested by a cribriform, microglandular pattern associated with fibrosis and new bone formation (Fig. 10.18). Occasionally, a macroglandular pattern is present (Fig. 10.19), also usually accompanied by fibrosis and neo-osteogenesis. Identification of breast carcinoma is usually based on its morphological resemblance to primary breast cancer, including duct formation and, particularly in lobular carcinoma, the presence of cell columns forming `Indian file' patterns (Fig. 10.20). Intracytoplasmic lumina are sometimes visible within individual breast carcinoma cells and these can be highlighted by combined alcian blue–PAS staining (Fig. 10.21). Most, but not all, bone marrow metastases from breast cancer are associated with fibrosis and new bone formation. Immunohistochemical staining is not usually helpful in confirming the primary site of suspected metastatic breast carcinoma. Because metastatic lobular carcinoma of the breast can produce an interstitial infiltrate with little cellular reaction, its detection can be difficult. Routine use of immunohistochemistry when a biopsy is carried out for staging purposes has therefore been advised [28]. Adenocarcinoma composed predominantly of ribbons or villous formations of columnar epithelium
with intracellular mucin (goblet cells) is usually of large bowel origin (Fig. 10.22). Clear cell carcinomas have large amounts of pale cytoplasm due to the presence of abundant glycogen or lipid; mucin stains are negative. Likely primary sites of metastatic clear cell carcinoma include kidney, ovary and lung. In rare cases when metastatic follicular carcinoma of the thyroid gland is present, it may be suspected on morphological grounds if follicles containing colloid are seen.

Metastatic small cell carcinoma of the lung commonly involves the bone marrow (see below). The cells are usually small with intensely hyperchromatic, round or oval nuclei and scant cytoplasm (Fig. 10.23). Necrosis is common and there is often smearing of nuclei which can render interpretation difficult. Morphological variants of small cell carcinoma also occur, in which the cells are slightly larger and have either a fusiform or polygonal shape. The principal differential diagnosis of metastatic small cell carcinoma is that of NHL. Metastases from other tumours with neuroendocrine differentiation, such as malignant carcinoid tumours, may also occasionally spread to bone.
marrow; these may have distinctive morphology, with nests and ribbons of monomorphic cells having nuclei with dense, evenly distributed chromatin (Fig. 10.24).

Malignant melanoma is found in the bone marrow in approximately 5% of patients with disseminated disease [29]. If melanin is present in the tumour cells (Fig. 10.25) or associated macrophages, the diagnosis is relatively easy although, if the patient is not already known to have melanoma, the nature of any pigment present should be confirmed by either a Masson–Fontana or a Schmorl stain for melanin. However, not infrequently, metastatic malignant melanoma is amelanotic (Fig. 10.26) and immunohistochemistry should then be considered for confirmation. Malignant melanoma should be suspected if metastatic tumour is composed of polygonal or spindle cells with prominent nucleoli.

The differential diagnosis of metastatic spindle cell tumour within the marrow includes carcinoma showing spindle cell differentiation, malignant melanoma and various sarcomas. Sarcomas rarely metastasize to the marrow and, when they do, the
primary tumour is usually readily apparent. Occasionally, Kaposi’s sarcoma and other angiosarcomas may present in the bone marrow or may be sampled when bone marrow is investigated for reasons other than staging in patients (e.g. individuals with AIDS) who have known primary tumours elsewhere. Bone marrow involvement by Kaposi’s sarcoma is rare but has been observed both in AIDS [30] and in occasional HIV-negative patients [31]. Genomic sequences of the human herpesvirus 8 (HHV8) have been demonstrated in many cases of Kaposi’s sarcoma and this virus is believed to be involved in the pathogenesis of the neoplasm [32]. The bone marrow may be extensively replaced by abnormal tissue composed of slit-like vascular channels lined by spindle-shaped endothelial cells (Fig. 10.27). Large, plump nuclei of neoplastic endothelial cells protrude into the abnormal vascular channels, some of which are engorged with erythrocytes. Haemosiderin-laden macrophages are increased. Angiosarcomas, including Kaposi’s sarcoma, show widely varying degrees of vascular differentiation and may form reticular or solid spindle cell areas (Fig. 10.28). Immunohistochemistry to
demonstrate expression of endothelial antigens may be helpful in cases lacking obvious vessel formation. However, it should be noted that one case of Kaposi’s sarcoma has been reported which lacked expression of von Willebrand factor although there was positive staining with *Ulex europaeus* lectin [31].

