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The diagnostic challenge of paediatric small round cell tumours

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INTRODUCTION

On writing about paediatric small round cell tumours (SRCTs) one cannot avoid the obvious question: 'Why another chapter on SRCTs of childhood?' There are three practical answers to this question. First, as diagnostic paediatric pathologists, we experience the challenge of correctly diagnosing these tumours on a daily basis.[1,2] Second, we acknowledge that many general pathologists received their training in rotations with limited exposure to paediatric material. Third, recent studies have drawn major conclusions regarding the diagnosis, prognosis and treatment of these tumours.

In the preparation of this chapter, every effort was made to provide a systematic and ordered approach to the differential diagnosis of paediatric SRCTs on biopsy specimens. We have tried to address problems and questions that commonly arise during the day-to-day practice and summarise information concerning the solid SRCTs published in generally available texts. A large body of information is available on leukaemias and lymphomas of childhood, which will not be considered further here. In fact, the next edition of this title, Progress in Pathology 7, will feature a comprehensive article on lymphoma/leukaemia in childhood.

SPECIMEN HANDLING

In dealing with the specimen, the pathologist should appreciate that taking a biopsy from a paediatric patient is not a trivial procedure. The clinical need is urgent for most of the cases and the sample provided is invariably smaller than

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Table 1.1 Samples required for the application of specialized techniques

Samples required in order of priority
Formalin fixed
Tissue culture for karyotyping
Trial-specific specimens
Frozen tissue for long-term storage (liquid nitrogen)
Glutaraldehyde for electron microscopy
Imprints
±Frozen section

the pathologists would like. The biopsy may have to be divided into even smaller samples to allow the application of specialised techniques. It is clear that if maximum benefit is to be obtained from such small samples, good communication is essential between pathologists, radiologists and surgeons.

Core or wedge biopsies must reach the pathology department fresh in an unfixed state, accompanied by a request form containing relevant information. According to clearly defined protocols,[3–8] the tissue submitted should provide several samples as seen in Table 1.1. These protocols include recommendations on the collection of clinical and pathological data with diagnostic and prognostic significance. The documents are evidence based and define the minimum standards for reporting each tumour group.

When the tissue is insufficient to cover all the options then flexibility and thought are required in deciding what to do rather than following prescribed lists of procedures. Light microscopy and cytogenetics are usually the most important, providing a diagnosis in the majority of the cases. Priority should always be given to the production of paraffin blocks for morphological and immunohistochemical assessment because SRCTs are undifferentiated and crush artefact is often present adding to the diagnostic difficulty. Furthermore, it is important to harvest tissue samples for specific procedures approved by the national bodies co-ordinating clinical trials of treatment. If there is strong clinical suspicion of neuroblastoma for example, then tissue needs to be submitted for N-myc analysis.[7] Little requirement is left for the urgent frozen section diagnosis today. Rapid processing facilities are available in most departments.

THE DIFFERENTIAL DIAGNOSIS

Paediatric SRCTs comprise a group of diverse primitive or undifferentiated neoplasms composed of sheets of small cells with round nuclei and inconspicuous cytoplasm.

The most common neoplasms presenting as SRCTs are lymphoblastic lymphomas/leukaemias, Ewing's sarcoma (ES)/peripheral neuroectodermal tumour (PNET), rhabdomyosarcomas and neuroblastomas. Most rhabdomyosarcomas and neuroblastomas show characteristic patterns of differentiation by light microscopy. However, a number of other paediatric tumours that are not usually problematic can on occasion present as SRCTs and cause diagnostic difficulty, particularly when they present in unusual sites or clinical context. The differential diagnosis, therefore, needs to be expanded beyond the traditional

Table 1.2 The differential diagnosis of SRCTs

Lymphoblastic lymphoma/leukaemia
Rhabdomyosarcoma
Neuroblastoma
ES/PNET
DSRCT
Small cell osteosarcoma
Synovial sarcoma
Wilms' tumour
Renal sarcomas

group to include peculiar variants, such as Wilms' tumour, renal sarcomas, desmoplastic small round cell tumour (DSRCT), small cell osteosarcoma and monophasic synovial sarcoma (Table 1.2).

