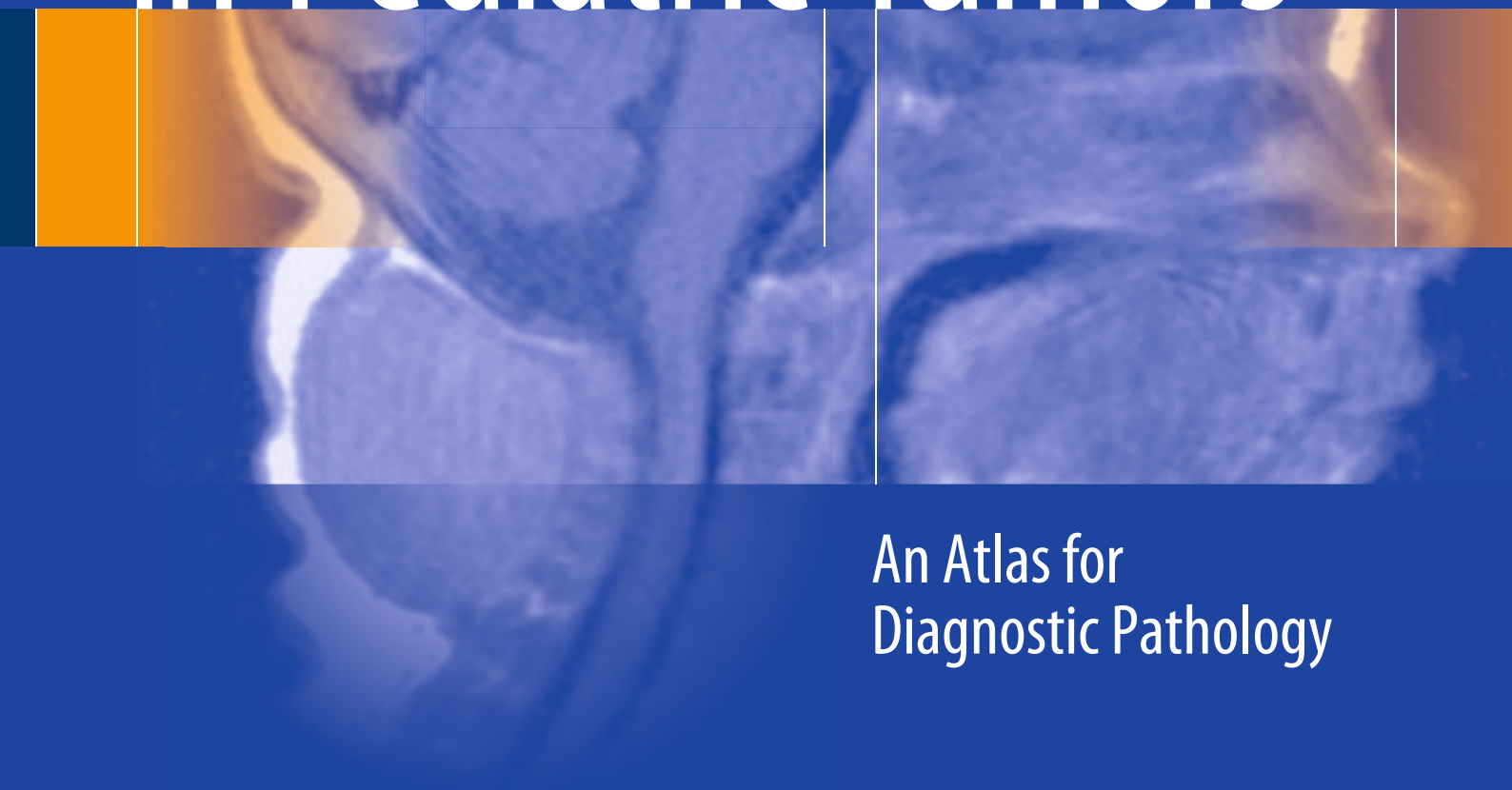
Jerzy Klijanienko
Živa Pohar Marinšek
Henryk A. Domanski
Editors

Small Volume Biopsy in Pediatric Tumors



An Atlas for Diagnostic Pathology

Springer

Small Volume Biopsy in Pediatric Tumors

Jerzy Klijanienko
Živa Pohar Marinšek
Henryk A. Domanski
Editors

Small Volume Biopsy in Pediatric Tumors

An Atlas for Diagnostic Pathology

Springer

Editors
Jerzy Klijanienko
Department of Pathology
Institut Curie
Paris Cedex
France

Henryk A. Domanski
Department of Pathology
Skåne University Hospital
Lund
Sweden

Živa Pohar Marinšek
Department of Cytopathology
Institute of Oncology
Ljubljana
Slovenia

ISBN 978-3-319-61026-9 ISBN 978-3-319-61027-6 (eBook)
https://doi.org/10.1007/978-3-319-61027-6

Library of Congress Control Number: 2017956933

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

In memory of my father, Tadeusz Klijanienko (1926–2015), a man of thousands of ideas

—Jerzy Tycjan Antoni Klijanienko

In memory of my mother, Klementa Pohar (1924–1981), a practical woman with great common sense

—Živa Pohar Marinšek

To my father Dr. Ignacy Domański who was my first mentor of pathology

—Henryk A. Domanski

Preface

In this book we present our international experience of 30 years in the diagnosis of pediatric tumors. Fine-needle aspiration was the principal method of diagnosis for a long time. More recently, core needle biopsy was introduced as a parallel diagnostic technique. Today, the combination of cytology with histology and molecular investigation completed by clinical and radiological information are the key to a successful diagnosis.

This Atlas is the fruit of collaboration among the important European, Russian, and Indian pediatric oncology centers: Institut Curie in Paris (France), Institute of Oncology in Ljubljana (Slovenia), Skåne University Hospital in Lund (Sweden), University of Padova (Italy), Hôpital Robert Debré in Paris (France), Goa Medical College in Bambolim (India), University of Athens (Greece), Centre de Pathologie Est, Hospices Civils de Lyon (France), and Railroad Clinical Hospital JSC "Russian Railways" (Russia).

We would like to acknowledge Prof. Antoine Zajdela, the former head of Clinical Cytopathology, and Prof. Jean-Michel Zucker, the former head of Pediatric Oncology, both from the Institut Curie in Paris. Prof. Zajdela has developed the fine-needle technique in France in the latter half of the twentieth century. Prof. Zucker and Dr. P. Vielh have successfully introduced fine-needle technique as an initial diagnostic method of pediatric lesions.

We all hope that this Atlas will be of help in the diagnosis of pediatric lesions. We address it to the confirmed surgical pathologists, but also to young beginners, fellows, medical students, pediatricians, molecular biologists, radiologists, and care personnel.

Jerzy Klijanienko, M.D., Ph.D., M.I.A.C.
Institut Curie
Paris, France

Contents

Contributors

Patsy Dominique Berrebi, M.D., Ph.D. Department of Pathology, Hôpital Robert Debré, Paris, France

Hervé J. Brisse, M.D., Ph.D. Department of Radiology, Institut Curie, Paris, France

Rocco Cappellesso, M.D. Department of Medicine (DIMED), Pathology and Cytophatology Unit, University of Padova, Padova, Italy

Cécile Cellier, M.D. Department of Radiology, Institut Curie, Paris, France

Marie-Louise Choucair, M.D. Department of Pediatric Oncology, Institut Curie, Paris, France

Sarah Cohen-Gogo, M.D., Ph.D. Department of Pediatric Oncology, Institut Curie, Paris, France

Henryk A. Domanski, M.D., Ph.D., M.I.A.C. Department of Pathology, Skåne University Hospital, Lund, Sweden

Emanuele S.G. d'Amore, M.D. Department of Pathology, San Bortolo Hospital, Vicenza, Italy

Ambrogio Fassina, M.D., Ph.D. Department of Medicine (DIMED), Pathology and Cytophatology Unit, University of Padova, Padova, Italy

Paul Frénaux, M.D. Department of Pathology, Institut Curie, Paris, France

Michel Huerre, M.D. Department of Pathology, Institut Curie, Paris, France

Jerzy Klijanienko, M.D., Ph.D., M.I.A.C. Department of Pathology, Institut Curie, Paris Cedex 05, France

Živa Pohar Marinšek, M.D., Ph.D. Department of Cytopathology, Institute of Oncology, Ljubljana, Slovenia

Lorenzo Nicolè, M.D. Department of Medicine (DIMED), Pathology and Cytophatology Unit, University of Padova, Padova, Italy

Daniel Orbach, M.D. Department of Pediatric Oncology, Institut Curie, Paris, France

Michel Peuchmaur, M.D., Ph.D. Department of Pathology, Hôpital Robert Debré, Paris, France

Roque Gabriel Wiseman Pinto, M.D., D.N.B., M.N.A.M.S., M.I.A.C. Department of Pathology, Goa Medical College, Bambolim, Goa, India

Tatiana Poloz, M.D., Sc.D Department of Pathology, Railroad Clinical Hospital, JSC "Russian Railways", Novosibirsk, Russia

Stamatios Theocharis, M.D., Ph.D. First Department of Pathology, University of Athens Medical School, Athens, Greece

Alexandre Vasiljevic, M.D. Center of Pathology and Neuropathology, Hospices Civils de Lyon, Lyon, France

General Considerations

1

Jerzy Klijanienko, Sarah Cohen-Gogo, Marie Louise Choucair, Daniel Orbach, Cécile Cellier, Hervé J. Brisse, Rocco Cappellesso, Ambrogio Fassina, Stamatios Theocharis, Patsy Dominique Berrebi, and Michel Peuchmaur

1.1 Introduction

Jerzy Klijanienko

The time when an excisional biopsy was a standard procedure for the cyto/histopatologic diagnosis is over. Very few indications still remain for an excisional biopsy. Such a procedure is necessary for a diagnosis of bone tumors or for low-grade, presumed benign lesions. Small volume biopsy, which is a combination of fine-needle aspiration (FNA), core needle biopsy (CNB), and molecular analyses, offers the new horizons in this specific and complicated field of pathology [1]. It is preferred that specific molecular analyses be performed on aspirates, knowing that histological material obtained by core needle is very precious for standard histological and immunochistochemical techniques. Today, in experienced hands, this combination is an extremely powerful and rapid diagnostic method.