Many of the malignant tumours that occur in childhood are composed of small cells with relatively uniform round nuclei. The differential diagnosis of bone marrow infiltration by such cells in a child includes NHL (usually lymphoblastic or Burkitt’s lymphoma), metastatic neuroblastoma, rhabdomyosarcoma, Ewing’s sarcoma, other PNET and retinoblastoma. In order to make a specific diagnosis, the clinical features, morphological findings and histochemical and immunohistochemical staining characteristics all need to be considered. The marrow findings in lymphoblastic and Burkitt’s lymphoma are described on pages 239 and 282; both express CD45 and B-cell immunophenotypic markers. Neuroblastoma is the most common malignant solid tumour in children and often metastasizes to the bone marrow. The majority of cases occur in children under 4 years of age. The neoplastic cells are slightly larger than small lymphocytes with regular, round hyperchromatic nuclei.

**Fig. 10.27** BM trephine biopsy sections, showing Kaposi’s sarcoma in a patient who did not have AIDS. Paraffin-embedded, H&E. (a) ×240. (b) ×960. (By courtesy of Dr RM Conran and Dr VB Reddy, Aurora, Colorado.)
Fig. 10.28 BM trephine biopsy sections, metastatic Thorotrast-induced angiosarcoma, showing refractile Thorotrast. Paraffin-embedded, H&E: (a) ×97; (b) ×390; (c) ×960.
and little cytoplasm [33] (Fig. 10.29). Rosettes are present in a minority of cases, consisting of tumour cells arranged around central fibrillary material which is pink in H&E-stained sections (Fig. 10.30). The cells of neuroblastoma may show focal PAS positivity but this is usually less marked than that seen in rhabdomyosarcoma and Ewing’s sarcoma. In all of these neoplasms, PAS positivity is difficult to detect in fixed tissue sections; it should be sought by staining of aspirate films, as described above.

In one series, rhabdomyosarcoma was found to have metastasized to the bone marrow in 16% of cases [1]. Several histological variants are recognized:

1. Embryonal, which may be further subdivided into myxoid, spindle cell or round cell patterns [34], with the round cell subtype being represented most commonly in bone marrow metastases;
2. Alveolar, characterized by a pattern of irregular spaces lined by tumour cells [35]; and
3. Pleomorphic, which is very rare and not usually seen in children.

In any of these subtypes there may be a few multinucleated rhabdoid cells with peripheral nuclei.
pseudo-alveolar pattern may be produced by cells adhering to the margins of vascular channels [19]. Erythrophagocytosis may be seen [36]. Diagnosis depends on the recognition of skeletal muscle differentiation. Rhabdomyoblasts may be oval, spindle, tadpole or strap-shaped. They have abundant pink granular cytoplasm which may show crossstriations (Fig. 10.31). The number of rhabdomyoblasts present is highly variable; in many patients the majority of cells are undifferentiated round or spindle cells. Ewing’s sarcoma and related PNET are malignant tumours that may arise in bone or soft tissue. Most patients are in the second decade of life and approximately 35% of cases develop bone marrow metastases [1]. The tumour cells are approximately twice the size of small lymphocytes and have round to oval vesicular nuclei (Fig. 10.32). They may show PAS-positive cytoplasmic staining for glycogen, either finely dispersed or forming large blocks of positively stained material (Fig. 10.33). Haemophagocytosis by malignant cells has been reported in rhabdomyosarcoma but is not a specific feature; it also occurs rarely in other neoplasms, including small cell carcinoma, medulloblastoma, breast carcinoma and haemangio-endothelioma [37,38].

Fig. 10.31 BM trephine biopsy section, rhabdomyosarcoma, showing elongated cells with plentiful eosinophilic cytoplasm (rhabdomyoblasts). Plastic-embedded, H&E x390.

Fig. 10.32 BM trephine biopsy section, Ewing’s sarcoma, showing irregular groups of cells in a fibrous stroma; the cells have ovoid nuclei with indistinct nucleoli and scanty cytoplasm. Plastic-embedded, H&E x390.
**Immunohistochemistry**

In bone marrow trephine biopsies containing metastatic poorly differentiated tumours, immunohistochemistry with a small panel of antibodies is useful to demonstrate lymphoid antigens (CD45, CD20 and CD3), epithelial markers (cytokeratins and EMA) and melanoma-associated antigens (S100 protein and the antigens recognized by antibodies HMB-45 and Melan A) [15,26,39,40].