THE CONTRIBUTION OF CLINICAL INFORMATION

Despite the effort of clinicians not to overload pathologists with clinical information, when one is confronted with an SRCT on an urgent core biopsy the contribution of clinical information cannot be overemphasised.

The minimum dataset required includes age, site, family history, radiological appearances and tumour markers.

Each neoplastic category has a distinct age distribution and this is particularly true for renal neoplasms. Anaplastic Wilms' tumours are virtually non-existent in infancy [9–13] while clear cell sarcoma of the kidney (CCSK) is unheard of in the first 3 months of life.[12–14]

Some anatomical sites are commonly associated with a specific type of tumour. For example, embryonal rhabdomyosarcomas typically arise in sites of embryonic tissue fusion; that is, the head and neck region and the genitourinary tract. A vaginal mass in an infant will most likely be an embryonal rhabdomyosarcoma. Imaging techniques are particularly helpful in establishing the actual site of the tumour and its extent. Information provided regarding the appearances of the tumour together with the differential diagnosis could be of great assistance in difficult cases. Multiple intra-abdominal masses in an adolescent male, for example, are suggestive of a DSRCT.[15]

Tumour markers also contribute to the differential diagnosis. Increased AFP levels in a patient with a hepatic tumour mass are suggestive of a hepatoblastoma while increased blood catecholamines associated with a suprarenal mass in an infant are diagnostic of a neuroblastoma.

Although most of the common childhood cancers occur sporadically, rare cases of host susceptibility are well documented. Familial and hereditary conditions associated with malignancy are important disorders to identify among paediatric patients because careful screening, examination and counselling can detect early signs of cancer and improve the chance of successful treatment. Twenty-five per cent of patients with Beckwith–Wiedemann syndrome (macrosomia, hemihypertrophy, exomphalos, neonatal hypoglycaemia, large tongue and creased ear lobes) develop a tumour (Wilms' tumour, hepatoblastoma and adrenal carcinoma) before the age of 5.[16,17]

MORPHOLOGICAL ASSESSMENT AND DIAGNOSTIC CLUES

The gross appearances are of limited value in facilitating the diagnosis of an SRCT on a small biopsy specimen. Imaging techniques, however, can provide relevant information regarding the macroscopical appearances (such as multicentricity, organ of origin, texture, calcification, extent of tumour, etc.).

RHABDOMYOSARCOMA

Rhabdomyosarcoma is the most frequent soft tissue tumour of childhood with a peak incidence during the first decade.[18] The tumour is non-familial in most cases. The familial forms are associated with the Li–Fraumeni syndrome and the siblings are at increased risk for the development of various types of neoplasms.[19] Although they occur more commonly in the soft tissues they have been described in virtually every organ.[20] Cutaneous rhabdomyosarcomas have been seen in association with patients having epidermal naevi and those with von Recklinghausen's disease.[18,21]

The histological subtyping in rhabdomyosarcoma began in 1958 with the description of four major groups, namely botryoid, embryonal, alveolar and pleomorphic, known as the conventional scheme.[22] The WHO adapted this classification in 1969[23] but in subsequent decades, schemes based on tumour architecture, histology, clinical behaviour and cellular differentiation had been introduced. It was not until 1994 that a systematic review of all the available schemes was conducted in order to compare their reproducibility and prognostic significance.[24] A modification of the conventional scheme achieved a fair to good observer agreement and demonstrated a highly significant correlation with relation to survival. As a result of this, the International Classification of Rhabdomyosarcoma was introduced (Table 1.3).[25]

The embryonal rhabdomyosarcoma is composed of rhabdomyoblasts at varying stages of muscle development. Usually, they consist of small, round- to spindle-shaped cells with hyperchromatic nuclei and indistinct cytoplasm. Cellularity is variable with alternating densely packed, hypercellular areas and loosely textured myxoid areas.[20] The botryoid rhabdomyosarcoma is a subtype of embryonal rhabdomyosarcoma seen in certain sites, such as vagina and