J. Klijanienko, M.D., Ph.D., M.I.A.C. (✉)
Department of Pathology, Institut Curie, Paris, France
e-mail: jerzy.klijanienko@curie.fr

S. Cohen-Gogo, M.D., Ph.D. • M.L. Choucair, M.D.
D. Orbach, M.D. • C. Cellier, M.D. • H.J. Brisse, M.D., Ph.D.
Departments of Pediatric Oncology and Radiology, Institut Curie, Paris, France
e-mail: sarah.cohen-gogo@curie.fr

R. Cappellesso, M.D.
Department of Medicine, Cytopathology Unit, University of Padova, Via 8 Febbraio 1848, 2, Padova 35122, PD, Italy

A. Fassina, M.D., Ph.D.
Department of Surgical Pathology and Cytopathology Unit, University of Padova, Via 8 Febbraio 1848, 2, Padova 35122, PD, Italy

S. Theocharis, M.D., Ph.D.
First Department of Pathology, National and Kapodistrian University of Athens, 75, Mikras Asias Street, Goudi, Athens GR11527, Greece

P.D. Berrebi, M.D., Ph.D. • M. Peuchmaur, M.D., Ph.D.
Hôpital Robert Debré, Paris 75019, France

There is no doubt that the use of "scant" cellular material will, in the future, play an important role, since small volume will mean: non-invasive or minimally invasive, repetitive, easy-to-perform, and rapid procedures providing sufficient material that will be destined for standard and ancillary techniques.

In pediatric pathology, there are many reciprocal relationships between clinical, radiological, and pathological aspects. The appearance of tumors in specific age groups is of great clinical importance for determining the prognosis. Some tumors, such as neuroblastoma, may occur in infants in the perinatal period. Detection at a very young age improves the parameter for a favorable prognosis. Moreover, rapidity of evolution and clinical symptomatology are the first important pieces of diagnostic information.

In some patients, the rapidity of tumor evolution and important tumor volume may restrict the use of general anesthesia or invasive diagnostic procedures. In such cases, "urgent" fine-needle aspiration is an excellent initial diagnostic method to confirm malignancy, sarcoma, or lymphoma.

Radiological evaluation is one of the capital elements of diagnosis [2]. Radiology informs the initial diagnosis and tumor "geography." Moreover, radiology helps define the optimal technique and anatomical position before taking a tumor sample. Sampling should be based on the collaboration between the radiologist and pathologist. Sampling by "technique of four hands" is, in our opinion, optimal. The radiologist takes samples while the pathologist evaluates specimen quality by macroscopic examination of core biopsies and microscopic examination of cellular smears. The pathologist is essential also for distributing cellular material for various uses: material to be smeared; material to be preserved in liquid media for cell-bloc preparation or immunocytochemistry; material to be embedded in paraffin; and material to be sent for molecular techniques. Furthermore, "rapid on site evaluation" (ROSE) may be performed for immediate diagnosis and for the decision on the ideal diagnostic material (smears versus core needle biopsy versus molecular studies, etc).

Fine-needle aspiration is one of the most challenging techniques in the pediatric age group [3–5]. Difficulty resides

J. Klijanienko et al. (eds.), *Small Volume Biopsy in Pediatric Tumors*, https://doi.org/10.1007/978-3-319-61027-6_1

in the fact that a large spectrum of tumors is possible in each anatomical location. Additionally, numerous tumors may have overlapping cytological patterns. Nodular fasciitis and low-grade spindle-cell sarcoma are the typical examples. In solid malignancies, cytology diagnosis becomes much easier compared to spindle-cell tumors. The differential diagnosis between blastemal tumors is mainly based on tumor localization. Similarly, in round-cell sarcomas, rhabdomyoblastic, rhabdoid, or rosette-like differentiations are easily detectable on smears. However, in the case of a superficial low-grade spindle-cell tumor, the technique of "let it run its course" is indicated and the final diagnosis may be made through prepared core needle biopsy or surgical excision.

Core needle biopsy is minimally invasive diagnostic technique, but when deeply located tumors are sampled, a general anesthesia is indicated. The quality of core needle biopsy depends on the radiologist's experience. This technique is judged to be safe and accurate but, surprisingly, the cellular material is less representative than the material obtained by fine-needle aspiration. This is particularly true in round-cell and blastemal tumors and less true in spindle-cell tumors.

Molecular diagnosis is one of the newcomers in the diagnosis in pediatric malignancies. Many tumors exhibit specific molecular alterations which may be used in the diagnosis. After an enthusiastic period in the development of molecular diagnosis, many alterations were shown to be "not so specific" and present in several types of tumors. Additionally, the constantly growing number of alterations requires a specialized technical team and systematic updating.

All these techniques may be successfully applied in children if the hospital or oncology center has a well-trained team. A multidisciplinary discussion informs the final diagnosis and appropriate medical or surgical treatment.

1.2 Clinical Aspects of Semi-Malignant and Malignant Tumors in Children and Adolescents

Sarah Cohen-Gogo, Marie Louise Choucair, and Daniel Orbach

Many parameters should be taken into account when forming hypotheses about a tumor in a child: age of occurrence, tumor site, natural history, and of course the tumor's clinical and radiological characteristics. Clinical syndromes predisposing pediatric tumors are listed in Table 1.1. Epidemiology of pediatric tumors is shown in Table 1.2.

Before any aspiration or biopsy, pediatricians, radiologists, and pathologists should always, as a group, discuss and define the best methods of sampling and the fate of the tumor samples. This first step is important because some tumors should not be biopsied at diagnosis due to their potential to spread, in case of rupture: adrenal carcinoma, pseudo-papillar pancreatic tumor, gonadal germ cell tumors, and sex stromal tumors, for instance, fall into this category. In this case, diagnosis should be based on the clinical and radiological presentation and confirmed by histological analysis of the resected tumor. Figure 1.1 displays the main possible malignancies according to the primary tumor site—the reader will then find detailed clinical information below. Figure 1.2 displays the main possible malignancies correlated to the age at diagnosis.

Table 1.1 Clinical syndromes predisposing pediatric tumors

Predisposing disease	Tumors
Neurofibromatosis type 1 (NF1 gene mutation)	Neurofibroma, Schwannoma, MPNST, GIST Rhabdomyosarcoma
Hereditary retinoblastoma (RB1 gene mutation)	Bone and soft tissue sarcomas
Antineoplastic treatment (radiotherapy, alkylating agents)	Sarcomas
Li-Fraumeni syndrome	Rhabdomyosarcoma, Adrenocortical carcinoma, Breast Cancer, GIST
Beckwith-Wiedemann syndrome	Rhabdomyosarcoma, Wilms tumor, Neuroblastoma, Hepatoblastoma
Gardner's syndrome	Fibromatosis, Gastro- intestinal tumors
Von Hippel Lindau	Clear cell renal carcinoma, Pheochromocytoma
Gorham, Maffucci, Blue rubber bleb nevus syndromes	Venous malformation
Turner, Noonan, Klippel-Trenaunay-Weber syndroms	Cystic lymphangioma
PHACE syndrome	Hemangioma

Table 1.2 Epidemiology of pediatric tumors according to the Slovenian cancer registry

Tumors	%
Leukemia	28.4
Brain	22.3
Lymphoma	12.4
Soft tissue sarcomas	8.4
Carcinomas	7.5
Nephroblastoma	5.5
Sarcomas of bone	3
Retinoblastoma	2.5
Malignant melanoma	2.5
Germ cell tumors	1.5
Hepatoblastoma	1
Miscellaneous	0.5

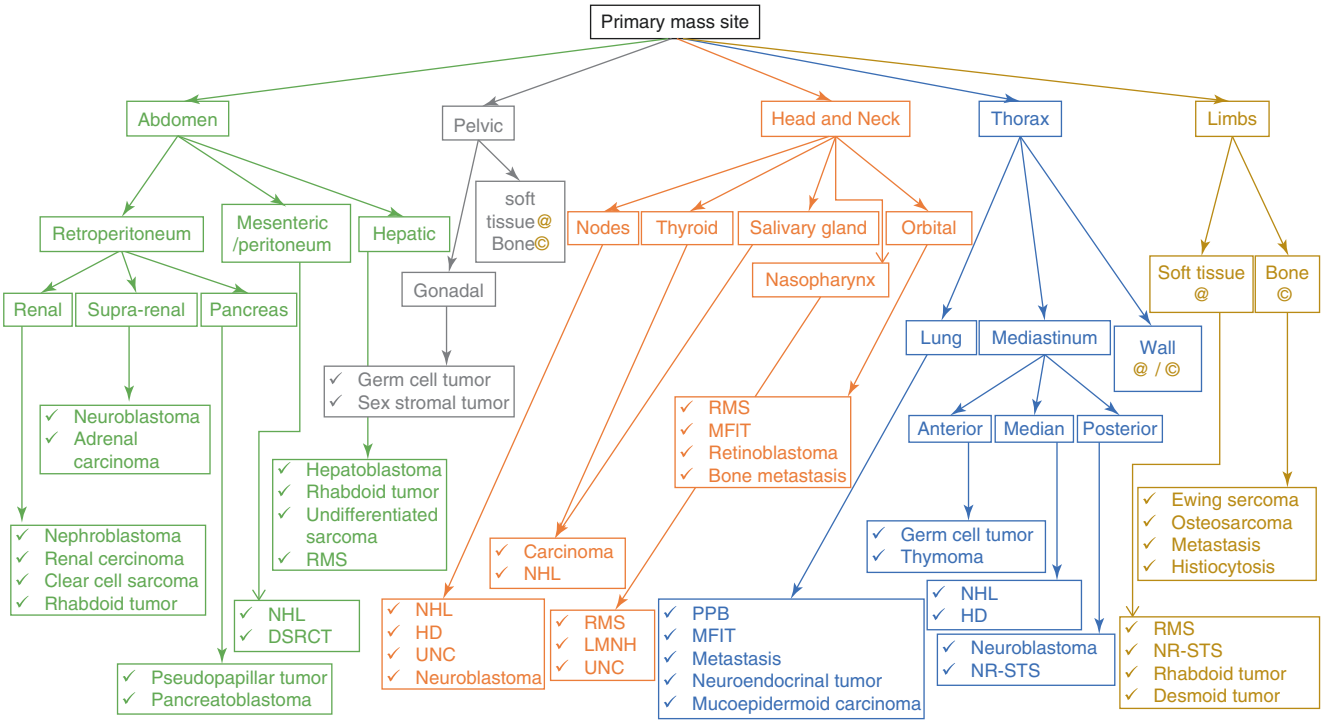


Fig. 1.1 Main malignancies occurring in children according to the primary tumor site. *DSRCT*, desmoplastic small round cells tumor; *HD*, Hodgkin disease; *MFIT*, myofibroblastic inflammatory tumor; *NHL*, Non-Hodgkin's lymphoma; *NR-STS*, non RMS soft tissue sarcoma; *PPB*, pulmonary pneumoblastoma; *RMS*, rhabdomyosarcoma; *UCN*, undifferentiated nasopharyngeal carcinoma