The prostatic origin of a metastatic adenocarcinoma may be confirmed by immunohistochemical staining with antibodies that react with prostate-specific antigen [41]. Since these antibodies may also react with some colonic tumours [42], the use of parallel immunostaining for prostate-specific acid phosphatase is recommended. Use of both antibodies considerably improves sensitivity and specificity in confirming the prostatic origin of metastatic cancer. Unfortunately, there are no antigens with equivalent tissue specificity for breast carcinoma. Of the markers available, α-lactalbumin (which is expressed in most primary breast cancers) has been claimed to be relatively specific [43] but is not widely used. Nuclear expression of oestrogen and progesterone receptors may be demonstrated immunohistochemically (Fig. 10.34) but these antigens are also expressed in a variety of other adenocarcinomas, particularly those arising in the uterus or ovary. It should be noted that human milk-fat globulin, despite its name, is another antigen that is expressed by many adenocarcinomas and has no specificity for those of breast origin. Immunohistochemical staining for thyroglobulin is useful in confirming the thyroid origin of metastatic tumour cells.

Small cell carcinoma reacts positively with antibodies directed against protein gene product 9.5 (PGP9.5), an antigen expressed by neuroectodermal cells, but it should be noted that polyclonal anti-PGP9.5 antisera cross-react with some lymphomas [44]. Small cell carcinomas also often express various sizes of cytokeratin filaments that can be detected by their distinctive intracytoplasmic dot-like or perinuclear ring patterns of immunohistochemical staining. Other tumours showing neuroendocrine differentiation, such as carcinoid tumours and medullary carcinoma of the thyroid gland, may metastasize to the bone marrow; immunohistochemical staining for chromogranin or for PGP9.5 identifies neuro-endocrine differentiation in these tumours [45,46]. In addition, medullary carcinomas of the thyroid may express calcitonin.

In cases of metastatic melanoma, immunohistochemical staining for S100 protein is usually positive [26]. Newer antibodies such as HMB-45 and Melan A also react well with metastatic malignant melanoma in bone marrow trephine biopsy sections. Spindle cell carcinomas and melanomas express cytokeratins and S100 protein (or other melanoma-associated antigens), respectively. Use

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**Fig. 10.33** BM trephine biopsy section, Ewing’s sarcoma, showing large granules of glycogen in the cytoplasm. Plastic-embedded, PAS ×390.
of vimentin to distinguish such spindle cell tumours from true sarcomas is unreliable, since many non-sarcomatous tumours with spindle cell morphology express vimentin; apart from variants of angiosarcoma, however, metastatic sarcomas are exceptionally rarely seen in bone marrow biopsies. In difficult cases of angiosarcoma or Kaposi’s sarcoma, the endothelial origin of malignant cells can be demonstrated by immunohistochemical staining for von Willebrand factor, CD31 and CD34. The *Ulex europaeus* lectin can also be used to stain endothelium.

Neuroblastoma cells usually express neuronal-specific enolase and PGP9.5; less consistently, chromogranin (Fig. 10.35) and the antigens detected by antibodies NB84 and NeuN are expressed. In rhabdomyosarcomas, immunohistochemical staining for desmin, myogenin and MyoD is usually positive [47] although staining for myoglobin, which is said to be more specific, is variable [34]. Ewing’s sarcomas with t(11;22)(q24;q12) express the MIC2 gene product, CD99 [48,49], but so do some ALL.

**The role of bone marrow examination in the staging of solid tumours**

Examination of the bone marrow by aspiration and trephine biopsy is an established part of the staging of neuroblastoma in children in most large centres.
The bone marrow biopsy is positive at the time of initial diagnosis in approximately half of all patients, most of whom have evidence of metastatic spread at other sites [50]. Discordance between marrow aspirate and trephine biopsy findings is common. In one reported series, the trephine biopsy alone was positive in 20% of cases whereas neoplastic cells were seen in the aspirate films when trephine biopsy sections appeared normal in 7%. Taking bilateral bone marrow aspirates and trephine biopsies from the iliac crests increases the sensitivity of the staging procedure by approximately 10%. Particularly careful examination of aspirate films and trephine biopsy sections is necessary because infiltration is often extremely focal [51,52]. In cases in which the marrow appearances are suspicious but not diagnostic of infiltration, immunocytochemical staining of aspirate films using antibodies reactive with neuro-ectodermal antigens (UJ13A and UJ127.11) may be of value in confirming marrow involvement [53,54]. Immunohistochemical staining of paraffin-embedded sections of trephine biopsies for neurone-specific enolase has not been found to increase the sensitivity of marrow biopsy as a means of detecting tumour cells [46]. Detection of PGP9.5 expression is considerably more reliable and should be performed in all cases; its use permits demonstration of tiny clusters and even single cells in patients with minimal bone marrow involvement (Fig. 10.36).