Table 1.3 International classification of rhabdomyosarcoma[25]

Superior prognosis
Botryoid rhabdomyosarcoma
Spindle cell rhabdomyosarcoma
Intermediate prognosis
Embryonal rhabdomyosarcoma
Poor prognosis
Alveolar rhabdomyosarcoma
Undifferentiated rhabdomyosarcoma
Subtypes whose prognosis is not presently known
Rhabdomyosarcoma with rhabdoid features

urinary bladder. The neoplasm extends to just beneath the overlying mucosa/epithelium where it is separated by a compressed layer of tumour cells called the cambium layer. A spindle cell variant with a better prognosis occurs primarily in the paratesticular region.[26]

The alveolar rhabdomyosarcoma tumour cells are poorly differentiated round to oval, forming aggregates, delimited by dense collagenous septa and showing central loss of cohesion. The solid variant of this tumour lacks an alveolar pattern entirely and it is composed of densely packed groups of neoplastic cells with little or no fibrosis.[27] Pleomorphic rhabdomyosarcoma occurs rarely in infants and children.[28]

The histological subtypes that most commonly cause diagnostic confusion are the solid variant of alveolar rhabdomyosarcoma and the most undifferentiated form of embryonal rhabdomyosarcoma. Cases of the former have been misdiagnosed as embryonal rhabdomyosarcoma before molecular genetics revealed the characteristic translocation. Reticulin is a reliable stain that will display a pericellular distribution in embryonal rhabdomyosarcomas in contrast to outlining cellular aggregates in alveolar rhabdomyosarcoma (Figs 1.1 and 1.2). The importance of this distinction lies with the much more aggressive behaviour and worse prognosis associated with alveolar rhabdomyosarcoma compared to the embryonal subtype.[29–32]

NEUROBLASTOMA

This neoplasm commonly presents as an abdominal mass in a patient with increased urine vanillyl mandelic acid (VMA) and homovanillic acid (HVA) levels. On imaging the tumour may show calcification and necrosis. Rarely it may invade or surround the kidney, mimicking a Wilms' tumour.[33]

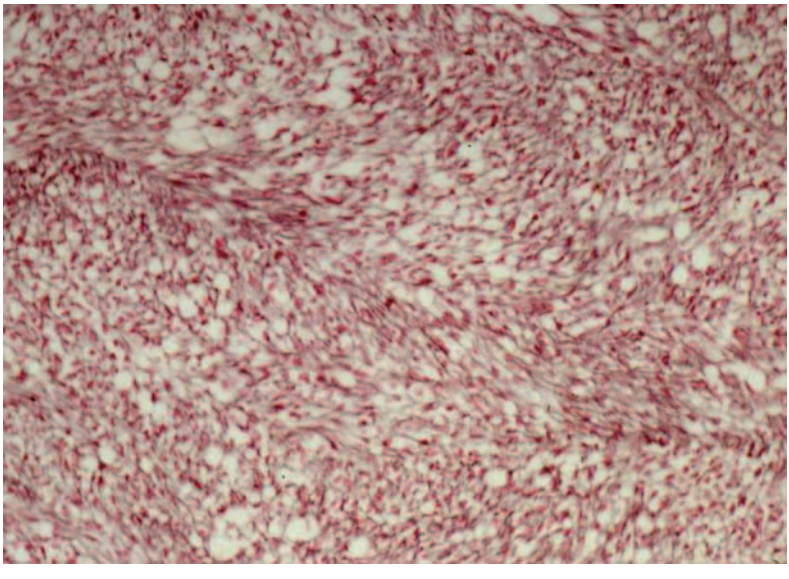

Fig. 1.1 Reticulin staining displaying a pericellular distribution in embryonal rhabdomyosarcoma.