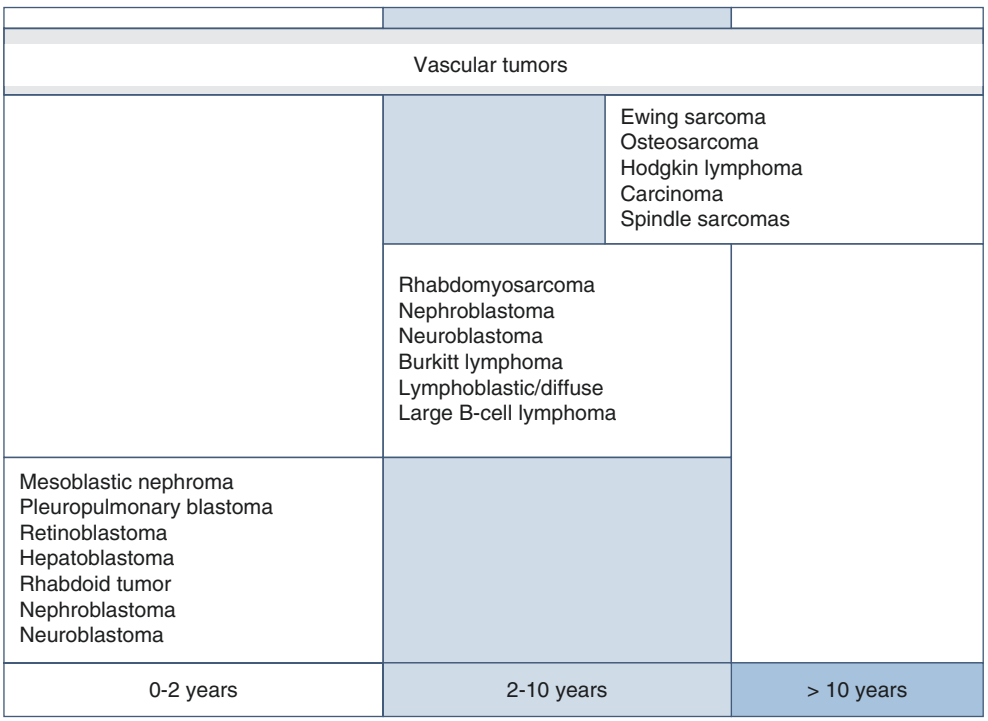


Fig. 1.2 Orientative distribution of tumors correlated to the age

1.2.1 Cervical Nodes

Enlarged cervical nodes are a frequent clinical finding in children and may arise from a wide variety of benign or malignant disorders. Clinical history, physical examination, and laboratory and radiological investigations may give some important clues for differential diagnosis (Fig. 1.3). In 90% of cases, the lymphadenopathies (LAPs) are benign and might arise from viral or bacterial infections (EBV, CMV, HIV, cat scratch disease, etc), tuberculosis, and autoimmune diseases [6]. Some symptoms can be suggestive of a malignant origin, such as unexplained fever, unintentional weight loss, night sweats, pruritus, dyspnea, and poor general condition. Physical examination should be complete and systematic. Each lymph node should be evaluated for its location (localized or generalized; supra-clavicular location is always highly suspicious for malignancy); size (LAP >3 cm are highly suspicious); and consistency, tenderness or skin inflammatory reaction. Further investigations are then recommended with complete blood count (CBC), erythrocyte sedimentation rate (ESR), lactate dehydrogenase concentration, and simple radiological exams, with a chest X-ray and an ultrasound examination of the affected site. Fine-needle aspiration (FNA) of the lymph node and/or excisional biopsy can then be scheduled if considered appropriate.

Leukemia is the most common childhood cancer; in leukemia, generalized lymphadenopathies can be a prominent feature. Acute lymphocytic leukemia accounts for about one-third of all pediatric malignancies. Treatment consists mainly of chemotherapy, and prognosis may vary depending on molecular characteristics. Lymphomas can present either with generalized or localized lymphadenopathy. They are divided into Hodgkin's lymphomas (HL) and non-Hodgkin's lymphomas (NHL). HL has a bimodal distribution with a peak in adolescence and adulthood. About 80% of HL patients present with asymptomatic cervical adenopathy [7].

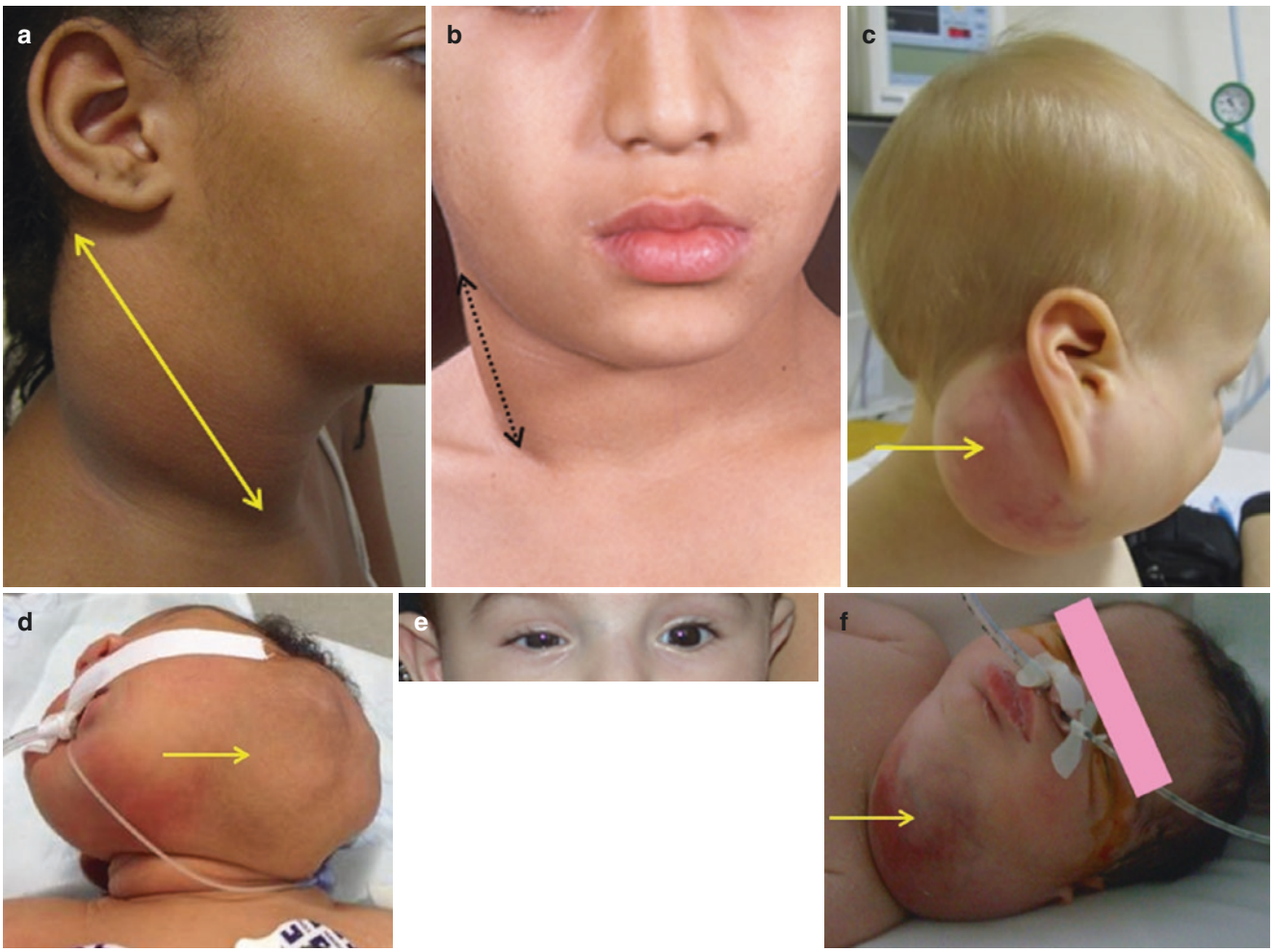


Fig. 1.3 Clinical aspect of children and adolescents with cervical mass. (**a**), 14-year-old female with undifferentiated nasopharyngeal carcinoma associated with bilateral cervical tumor nodes; (**b**), 13-year-old male with cervical Hodgkin disease; (**c**), 8-month-old male with a parotid desmoid tumor; (**d**), newborn male with a parotid sialobastoma; (**e**), 9-month-old boy with a right cervical localized neuroblastoma revealed by a Claude Bernard Horner syndrome (ptositis, myositis); (**f**), newborn with a stage IV cervical rhabdoid tumor

NHL is a heterogeneous group of lymphoid malignancies. In the pediatric setting, the tumor is often a Burkitt's lymphoma or large B cell NHL, but anaplastic large-cell lymphoma (ALCL) and T-lymphoblastic lymphoma can also occur. Treatments of lymphoma have improved a lot over the last decades and consist in short but intensive chemotherapy sometimes associated with immunotherapy. More detailed information on lymphomas will be available in the following sections about thoracic and abdominal tumors [8].

Finally, malignancy can be due to nodal metastasis of solid tumors such as cervical neuroblastoma, nasopharyngeal rhabdomyosarcoma, or undifferentiated carcinoma of nasopharyngeal type (UCNT). UCNT is rare in Europe and the USA and represents 1% of all childhood cancer. It mainly concerns adolescents and young adults. These tumors are usually revealed by their cervical nodal involvement and also by nasal obstruction, epistaxis, trismus, and headache. The nasopharyngeal mass can be discovered during an ear, nose, and throat examination and confirmed with medical imaging investigations. UCNT has a high chemo- and radio-sensibility in children. The global prognosis is satisfactory with an overall survival approaching 90% after treatment with a chemo-radiotherapy association [9].

1.2.2 Thoracic Tumors

The discovery of a thoracic lesion in a child can lead to a wide possibility of benign or malign lesions, but can also correspond to pseudo tumoral images secondary to infectious or malformative diseases. The physician will need more data to give a more precise diagnosis: age, clinical presentation, genetic predisposition context, anatomical location of the lesion as defined by imaging, and, eventually, specific cytology or histology samples.