Fig. 10.35 Bone marrow trephine biopsy section, neuroblastoma. Immunoperoxidase for chromogranin ×390. (By courtesy of Professor D Evans, London.)

The additional utility of immunohistochemical staining with newer antibodies, such as NB84 [55,56] and NeuN [57], has yet to be established. Assessment of marrow infiltration by neuroblastoma is more difficult in patients who have been treated with chemotherapy; it is essential that trephine biopsies are of adequate size and quality [58–60]. It has been suggested that the appearances in post-treatment marrow trephine biopsy sections should be divided into four grades:

1. grade 1, normal (or hypocellular) marrow;
2. grade 2, marrow with reticulin fibrosis as the only abnormality;
3. grade 3, distorted architecture with collagen fibrosis; and
4. grade 4, marrow with obvious tumour cells, with or without other abnormalities [33].

Grades 2–4 were all considered by Reid and Hamilton [33] to be compatible with continued bone marrow involvement by tumour, even when individual neoplastic cells could not be identified with certainty. It remains unclear whether this is really true for grades 2 and 3 [61] and whether the different grades correlate with differences in clinical outcome. An important additional feature to recognize in follow-up bone marrow samples obtained during treatment of neuroblastoma is differentiation of the primitive small cells to produce large, ganglion-like cells and areas of pink, fibrillary tissue resembling neuropil (Fig. 10.37). Ganglion-like
cells may show superficial resemblance to mega-karyocytes and the neuropil-like tissue requires differentiation from fibrous tissue. Immunostaining for PGP9.5 is positive in both components of differentiated neuroblastoma tissue and is very helpful in confirming their true nature.

In adults, examination of the bone marrow is not a routine staging procedure for most solid tumours and, although it has been advocated for small cell lung carcinoma and breast cancer, it is by no means practised universally, even for these tumours. In small cell carcinoma of the lung, bone marrow trephine biopsy is positive in 25–30% of cases [10,62,63]; the aspirate is only slightly less sensitive for detecting marrow involvement. It has been suggested that bone marrow examination is indicated in patients with small cell lung carcinoma in order to identify patients who may be suitable for attempted curative therapy [64]. However, some studies have shown no difference in survival between patients with and without marrow involvement [10,64], and the value of routine bone marrow examination has therefore been questioned [10]. Imaging techniques such as magnetic resonance imaging have been proposed as more sensitive alternative staging procedures [65].

Several studies have evaluated the use of marrow aspiration and trephine biopsy in the staging of breast cancer. Detection of subclinical metastatic disease may be useful in identifying patients with
apparently localized disease who might benefit from adjuvant chemotherapy. Bone marrow biopsy is positive in 25–55% of patients with positive radio-isotope bone imaging, but only 4–10% of patients with negative radio-isotopic imaging have tumours detected by bone marrow biopsy [8,66]. In one reported series, 23% of all breast cancer patients had positive bone marrow biopsies at the time of first recurrence [66]. In an attempt to increase the sensitivity of bone marrow examination as a staging procedure, some studies have used immunocytochemical staining to identify micrometastases that would not be detected by conventional techniques. The method used has involved aspirating bone marrow from multiple (up to eight) sites under general anaesthesia at the time of initial surgery, pooling the material and preparing several films. Tumour cells are then detected by immunostaining with antibodies reactive with EMA [67,68], cytokeratins [69] or a cocktail of antibodies recognizing these antigens [70]. Using this approach, micrometastases have been found in 27–35% of cases at the time of diagnosis and correlation has been shown between their presence and the size of the primary tumour [67,68]. The presence of bone marrow micrometastases is a predictor of early relapse in bone. Despite these findings, application of immunocytochemistry to detect micrometastases in bone marrow aspirates performed for staging of breast cancer has not yet become standard practice. A similar approach has been suggested for: (i) detection of oral, oesophageal and gastric cancers [71–74]; (ii) pancreatic cancer [75,76]; (iii) non-small cell lung cancer [77]; (iv) urological cancers [78]; and (v) malignant melanoma [79]. Use of reverse transcriptase polymerase chain reaction (RT-PCR) directed at carcino-embryonic antigen mRNA to detect occult involvement of aspirated bone marrow by carcinomas arising from breast or colon [80,81], and directed at cytokeratin mRNA in cases of breast cancer [82], has also proved successful in initial studies. The clinical value of detecting occult bone marrow micrometastases in such tumours by these techniques remains unproven [83]. In all cases, including breast carcinoma detection, technical problems with the immunocytochemical approach, including false-positive results due to skin contamination or non-specific haemopoietic cell reactivity, limit its applicability in routine practice [84,85].