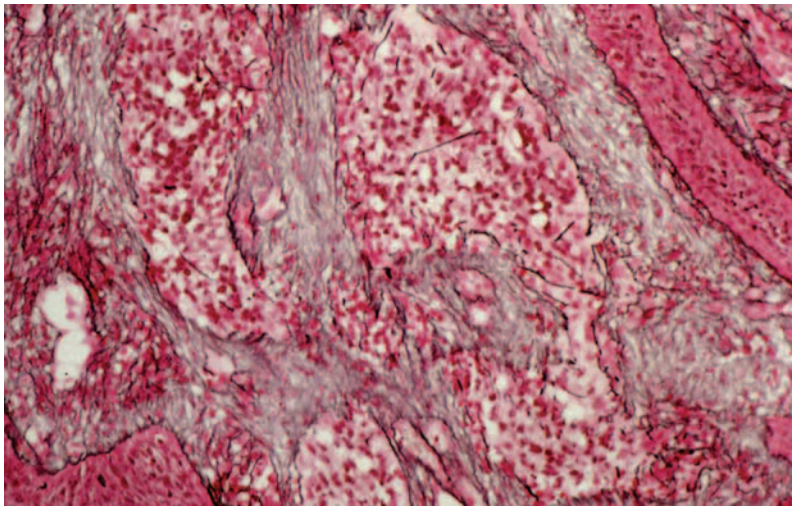

Fig. 1.2 Reticulin stain outlining cellular aggregates in alveolar rhabdomyosarcoma.

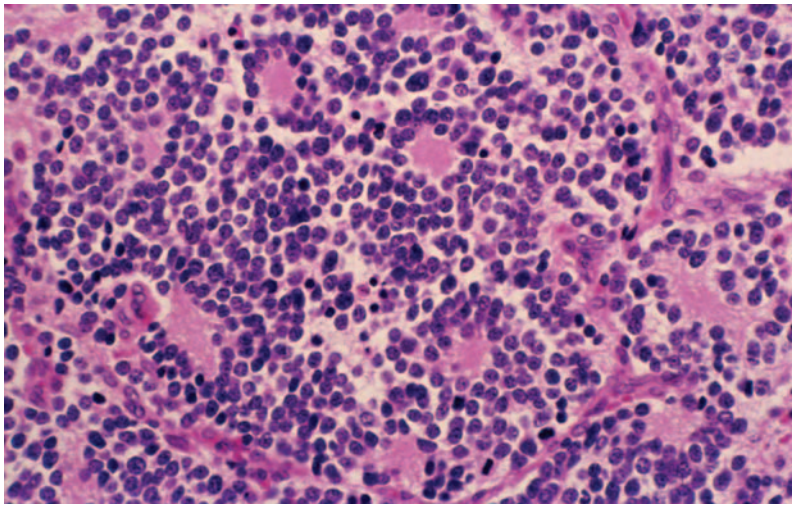

Fig. 1.3 Neuroblasts polarised towards a central point forming a Homer–Wright rosette with a solid fibrillary central core.

The tumour is composed of small primitive neuroblasts, with densely speckled nuclei and little cytoplasm, sometimes admixed with larger cells with features of ganglion cell differentiation. Neuroblasts may be polarised towards a central point forming a Homer–Wright rosette with a solid fibrillary central core (Fig. 1.3). An eosinophilic, acellular fibrillary matrix is often present and fibrovascular septa commonly divide the neoplasm into lobules. Necrosis and calcification are so common as to be helpful in the differential diagnosis of SRCTs.

EWING'S SARCOMA/PRIMITIVE NEUROECTODERMAL TUMOURS

ES/PNET and Askin tumour (thoracopulmonary PNET) represent a group of SRCTs with overlapping clinical and pathological features.