Chest radiograph and most often CT scan will be mandatory to assess where exactly the thoracic mass is located. The majority of intra-thoracic malignant tumors will be found in the anterior or middle mediastinum and will correspond to hematopathies. Diagnosis and treatment are then an emergency. Classical radiological presentation is a mediastinal enlargement initially diagnosed on a chest radiograph performed for a number of different reasons: cough, dyspnea, chest pain, or other symptoms such as cervical adenopathies or and abdominal mass. Pathology assessment has to be done quickly and treatment will rest upon steroids and polychemotherapy.

In addition to hematopathies, many other tumors can be found in the thorax. A monocentric study conducted in 2005 included 205 children presenting with thoracic mass; 38% of the subject had, in fact, chest wall tumors, and 62% intrathoracic tumors. The most frequent diagnoses were neuroblastoma (41%), Ewing sarcoma family of tumors (17%), rhabdomyosarcoma (RMS) (9%), malignant germ cell tumors (8%), thymomas (4%), and Langerhans cell histiocytosis (4%) [10]. Other tumors with intermediate malignancy, such as pulmonary pneumoblastoma or inflammatory myofibroblastic tumors, are rarely found. Tumors arising from the lung or the pleura are exceptional in childhood [11]. Neuroblastomas are located mainly in the posterior mediastinum. These tumors can have two extreme clinical presentations: either strictly asymptomatic or responsible for medullar compression due to paravertebral endocanalar extension (dumb-bell tumors). In case of severe initial paraplegia, immediate chemotherapy should be discussed in emergency before any tumor sampling. In this case, tumor biopsy will be planned after reduction of the spinal cord compression. Chest wall sarcomas (Ewing's or rhabdomyosarcoma) may be revealed by pain and swelling.

Secondary malignant pulmonary tumors can be seen in pediatric oncology, but are rarely the initial symptom. They arise from solid tumors that are different than those found in adults: Wilms' tumor, bone sarcomas, or soft tissue sarcomas. Pulmonary involvement is also rare in Hodgkin's lymphoma [12].

1.2.3 Mesenteric and Peritoneal Tumors

The discovery of an abdominal mass revealed by abdominal pain, a mass, or an intussusception is relatively frequent in pediatric oncology. This situation is frequent for non-Hodgkin's lymphomas (NHL), which represents about 10% of all childhood cancers. NHLs usually occur in previously healthy children, although some might appear within rare immunodeficiency disorders such as AIDS, ataxia telangiectasia, Wiscott-Aldrich syndrome, or after organ transplants. Abdominal lymphomas account for approximately 40% of all NHL. The median age of onset is 7–8 years. In this situation, the tumor is a highly malignant B cell proliferation, corresponding to a clonal proliferation of immature lymphoid precursors. The Epstein Barr virus often has a role in malignant transformation, even in immuno-compromised children.Tumor proliferation is centered on the ileocaecal area, Peyer's patches, and mesenteric lymph nodes, explaining the frequent symptoms of secondary intussusception. Abdominal NHLs are frequently associated with poor general condition. Ultrasound and abdominal CT often find a large intraperitoneal tumor mass combined with thickened bowel loops, mesenteric lymphadenopathies, or ascites. It is essential to avoid extensive initial surgery. In the absence of major gastrointestinal symptoms due to intestinal perforation, medical care can quickly lever the intestinal compression and avoid extensive surgery. Diagnosis is confirmed by FNA of the affected sites, usually by transcutaneous way.

B-NHL may be life-threatening and need an urgent diagnosis. Prognosis of these lymphomas is primarily related to lactate dehydrogenase (LDH) level, initial disease extension, response to induction chemotherapy, and the possibility to reach complete remission at the end of treatment. Currently, overall survival is between 70 and 90% with multichemotherapy regimen ± rituximab [12].

Other peritoneal tumors are very rare and include desmoplastic small round-cell tumors (DSRCT) or peritoneal mesotheliomas. DSRCTs are rare tumors that occur mainly in adolescents and young adults. The diagnosis is suggested by the presence of a mass located primarily on the peritoneum, most often associated with liver metastases. Tumor biopsies are usually performed during an exploratory laparoscopy or by radiological trans-peritoneal route. Despite treatment using prolonged poly-chemotherapy, an extensive peritoneal surgery, sometimes in association with whole abdomen radiotherapy, the prognosis remains very severe with a survival rate of 20% after 5 years [13].

1.2.4 Hepatic Tumors

Malignant hepatic tumors are rare and represent 2% of childhood cancers. Two-thirds of pediatric liver tumors are malignant. The two most common malignant tumors are hepatoblastoma (HB) and hepatocellular carcinoma (HCC), which together represent 90% of all hepatic malignant tumors. HB is seen in younger children and HCC in older ones. Other malignant liver tumors are quite rare and include hepatic rhabVd tumor, embryonal undifferentiated sarcoma, and biliary rhabdomyosarcoma. Ultrasonography is the first line of examination. Once the hepatic origin of the mass is confirmed, the main aim is to assess disease extension according to the PRETEXT classification (PRE-Treatment-EXTension of the disease). Eighty percent of hepatoblastoma cases occur before 2 years of age and the median age at diagnosis is 18 months. Many risk factors have been identified: Beckwith-Wiedemann syndrome, familial adenomatous polyposis, very low birth weight (<1000 g) and prematurity (<33 weeks). HB mostly presents as an asymptomatic abdominal mass. Laboratory investigations usually show normal liver tests and a very high blood alphafoetoprotein (AFP) level (AFP > 10^4 to 10^7 ng/mL). If the hepatic mass looks malignant but there is no AFP elevation, then, depending on the clinical and radiological findings, several differential diagnoses must be discussed relative to the child's age. In infants, differential diagnosis would be a rare form of HB without AFP secretion or a hepatic rhabdoid tumor, both of which have a very poor prognosis. Molecular analysis, with testing for loss of SMARCB1/INI1 expression, will help to rule out rhabdoid tumor diagnosis. In older children, it could be a HCC or an embryonal undifferentiated sarcoma. Of note, HCC is more frequent in children with an underlying liver condition such as chronic B hepatitis, tyrosinemia, type 1 glycogen storage disease, and biliary atresia. A biopsy should always be performed, except in case of tumoral rupture, to differentiate between HB and HCC. In Europe, treatment consists in neoadjuvant chemotherapy followed by surgery and adjuvant chemotherapy. The intensity of the treatment depends on initial AFP level, the PRETEXT classification, and the presence of metastases [14–17].

1.2.5 Pancreatic Tumors

Pancreatic tumors are very rare in pediatrics, the most frequent one being the pseudopapillary and solid tumor of the pancreas. Further diagnoses are nevertheless possible: pancreatoblastoma in young children and neuro-endocrine tumors in adolescents. The diagnosis is mainly evoked by the discovery of a mass on CT or MRI localized in the retroperitoneum in the pancreas. Pseudopapillary and solid tumor of the pancreas or Frantz tumor occurs mainly in young women (sex ratio of 1:9), at an average age of 22 years. Ultrasound and abdominal CT show a heterogeneous mass, solid and cystic, well-encapsulated, sometimes with calcifications. Biopsy should be avoided because it might be a risk factor for relapse. Complete surgical resection can be performed when clinical and radiological orientation is strong. The long-term prognosis is excellent (survival >95%) [18].

Pancreatoblastoma (PB) is an extremely rare pancreatic tumor seen in young children, with a male predominance. PB may arise in the context of Wiedemann-Beckwith syndrome. The telltale sign is usually the discovery of an abdominal mass sometimes associated with abdominal pain, asthenia, or jaundice. PPB is a solid mass, rather well-encapsulated, round, of soft consistency, and often large, exceeding 10 cm in major axis and extending beyond the limits of the pancreas. It can show necrosis, hemorrhage, or cystic changes. Metastases are rare. Diagnosis of PB is strongly suspected after detection of elevated serum AFP levels. Final diagnosis is confirmed at the time of tumor resection, or a biopsy performed if resection is not immediately possible. Treatment of PB is primarily surgical. Neoadjuvant chemotherapy is sometimes given with the objective of reducing the tumor volume to allow complete surgical excision and perform prophylaxis of metastasis. Survival depends on the initial spread of the disease. Relapse-free survival at 5 years is 59% and overall survival 79%. The only known prognostic factor, besides initial extension, appears to be the possibility of complete resection with or without chemotherapy [19].

Neuro-endocrine tumors of the pancreas are very rare in children, and are more frequent after puberty. Insulinomas and gastrinomas are the most frequent and may occur in association with multiple endocrine neoplasia type I or II.

1.2.6 Adrenal Tumors

In childhood, neuroblastoma accounts for more than 90% of adrenal tumors, while adrenal cortical tumors account for 6% of adrenal cancers in children. Even if adrenal adenomas and carcinomas occur also in childhood, these tumors are indistinguishable on imaging from neuroblastoma. Usually, radiologic criteria for the diagnosis of adrenal carcinoma include size larger than 5 cm, a tendency to invade the inferior vena cava and to metastasize, but none of them are specific to adrenal carcinoma and are frequently seen in adrenal neuroblastoma as well. Furthermore, chromaffin-cell proliferation contributes to pediatric neoplastic processes in the form of an adrenal pheochromocytoma [20]. Neuroblastoma (NBL), along with ganglioneuroblastoma and ganglioneuroma, constitute a group of ganglion cell-origin tumors that originate from primordial neural crest cells, which are the precursors of the sympathetic nervous system [21]. Neuroblastoma accounts for 8–10% of childhood cancers. The most undifferentiated and aggressive NBL presents in young children (median age ≤2 years). The more mature tumor type is ganglioneuroma, which affects older age groups. Approximately 50% of NBL occurring in children older than 18 months of age are metastatic at diagnosis. The main prognostic factors in NBL are age, stage of disease at presentation, and molecular abnormalities such as MYC-N amplification or segmental chromosome alterations [22]. Localized neuroblastoma and those arising in infants have a 90% survival rate except in cases with myc-N amplification, where survival is below 30% [18, 19]. Risk-stratified therapy has facilitated the reduction of therapy for children with low-risk and intermediate-risk disease. Advances in therapy for patients with high-risk disease include intensive induction and myeloablative chemotherapies, followed by the treatment of minimal residual disease using differentiation therapy and immunotherapy; these have improved 5-year overall survival to 50% [23].