Problems and pitfalls

Normal components of bone marrow may be mistaken for malignant non-haemopoietic cells in aspirate films and biopsy sections. These include megakaryocytes, crushed erythroid cells, osteoblasts (Fig. 10.38), osteoclasts, stromal macrophages, endothelium and fibroblasts. Awareness of the appearances of stromal components in aspirate films is particularly important in avoiding confusion with malignant cells.
Artefactual inclusions of extraneous tissue in trephine biopsy specimens (e.g. skin, sweat glands, hair follicles or skeletal muscle) may mimic malignant non-haemopoietic cells (Fig. 10.39 and see also Figs 1.64, 1.66 and 1.67). Care should be taken to avoid such inclusions by making a small skin incision prior to insertion of the biopsy needle and using disposable needles to ensure a sharp cutting edge. Carry-over from other specimens into the paraffin block during tissue processing should be avoided by good laboratory practice with regard to preparation of small or friable biopsy specimens; such specimens should be wrapped in tissue paper or sponge, or placed in a wire mesh insert, before processing. If carry-over is suspected, it can be confirmed by molecular analysis of major histocompatibility antigen genes to demonstrate the different patient origins of separate tissue fragments in a single wax-embedded block [86]. However, formic acid decalcification limits the quality of DNA obtainable from trephine biopsy samples and this approach may therefore be unsuccessful in specimens decalcified in this way. Extraneous tissue may also appear to be present in histological sections due to contamination by a ‘floater’ from another tissue block as sections are cut, floated on a waterbath and picked up on individual glass slides (see Fig. 1.70). Good laboratory practice will ensure that
fragments of previous sections are not allowed to contaminate the water-bath between cases. If such contamination is suspected, examination of the complete set of stained trephine biopsy sections should show that other sections are free of extraneous material. If doubt persists, new sections cut from the trephine biopsy specimen will be free of contamination.

Other pathological components in trephine biopsy samples may occasionally be mistaken for malignant non-haemopoietic cells. These include macrophages (present singly or within granulomas), lymphoid cells in some types of NHL, Reed–Sternberg cells in Hodgkin’s disease, neoplastic mast cells in systemic mastocytosis and the cells of Langerhans cell histiocytosis. Immunohistochemistry will establish the nature of each of these types of cell. Macrophages are best demonstrated by the CD68 antibody PGM1 and lymphoid cells by their expression of CD3 (T cells) or CD20 (B cells). Reed–Sternberg cells express CD30, mast cell tryptase can be demonstrated with the monoclonal antibody AA1 and cells in Langerhans cell histiocytosis express CD1a.

Malignant cells may be confused with normal bone marrow constituents or with haemopoietic malignancies. Examples include undifferentiated carcinomas that may infiltrate bone marrow without any stromal reaction, clear cell carcinoma, signet ring carcinoma (Fig. 10.40), malignant

**Fig. 10.40** BM trephine biopsy section, signet ring carcinoma of unknown primary origin. (a) In H&E-stained sections, malignant cells are indistinct and resemble macrophages. (b) Immunostaining for low molecular weight cytokeratins confirms their epithelial nature. Paraffin-embedded, both ×376.
melanoma and the small cell solid tumours of childhood. Deposits of metastatic carcinoma eliciting a fibrotic response may also be confused with myelofibrosis, Hodgkin’s disease or NHL. Among the NHL, those of T-cell lineage are most likely to produce significant stromal fibrosis. Immunohistochemistry will assist in making a correct diagnosis. Expression of low and high molecular weight cytokeratins is present in almost all carcinomas, S100 protein in melanomas and CD45 (plus CD3 or CD20) in T- or B-cell lymphomas, respectively. Neoplastic plasma cells frequently lack CD45 and CD20 expression but their nature can be confirmed by p63 expression (detected using monoclonal antibody VS38c) in the absence of expression of cytokeratins; CD79a is expressed in many cases, but not all, and a negative result can therefore be misleading. The diagnosis of most solid small cell tumours of childhood can be confirmed on the basis of expression of PGP9.5 (neuroblastoma), CD99 (Ewing’s sarcoma) or desmin (rhabdomyosarcoma).

Detection of malignant non-haemopoietic cells in necrotic deposits can be very difficult. Reticulin staining may demonstrate a preserved pattern of necrotic deposits can be very difficult. Reticulin staining may demonstrate a preserved pattern of necrotic tissue and can be misleading due to nonspecific false-positive results, as well as loss of antigen expression by dead or dying cells [87].

References


54 Carey PJ, Thomas L, Buckle G and Reid MM (1990)