These neoplasms typically arise in the same age group; that is, childhood to young adulthood. ES is classically a primary bone neoplasm where PNET usually arises in soft tissues. ES, however, may present at an extra-osseous site and rare cases of PNET arise in bone.[34,35] These features suggest that the two tumours are related. Indeed the most notable feature shared by ES and PNET is a unique chromosomal translocation. The t(11;22)(q24;12) translocation was identified in ES in 1983[36] and the identical translocation was identified in PNET and Askin tumour in 1984[37] and 1985,[38] respectively. Nevertheless, there is no universal agreement as to whether there is any clinical significance in differentiating between these entities. Some have suggested rosette formation as a diagnostic feature of PNET while others require immunohistochemical evidence of neural differentiation, with or without rosettes.[35] The criteria used, however, appear random and will become irrelevant if future studies fail to reveal prognostic significance. Weiss suggests the classification of these tumours as members of the ES/PNET family, followed by a comment on the presence or absence of morphological, immunohistochemical or ultrastructural features supporting neural differentiation.[39]

ES comprises about 5% of all primary bone tumours but together with osteogenic sarcoma, encompasses the majority of bone tumours in children. The tumour occurs in the medullary cavity of the metaphyseal region of long bones and shows areas of necrosis. Correlation with radiological imaging studies is essential in reaching the diagnosis. The tumour typically presents as a poorly defined laminated lesion with periosteal reaction, sometimes associated with a soft tissue mass. The differential diagnosis includes osteomyelitis and Langerhans cell histiocytosis.[40] Microscopically it is composed of tightly packed round cells with clear or speckled nuclei, without nucleoli. A two-cell population is sometimes present composed of large viable cells and small hyperchromatic necrotic cells (Fig. 1.4). Mitotic count is usually low (<1/10 hpf). Necrosis is often seen, but calcification is not prominent, a feature that may be helpful in the differential diagnosis with neuroblastoma.[39] Cytoplasmic glycogen can be demonstrated on PAS and a lack of background reticulin fibres on reticulin.

PNET is a tumour showing a lobular diffuse and discohesive growth pattern with haemorrhage but no calcification. The tumour lobules are separated by dense fibrovascular septa which may undergo hyalinisation. Both Homer–Wright and perivascular pseudo-rosettes may be seen.[41] The cells are small containing a round to oval nucleus with coarse chromatin and small nucleoli, surrounded by a small rim of eosinophilic cytoplasm. The presence of intercellular fibrillary material is extremely unusual as is the presence of ganglion cells.[42]

DESMOPLASTIC SMALL ROUND CELL TUMOUR

DSRCT is a highly aggressive tumour of uncertain origin. The tumour occurs more commonly in males, presenting with abdominal distension and pain associated with an abdominal mass. At laparotomy, a variably sized mass associated

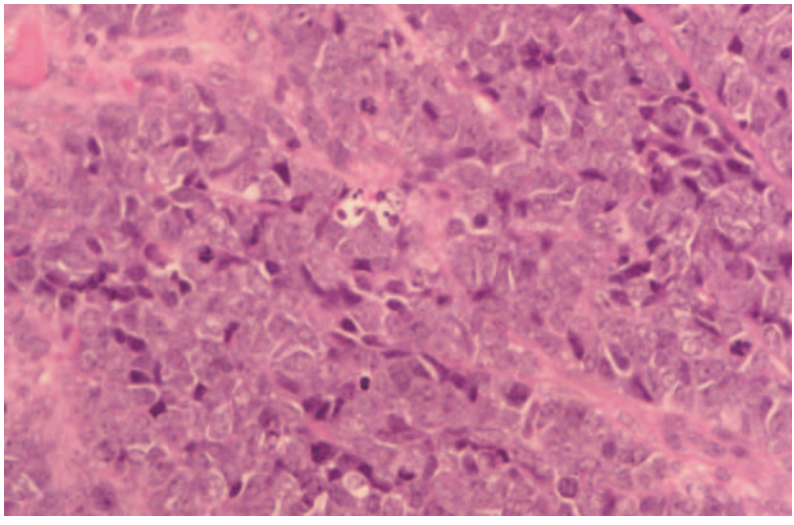

Fig. 1.4 A two-cell population is sometimes present in ES, composed of large viable cells and small hyperchromatic necrotic cells.