Adrenocortical tumors (ACT) are very rare in children, with a worldwide annual incidence of 0.3 per million children below the age of 15 years [24]. This tumor is frequently associated with p53 germline mutations [25]. The incidence is higher in young girls, with a female/male ratio of 2:1, whereas in adolescence the sex ratio is equal. Virilization, with early onset of pubic hair, hypertrophy of the clitoris or penis, accelerated growth, gynaecomastia or acne, is the most common presentation. The second most common manifestation is with hypercortisolism (Cushing's syndrome), while presentation with a palpable abdominal mass is unusual. Diagnosis should be evoked on the clinic-biologic-radiological presentation. In order to avoid the tumor spreading and therefore deteriorating outcome, this tumor should not be biopsied at diagnosis. Immediate surgery is the gold standard for localized tumors: surgical resection is the mainstay of treatment. The role of radiotherapy is uncertain. Similarly, the role of perioperative chemotherapy in association with mitotane (O'PDDD) is limited, as in adult ACTs, and its efficacy in children has not been well-studied prospectively [26].

Pheochromocytomas arise from the adrenal medulla. However, up to one-third of pheochromocytomas may occur outside of the adrenal gland. They are generally sporadic in childhood, usually occurring in adolescence. High blood pressure is often associated and should be controlled before any type of tumor sampling. Pheochromocytoma could also be associated with multiple endocrine neoplasia syndromes (mostly type 2), von Hippel–Lindau syndrome, or neurofibromatosis. Biopsy or tumor sampling should be avoided. Immediate surgery is required in treating this tumor.

1.2.7 Pelvic Tumors

Pelvic tumors can be diagnosed in connection with an abdominal or perineal mass discovered during a routine examination, or by parents. Patients may also present with pain related to a pelvic nerve root compression, vesicosphincter dysfunction, inguinal lymphadenopathies, or poor general condition. Pelvic masses can be benign, especially when of ovarian origin. Still, many cancer types can be diagnosed in this area, such as germ cell tumors (GCT), sex cord stromal (SCT) tumors, rhabdomyosarcomas, or neuroblastomas. Inflammatory myofibroblastic tumors (IMT) can also be found. Imaging is mandatory and ultrasound should be the first step.

Pediatric GCTs are very diverse. They can be diagnosed from *in utero* to adolescence, at gonadal and non-gonadal sites, and from the head to the sacro-coccygeal region. Some of them secrete alpha-fetoprotein (AFP) or hCG, which can then be used as a marker for disease. GCTs remain rare and represent approximately 3% of all childhood cancers [27, 28]. The most frequent primaries are gonadal GCTs, as well as sacro-coccygeal teratoma.

Eighty percent of ovarian masses are benign, and 90% of tumors are non-malignant GCT (mature teratoma). Females present with pain, lower abdominal fullness, and, less commonly, acute abdomen caused by torsion or tumor rupture. Early pubic hair and breast enlargement can occur in case of β-hCG or, more frequently, estrogen secretion (granulosa cells tumors or SCT). An ovarian malignant tumor is a germ cell tumor in 85% of the cases. Serologic markers (AFP, β-hCG, HCG) are essential to assess the nature of the tumor. All the histological subtypes of GCTs may be represented in the ovary tumor. The most common malignant entity is the yolk sac tumor (AFP+) but choriocarcinoma (β-hCG, HCG +) also occurs.

Two age peaks are seen for testicular GCTs: (a) children under 3 years old, who may experience mature teratoma or

yolk sac tumors; and (b) adolescents and young adults who may also have seminomas or other mixed tumors. Most often, those tumors will present as painless scrotal masses. Differential diagnosis includes para-testicular rhabdomyosarcoma, leukemia, and lymphoma.

Sacro-coccygeal teratoma is the most common extragonadal GCT and occurs in newborn and infants. Generally, this tumor presents with either one of two distinct clinical patterns: (a) large, predominantly external lesions that are detected prenatally or at delivery, rarely malignant, and with a favorable evolution after surgery; or (b) older infants who present with less apparent pelvic tumors with a very high rate of malignancy. In both cases, serial blood AFP levels must be performed.

Surgical resection remains the main step in management of GCTs. No biopsy should be performed in ovarian and testicular tumors and orchidectomy should be performed by inguinal approach. The management of all benign tumors, and of localized and completely resectable malignant tumors, is surgery alone. Chemotherapy is very effective in infants and children with unresectable or metastatic disease and allows a high survival rate (>90%).

Rhabdomyosarcoma can also occur in the pelvis, mostly as bladder/prostate, paratesticular, and vaginal RMS. Inflammatory myofibroblastic tumors (IMT) can also occur in this setting [29]. Those tumors have intermediate aggressiveness, with a very low metastasis rate but a tendency for local recurrence. The pelvic mass can be associated with fever, weight loss, anemia, thrombocytosis, polyclonal hyperglobulinemia, and an elevated erythrocyte sedimentation rate. Transcutaneous or bladder/vagina per-endoscopy biopsy is needed to allow diagnosis.

1.2.8 Renal Tumors

Renal tumors in children are rare and account for 6–7% of all cancers in children. Initial diagnosis is most often made when an abdominal mass is discovered by the parents or the physician. There might be hematuria, hypertension, or abdominal pain. These tumors can also be found during ultrasound follow-up of children with a genetic predisposition (Wiedemann-Beckwith, Drash, WAGR, etc.). The gender ratio is rather balanced. Among children aged 6 months to 5 years, Wilms' tumor is by far the most common diagnosis [30]. When confronted with a patient who exhibits a renal mass, the current European SIOP (*Société Internationale d'Oncologie Pédiatrique*) strategy is to assess how probable Wilms' histology might be. The clinician is helped by clinical, biological, and radiological criteria. When the presentation is atypical, the SIOP recommends confirm diagnosis through a biopsy. Moreover, immediate tumor biopsy (or immediate nephrectomy) is recommended when (a) the child is older than 5–6 years old or younger than 6 months old: Wilms' tumors are less frequent and other tumors may be found, such as congenital mesoblastic nephroma, hypercellular renal fibrosarcoma, and aggressive rhabdoid tumors, in the youngest patients, and renal carcinomas or clear cell renal sarcomas in the oldest ones; (b) when urinary tract infection cannot be ruled out easily: pseudotumoral pyelonephritis or abscess might be a differential diagnosis; (c) when abdominal adenopathies can be seen: they are not frequent with Wilms' histology; and (d) when the tumor is not obviously intra-renal: neuroblastoma can be evoked.

Different techniques can be used to perform diagnosis: cytology through fine-needle aspiration, or histology through core needle biopsy, but always through a posterior retroperitoneal approach, with the help of an ultrasound scan. Surgical biopsies are not recommended. As diagnosis of Wilms' tumor is mainly presumptive, urinary catecholamines tests should be systematic to help rule out neuroblastoma, which may mimic nephroblastoma.

Prognosis of Wilms' tumors is now good with survival rates above 90% (regarding all stages altogether) [31]. These results have been reached with two very different initial approaches: (a) in the USA, the National Wilms' Tumor Study recommends total nephrectomy as a first step, and then adjuvant treatments based on stage and histology; (b) elsewhere, the SIOP recommends neo-adjuvant chemotherapy, then surgery, then adjuvant treatment based on stage and histology [32, 33]. Overall prognosis of renal tumors is linked to histology (high-risk tumors are Wilms' tumors with anaplastic or blastemal predominant components and clear cell sarcomas), locally spread disease and metastatic presentation at diagnosis.

1.2.9 Soft Tissue Lesions

Sarcomas in children and adolescents are rare diseases and include various histological types that could be classified as soft tissue sarcomas of "pediatric-type" (i.e., rhabdomyosarcoma), "adult type" (i.e., synovial sarcoma, malignant peripheral nerve sheath tumor), specific entities (infantile fibrosarcoma, desmoid tumor, dermatofibrosarcoma protuberans), and bone sarcomas. Clinical presentation frequently associates a rapidly growing mass with signs depending on the primary location (Fig. 1.4). The group of "non rhabdomyosarcoma soft tissue sarcomas" (NR-STS) gathers all soft tissue sarcomas, except rhabdomyosarcoma and Ewing sarcoma, occurring during childhood and adolescence. Median age of patients with RMS at diagnosis is 5 years, versus 9 years for NR-STS. These sarcomas may occur in every part of the body, but some sites are more frequent: head and neck location for RMS and limbs for NR-STS. Sensitivity to medical therapy depends on the disease type, which must be taken into account

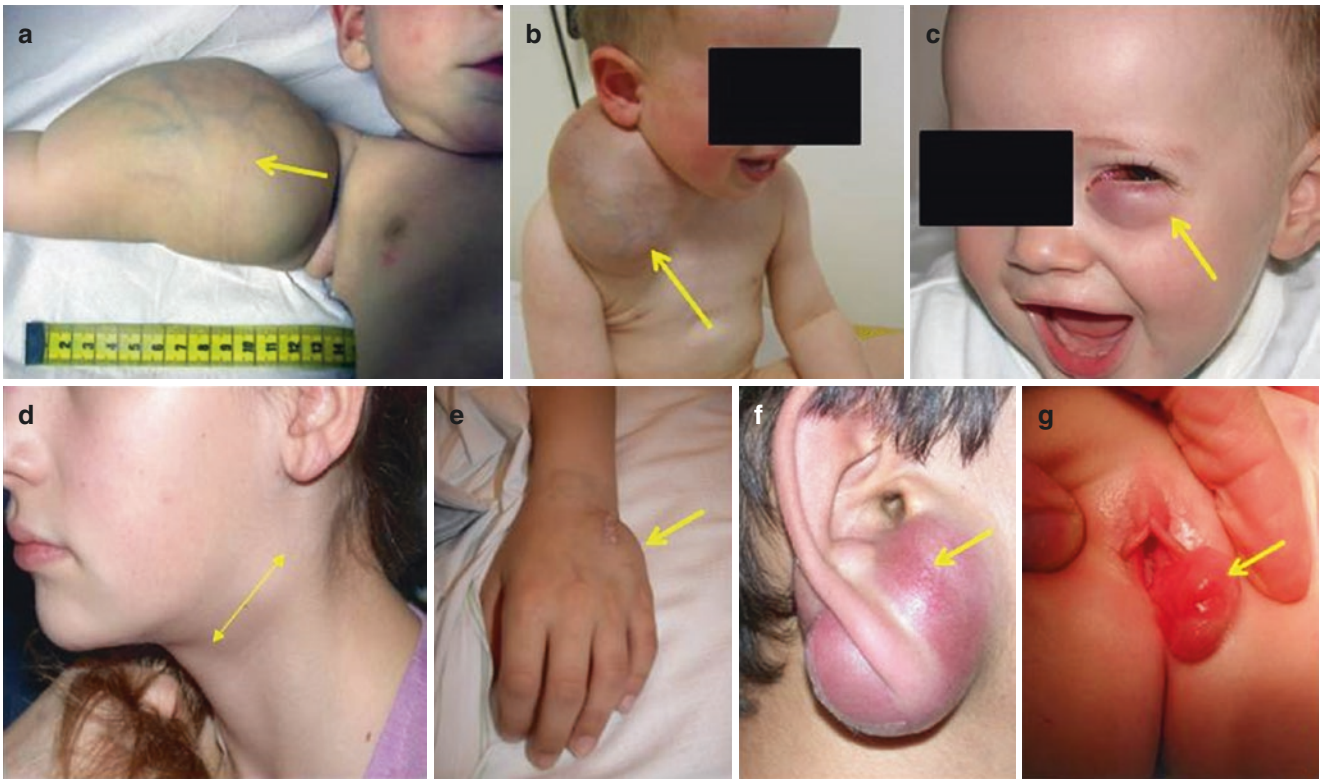


Fig. 1.4 Clinical aspect of children with soft tissue tumors. (**a**), 6-month-old infant with an arm localized infantile fibrosarcoma; (**b**), 14-month-old child with a cervical localized rhabdoid tumor; (**c**), 7-month-old infant with an orbital localized alveolar rhabdomyosarcoma; (**d**), 14-year-old adolescent with a cervical localized synovial sarcoma; (**e**), 13-year-old adolescent with an arm alveolar rhabdomyosarcoma associated with regional nodal extension; (**f**), 12-year-old preteenager with an ear embryonnal localized rhabdomyosarcoma; (**g**), 5-month-old infant with a bifocal vaginal and bladder embryonnal rhabdomyosarcoma

in the therapeutic strategy. RMS are very chemosensitive tumors [34]. The age of the patient, the tumor extension, and the potential resectability of the primary tumor also play an important role. Survival for most of these sarcomas is favorable, although lower in adolescents than in younger patients and in certain histological types and metastatic presentations that are difficult to cure with current treatments [35, 36].

1.2.10 Bone Tumors

Malignant bone tumors are most often primary in children. Bone metastases can be seen in neuroblastoma, Wilms' tumors, and in primary bone tumors—but in these cases, clinical context is obvious [37]. This chapter will therefore focus on primary bone tumors only. Primary bone tumors are the sixth most common neoplasm occurring in children and constitute approximately 6% of all childhood malignancies [38]. There is a peak incidence in 15–19-year-old individuals with these lesions being the third most common tumors in adolescents and young adults, exceeded only by leukemia and lymphoma [39]. Osteosarcoma and Ewing sarcoma are the most common malignant primary bone tumors in this age group [40–42]. Although the overall incidence of osteosarcoma is higher than Ewing sarcoma in adolescents younger than 20 years, Ewing sarcoma is more common in children younger than 10 years of age. Of note, focal bone Langerhans cell histiocytosis can be a differential diagnosis.

Patients carrying a bone tumor are most often symptomatic with pain, swelling, pathologic fractures, and, sometimes, constitutional symptoms such as fever or weight loss. Osteosarcoma occurs mostly in the extremities such as the knee, femur, and humerus; Ewing sarcoma's most common sites are pelvis, femur diaphysis, and chest wall. Metastases to the lungs or other bones are not rare, with Ewing sarcoma also leading to possible bone marrow involvement. Local radiological assessment most often comprises local X-rays and MRI.

Formal diagnosis will need a surgical bone tumor biopsy, but cytology or tru-cut biopsy can be of help in case of soft tissue involvement, which is frequent in Ewing sarcoma. Detection of the specific *EWS-FLI1* transcript in molecular biology is helpful to confirm Ewing's sarcoma diagnosis [43]. Treatment consists of a combination of chemotherapy and limb-sparing surgery. Adjuvant radiotherapy is also discussed in Ewing sarcoma. Overall survival can reach 60–70% for patients with a localized disease [39, 42].

1.3 Radiological Diagnostic Approach in Extracerebral Pediatric Tumors

Cécile Cellier and Hervé J. Brisse

It should always be kept in mind that the aim of radiological examinations is not to define a single diagnosis, but to define a "group of possible diagnoses" and consequently to propose the appropriate management.

The radiological patterns of the various tumor types have been already largely described in review articles [44–49] or reference books [50, 51]. Depending on the anatomical localization of the lesion, it is generally agreed that the radiological diagnosis should consist of initial X-ray and ultrasound examination followed by CT scan or MRI. The role of imaging is essential in these cases, either to confirm its benign nature or, on the contrary, to guide the indication for biopsy if the lesion is potentially aggressive or of a nonspecific appearance.

The nature of the sample and its modality should always be previously discussed in a multidisciplinary team. Clinical examination is still the first step of diagnosis. Age, sex, and site of the lesion are useful pieces of information leading to diagnosis [48, 49]. Tumors can occur anywhere, but some sites can help to guide the diagnosis. Genetic-predisposing diseases such as type-1 neurofibromatosis, Beckwith-Wiedemann syndrome, Hereditary retinoblastoma (RB1 gene mutation), and so on must be clinically investigated because they may orientate on tumor type (Table 1.1).

Diagnostic samples may be obtained via simple palpation-guided cytology without aneasthesia, especially in infants presenting superficial lesions. In deep lesions a combination of cytology and core needle biopsy is preferred.

1.3.1 Imaging Techniques

Conventional radiography is of diagnostic value as first-line investigation, particularly for limb lesions. Added to plain films, ultrasound is part of the simple first-line examination for cases clinically and radiologically suggestive; the radiography-ultrasound establishes the diagnosis of some pseudotumors (adenitis, abscess), benign tumors (lipomas, infantile hemangioma, fibromatosis colli) or malformations (cystic lymhangioma, venous malformation). Doppler analysis confirms the avascular nature of cystic lesions or, conversely, assesses the type of blood supply of solid lesions.

MRI technique is currently the gold standard for evaluation of soft tissue and bone tumors due to its excellent tissue contrast [47, 49, 52–56] and is mandatory before biopsy. If paravertebral neuroblastoma is suspected, MRI is a mandatory technique to detect an endocanalar extension, but in other localizations, CT scan may be sufficient [57, 58]. All other thoraco-abdominal tumors are diagnosed using MRI or CT scan if MRI is not available [59–61].

Figure 1.5 illustrates clinic-radiologic strategy to obtain pathologic samples in pediatric tumors. Figures 1.6, 1.7, 1.8, 1.9 and 1.10 provide examples of radiological evaluation of pediatric masses.

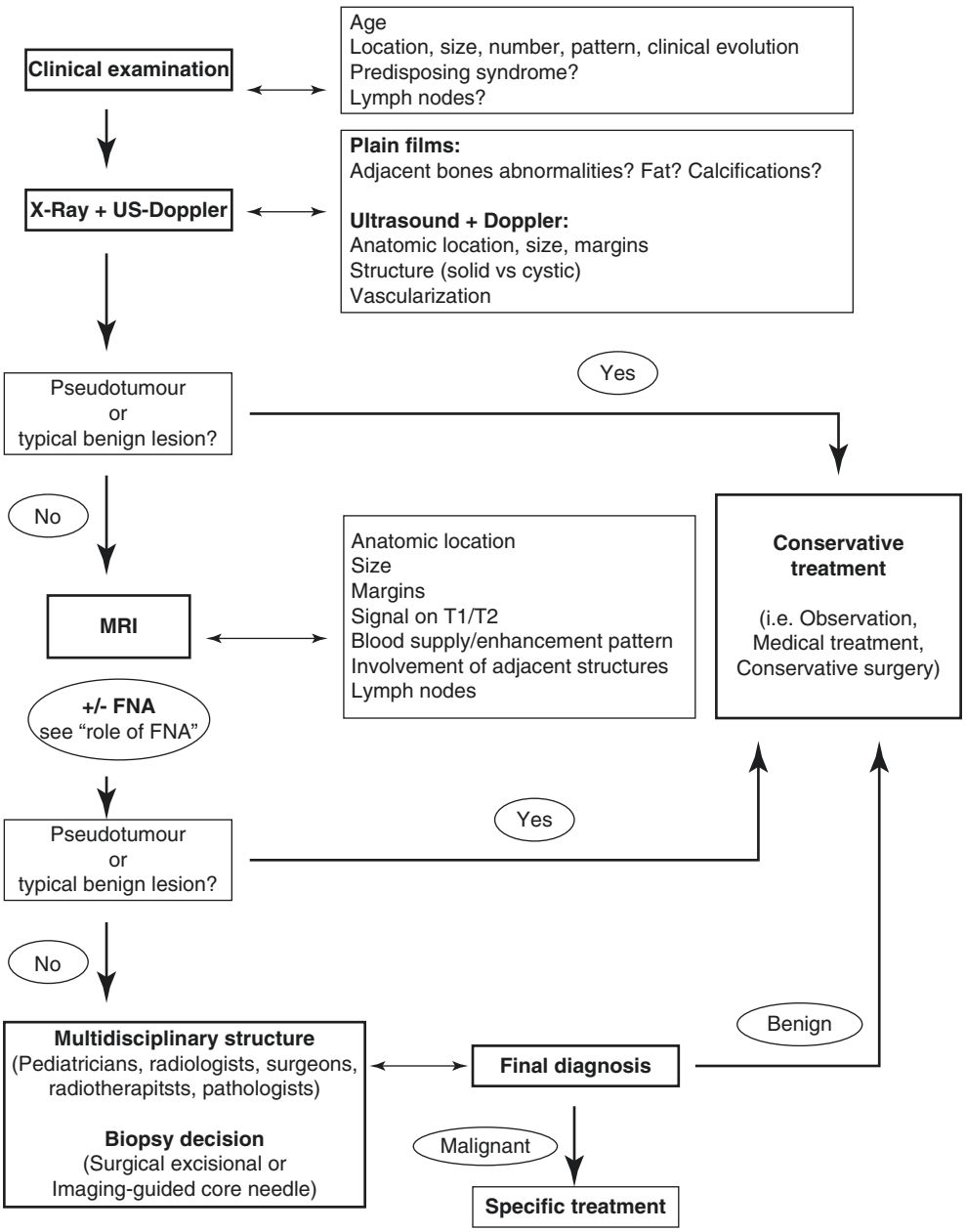


Fig. 1.5 Diagnostic clinico-radiologic strategy in pediatric tumors

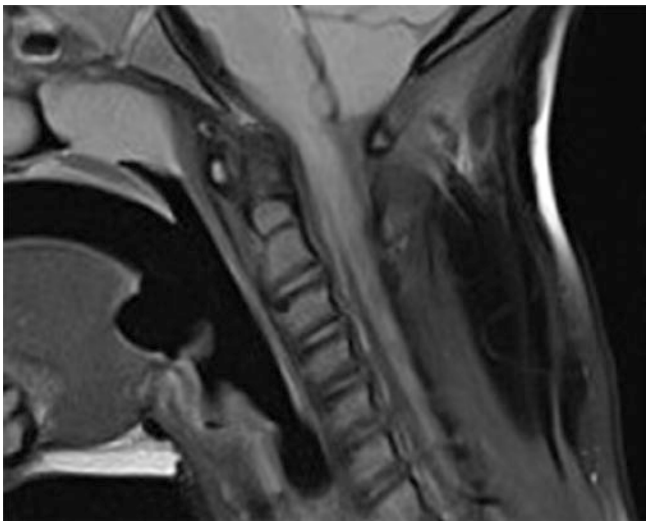


Fig. 1.6 Boy, aged 5 years, with Gardner syndroma presenting a cervical aponeurotic fibroma with very low signal on MRI