with numerous smaller peritoneal implants may involve any portion of the peritoneal cavity.[15]

DSRCT has a distinctive low-power appearance with sharply outlined trabeculae of tumour cells delimited by a desmoplastic stroma (Fig. 1.5). Central necrosis is common and may be associated with calcification in some of the larger trabeculae. The tumour cells are uniform with scanty cytoplasm, hyperchromatic nuclei, indistinct nucleoli and frequent mitoses. Diagnostic confusion tends to arise when the clinical presentation is atypical as in an extra-abdominal location (such as pleura,[43] central nervous system[44] and lymph node[45]).

OSTEOSARCOMA

Osteosarcoma is the commonest malignant bone tumour of childhood, displaying a diversity of histological subtypes, which is beyond the scope of this chapter. The subtype of osteosarcoma that enters the differential diagnosis of SRCTs is the small cell variant. The tumours are usually large (>6 cm) showing a mixed pattern of blastic and sclerotic changes with cortical distortion and often extension through the periosteum. Subperiosteal new bone formation results in either the classical 'Codman's triangle' or bony spiculation. The bone marrow cavity is often involved with transepiphyseal spread. 'Skip lesions', composed of tumour nodules separated by apparently normal marrow, are not uncommon.[40]

The small size and uniformity of the tumour cells as well as their diffuse growth pattern closely simulate the appearance of ES and lymphoma. The histological diagnosis of osteogenic sarcoma is dependent upon the identification of tumour osteoid in the tissue of interest, associated with the appropriate background. In a review of 72 cases of osteosarcoma of bone, in 1997, the investigators suggested making the diagnosis of osteosarcoma only if mineralized

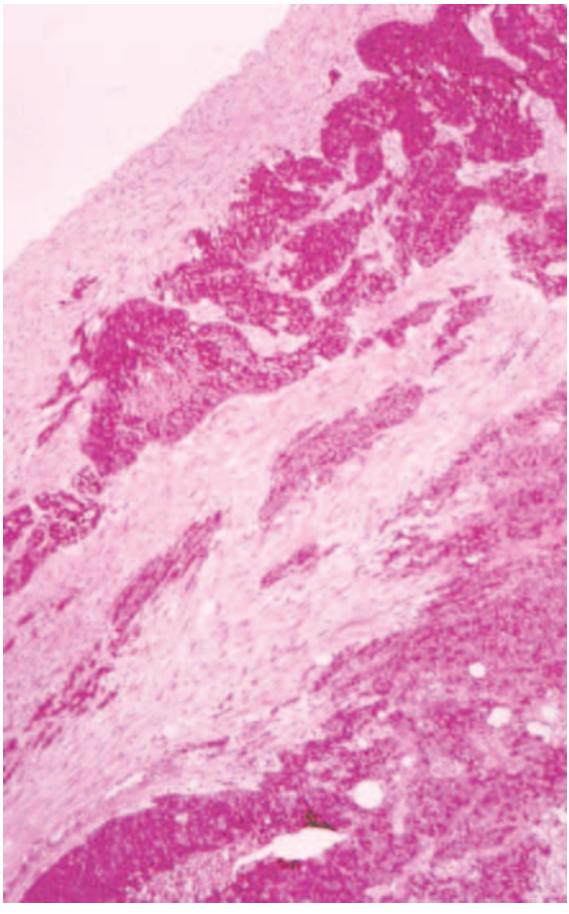

Fig. 1.5 DSRCT has a distinctive low-power appearance with sharply outlined trabecula of tumour cells delimited by a desmoplastic stroma.

matrix is identified.[35,46] Some authors[35] actually suggest that if the diagnosis is doubtful then it is better to err by making the diagnosis of ES. Indeed, we agree that the relationship between ES and the small cell variant of osteosarcoma needs additional study and clarification. Could they represent unusual examples of a bone-forming ES?