1.3.2 Tumor Biopsy

Deep-sited lesions should be sampled under radiological guidance. Superficial tumors may be sampled under either radiological or palpatory guidance.

1.3.2.1 Surgical and Core Needle Biopsies

In the absence of definite signs of benign lesion, a biopsy should always be performed. Consultation with the radiologist, the surgeon, and the pathologist allows them to define the biopsy tract using compartmental anatomy definitions [62, 63], the biopsy site (especially the most suspicious portion), and appropriate processing of biopsy specimens (tissue preservation for genetic studies). A surgical excisional biopsy or a percutaneous procedure (core needle biopsy) is decided according to the size and location of the mass. Although large lesions of the limbs can easily be biopsied without image guidance, deep-seated

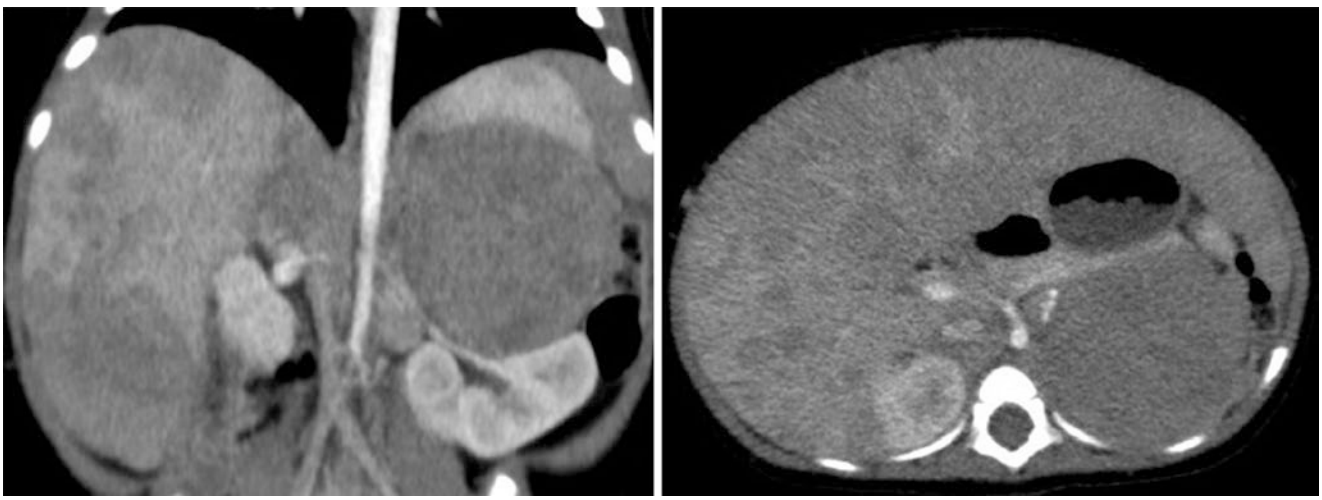


Fig. 1.7 Girl, aged 2 months, presenting a left adrenal neuroblastoma with liver metastasis on CT scan

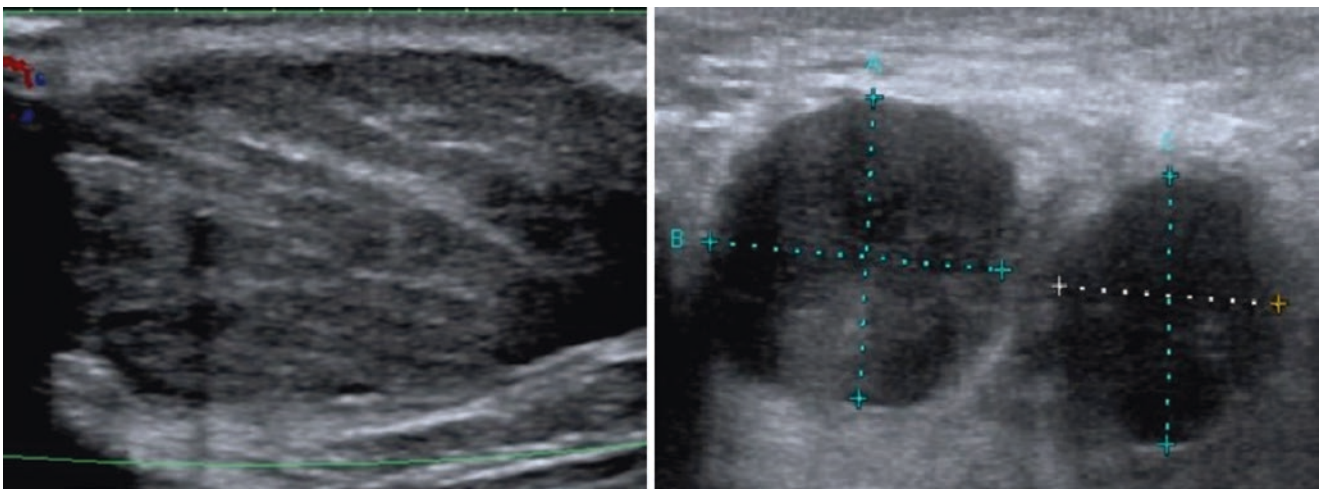


Fig. 1.8 Girl, aged 15 years, with a left hand intramuscauar mass and homolateral axillary lymphadenopathies on ultrasound examination corresponding to an alveolar rhabdomyosarcoma

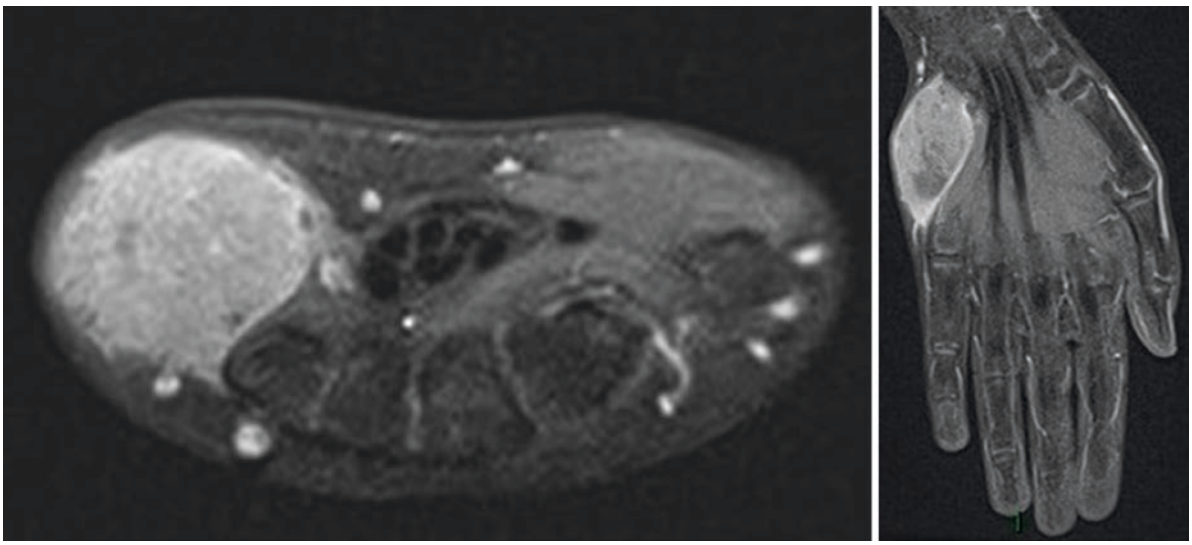


Fig. 1.9 Girl, aged 15 years, with a left hand intramuscular mass on MRI corresponding to an alveolar rhabdomyosarcoma

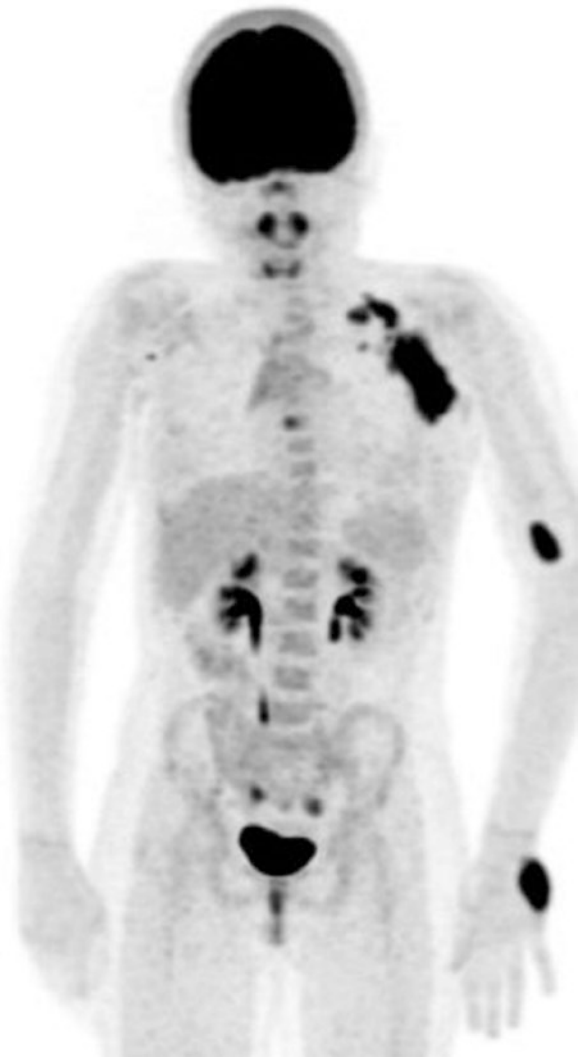


Fig. 1.10 Girl, aged 15 years, with a left hand intramuscular mass and homolateral supra trochlear, axillar y and supra clavicular lymphadenopathies on TEP scanner corresponding to an alveolar rhabdomyosarcoma

musculoskeletal lesions are difficult to target, and benefit from CT or US guidance. Imaging-guided percutaneous core needle biopsies are performed by trained radiologists, under local or general anesthesia, using CT or US guidance [64–68]. The procedure should ideally be performed by both the radiologist and the pathologist, the latter being the most qualified to evaluate the specimen quality and to separate the tissue for morphological and biological studies. If the specimen cannot be frozen immediately, it must not be fixed directly, but should be temporarily placed in culture medium such as Roswell Park Memorial Institute medium, RPMI.