SYNOVIAL SARCOMA

Synovial sarcoma is a well-defined entity that has been described extensively in the literature.[47–50] It is seen in adolescents and young adults, presenting as a palpable, deep-seated swelling or mass associated with pain or tenderness, usually arising in the vicinity of large joints, especially the knee. Since it is a slow-growing mass it tends to be sharply circumscribed, round or multilobular.[47]

Unlike most other types of sarcoma, the tumour is composed of two morphologically distinct cell populations: epithelial and spindle cells. Depending on the relative prominence of the two cellular components, synovial sarcomas have been broadly classified into: biphasic, monophasic (fibrous and epithelial) (Fig. 1.6) and poorly differentiated types. The poorly differentiated type is the one that enters the differential diagnosis of SRCTs and is characterised by a

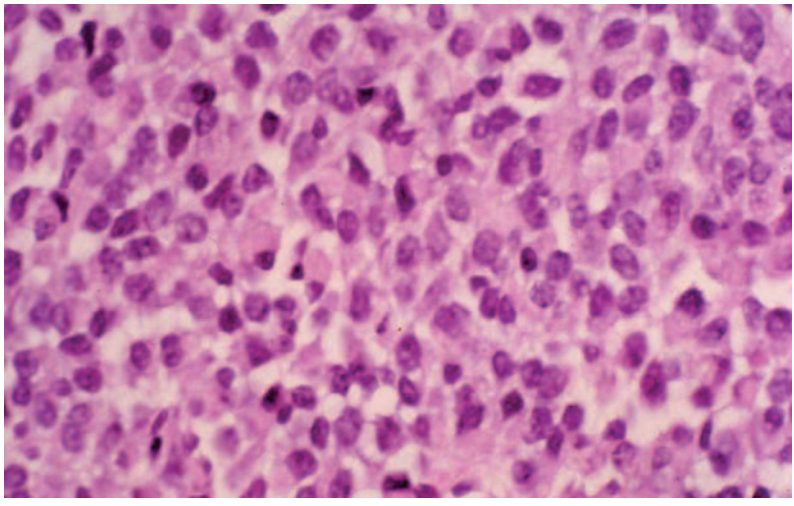

Fig. 1.6 Monophasic synovial sarcoma composed of small cells.

small cell population. The tumours often have a rich vascular background with dilated thin vascular spaces resembling hemangiopericytoma. The diagnosis can be very difficult but the identification of a low-grade component that is typical of monophasic or biphasic synovial sarcoma may be helpful.[47]

WILMS' TUMOUR

Wilms' tumour is one of the commonest malignant, solid, extracranial tumours of childhood, with an incidence of 1 in 10 000 children.[12]

The tumour usually displays the classic triphasic appearance including blastema, epithelial and stromal elements but not all Wilms' tumours are triphasic. Biphasic and monophasic cases are by no means rare. The tumours display histological diversity with variable degrees of differentiation (purely blastemal to highly differentiated stromal and epithelial tumours) and patterns of differentiation (diverse spectrum of cell types and organisation patterns). The pattern that is more likely to be included in the differential diagnosis of SRCTs is the diffuse blastematous one.

The blastematous subtype comprises monomorphous sheets of intermediate-sized cells, often with non-cohesive infiltrative margins resembling those of lymphoma. Under such circumstances the identification of any of the three organoid blastemal patterns may provide a clue to the differential diagnosis. The serpentine pattern is most distinctive for Wilms' tumour, characterised by anastomosing cords of blastemal cells separated by myxoid stroma. The nodular pattern is similar to the serpentine but with rounded nests and the basaloid pattern displays an outlining of a layer of cuboidal or columnar cells.[13]

The identification of an epithelial (tubular or glomeruloid) or a stromal (myxoid, fibrous, smooth muscle or adipose cells) component is diagnostic of the triphasic pattern. However, glomeruloid and tubular structures should be differentiated from entrapped renal parenchyma.