1.3.2.2 Fine-Needle Aspiration

Fine-needle aspiration (FNA) usually does not replace biopsy but, in our experience, constitutes an excellent first-line and reliable diagnostic procedure, provided that it is performed and examined by trained pathologists. It is an inexpensive technique, almost without morbidity [69], and it can be performed under local anaesthesia. Using fine needles (23 Gauge, 0.6 mm of outer diameter), with ultrasound guidance if necessary, provides highly contributive cell aspirates [70, 71]. In the diagnostic strategy, FNA may be used right after initial imaging when the decision to perform or not perform a biopsy is pending, e.g., in case of clinically and radiologically presumed benign or pseudotumoral lesion, in case of a highly vascularized lesion at risk for a biopsy, or for suspected relapses. FNA material is excellent for ancillary techniques (this material is tumor-cell rich and stroma-cell poor) and allows karyotyping and molecular analyses. Cells should be stored in ethylenediaminetetraacetic acid (EDTA) (Figs. 1.11 and 1.12).

Fig. 1.11 Four hands procedure using ultrasound-guided cytological and histological samples

Fig. 1.12 Portative material necessary for palpation-guided or radiologically guided samplings

1.4 Ancillary Methods

Rocco Cappellesso and Ambrogio Fassina

For decades, open biopsy has been the gold standard for the diagnosis of pediatric tumors. Only after the demonstration of similar diagnostic accuracy, core needle biopsy (CNB) has been accepted as less invasive valuable diagnostic alternative. Fine-needle aspiration (FNA), on the other hand, was not used in routine diagnostics until few years ago, mainly because of the publication of earlier studies showing it achieved lower diagnostic sensitivity and specificity than CNB. A great contribution in the recent success of FNA was related to the possibility of performing ancillary techniques on the aspirates. These tests enormously enhanced the overall diagnostic performances of FNA and nowadays its reliability is almost unanimously accepted. FNA yields material suitable for immunocytochemistry (ICC), flow cytometry (FC), and molecular analyses. Moreover, the amount of collected cells is almost the same as in CNB. Furthermore, FNA material can be more representative than CNB in small or heterogeneous tumors, since the movements of the needle allow for sampling of different areas of the neoplasm. This chapter covers the main issues related to the application of ancillary methods in the cytological diagnosis of pediatric tumors [72–77].

1.4.1 An Overview of Molecular Alterations in Pediatric Tumors

The vast majority of pediatric tumors are represented by mesenchymal neoplasms and lymphomas. The diagnosis and classification of both these categories of tumors are based on morphology, immunophenotype, and demonstration of specific molecular alterations in the appropriate clinical and radiological context. Thus, the cytologist who is faced with a tumor likely belonging to one of these groups of malignancies is required to know which molecular modifications must be investigated and how the aspirate must be handled.

From a molecular point of view, these tumors can be divided in two main broad categories:

1. Neoplasms with stochastic, multiple, and complex molecular alterations
2. Neoplasms with recurrent simple molecular alterations

The first category encompasses all those tumors harboring random transfer, gain, or loss of large parts of chromosomes or DNA resulting in aneuploidy, composite and nonspecific karyotype, or multiple gene aberrations. These neoplasms are usually characterized also by a low degree of differentiation and marked pleomorphism. Molecular analyses in such cases are applied only to achieve a diagnosis of exclusion. Undifferentiated and pleomorphic sarcomas are clear examples. In the latter group, instead, are included all those tumors strongly related to a frequent definite cytogenetic modification or single gene mutation. The detection of the molecular alteration in such cases is often mandatory to attain a correct diagnosis. For instance, Burkitt lymphoma is characterized by a chromosomal translocation combining the oncogene MYC on chromosome 8 with immunoglobulin locus regulatory elements in chromosomes 2, 14, or 22, and the cytogenetic demonstration of MYC rearrangement is the gold standard for the diagnosis. Other examples are pediatric anaplastic large cell lymphoma and inflammatory myofibroblastic tumor, both harboring a chromosomal rearrangement involving the ALK gene. A cytologist should be aware of the specific molecular abnormalities of each tumor and how these can be identified. There are three levels at which the molecular alteration can be detected:

1. Chromosome
2. DNA/RNA
3. Protein

Each level corresponds to a technique to be applied. The example of Ewing sarcoma (ES)/primitive neuroectodermal tumors (PNET) is useful to clarify this issue (Fig. 1.13). For what concerns the first level, the translocation involving the chromosomal region 22q12 can be readily demonstrated by fluorescence in situ hybridization (FISH). In turn, the chromosomal modification causes the juxtaposing of the EWSR1 gene contained in the translocated material with another gene and this is detectable by amplification through polymerase chain reaction (PCR) of the fused genetic region and sequencing. However, this method is not used in routine diagnostics for technical reasons and it is preferred to identify the transcript by reverse transcriptase-polymerase chain reaction (RT-PCR) and sequencing. Indeed, the resulting combined gene is normally transcribed to RNA as any other gene and then translated into a chimeric protein that, in turn, can be detected by ICC, if a specific antibody is already available. Further examples of pediatric tumors with known chromosomal abnormalities that lead to genetic alteration and aberrant protein product are listed in Table 1.3.

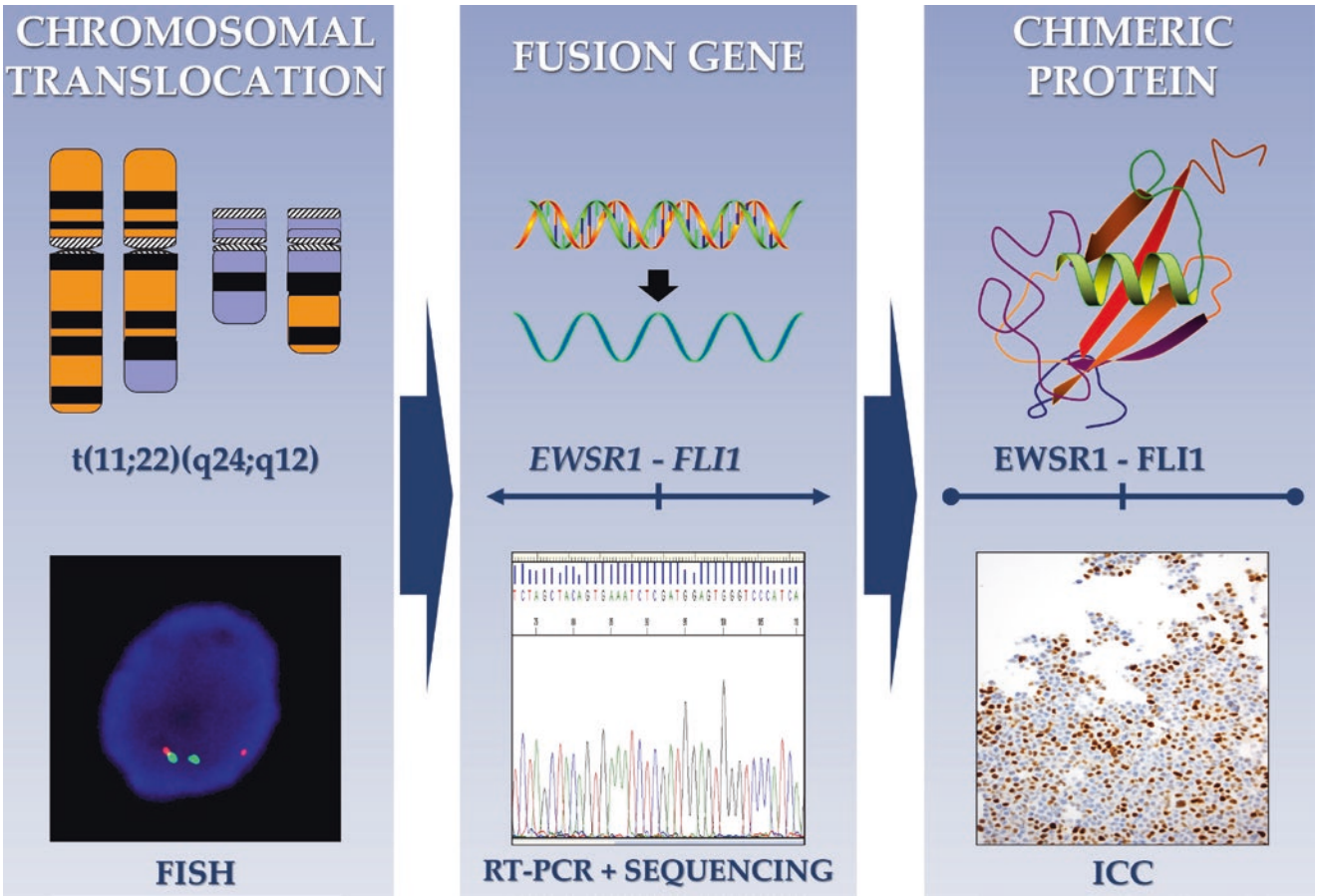


Fig. 1.13 The figure shows the three levels at which this molecular alteration of Ewing sarcoma (ES)/primitive neuroectodermal tumors (PNET) can be identified and by what method