teratoma; immature teratoma; and teratoma with malignant elements. Mature teratoma comprises only mature elements such as the skin, hair, fat tissue, cartilage, bone, glands, etc. Immature teratoma contains immature elements such as neuroepithelial tissue and immature mesenchyme. The presence of microscopic foci of yolk sac tumor, rather than the histological grade of immaturity, is a valid predictor of recurrence, and grading of immature teratoma is unnecessary in children.[5] In rare instances, mature teratoma also recurs as a malignancy, but a careful review of the original tumor usually reveals occult malignant elements.[6] The biological behavior of teratomas with malignant elements (yolk sac tumor, choriocarcinoma, seminoma, and dysgerminoma) is determined by the most malignant element in the tumor. Elevations of serum alpha-fetoprotein (AFP) levels usually indicate the presence of foci of yolk sac tumor somewhere in the tumor (Table 27.3). Therefore, when the serum AFP is high, the tumor should be extensively sampled and carefully examined before the patients' postoperative treatment is determined. The majority of children with teratoma, however, usually do well with surgery alone regardless of primary tumor location, and salvage chemotherapy is successful in the few instances of malignant recurrence.[7]

Mature Teratomas

These are usually multicystic tumors that may have bone, cartilage, teeth, and hair. The cysts have thick clear, mucoid, viscid or yellow material with intervening gray-tan tissues.[7] While those in the gonads are well encapsulated, the extragonadal one do not have a capsule. Histologically they have representative tissues from all the three germ cell layers, namely the ectoderm, mesoderm and endoderm. Structures most commonly found are skin and its appendages, adipose tissue, brain, intestinal epithelium and cystic structures lined with squamous, cuboidal or flattened epithelium. In addition the mediastinal teratomas frequently contain hematopoietic, pancreatic or pituitary tissue.[8] In infants and young children, mature teratomas always behaves in a benign fashion. Many ovarian mature teratomas are associated with nodules of mature glial tissue implanted throughout the peritoneum- gliomatous peritonei,[9] or in the lymph nodes.[10] These mature glial implants do not alter the stage or prognosis but the same can not be said when there are implants of immature tissues.

Table 27.3: Normal ranges of serum AFP in infants

Age	Mean ± SD (hg/mL)
Premature	134,734 ± 41,444
Newborn	48,406 ± 34,718
0-2 weeks	33,113 ± 32,503
2 week-1mth	9,452 ±12,610
2 month	323 ± 278
3 month	88 ± 87
4 month	74 ± 56
5 month	46.5 ± 19
6 month	12.5 ± 9.8
7 month	9.7 ± 7.1
8 month	8.5 ± 5.5

Immature Teratomas

These are grossly and histologically similar to the mature teratomas, but in addition they have various immature tissues derived from the ectoderm, mesoderm or the endoderm. The grading of immature elements is done using the Dehner's modification[11] of the Thurlbeck and Scully system[12] (Table 27.4), on slide with the largest amount of aggregated immature tissue. This grading system basically uses the number of low power fields of primitive neuroepithelium per slide.[13] In children the immature teratomas are usually benign and are known to behave in a malignant fashion only if foci of malignant

Table 27.4: Histologic grading system of ovarian immature teratomas (Dehner's)

Grade	Microscopic appearance
0	Mature tissue only
1	Mainly mature tissue, but some immature also Neuroepithelium or other immature tissue Limited to 1 low power field per slide
2	Moderate amount of immature tissue present Neuroepithelium or other immature tissue Occupies 1 to 3 low power field per slide
3	Abundant immature tissue Neuroepithelium or other immature tissue Occupies more than 4 low power field per slide.

germ cell element is present in which case the tumor is actually a mixed malignant germ cell tumor. The malignant element is usually endodermal sinus (EST or yolk cell tumor-YST) or rarely neuroblastoma or meduloblastoma. The malignant germ cell element can easily be missed histologically as they are small and are associated with immature neural elements and frequently do not stain positive for AFP. In a recent report from the POG/CCG in 1998,[5] it was noted 55.6 percent of the immature teratomas registered in this combined study of 135 immature teratomas were actually mixed tumors and all of these had yolk sac component with a few having additional malignant components as well. The remaining 44.4 percent were pure immature teratomas. Though 47 percent of the immature teratomas were of grade 3, but it was reported that the grade did not correlate with the age or the outcome. In this study[5] it was noted that there was significant correlation between the stage and the presence of foci of EST (YST) and that of grade and the presence of a foci of EST (YST). The study concluded that the presence of microscopic foci of EST rather than the grade of immature teratoma, was the only valid predictor of recurrences[5].

Malignant Teratomas

The teratomas containing yolk sac element grossly appear as mature teratomas but may be more solid depending on the quantity of malignant component tissue present. Microscopically the foci of yolk sac tumor in the teratomas appear as small glandular or reticular structures with large, vesicular nuclei within loose myxoid background. These yolk sac tumor elements do not stain positive for AFP.

Teratomas are described below according to their original sites.

SACROCOCCYGEAL TERATOMA

Sacrococcygeal teratoma is a tumor that frequently occurs in neonates and infants and affects predominantly female children.[15,16] Classically, tumors are classified into four types:[15] Altman type I (46.7%), the most common type are tumors that are predominantly external with a minimal presacral component; type II (43.7%) tumors are external but have a significant intrapelvic component; type III (8.8%) tumors are external but pelvic and extend significantly into the abdomen; and type IV (9.8%) tumors are entirely presacral.

Sacrococcygeal teratoma is a troublesome condition if the tumor is diagnosed after delivery has started. Dystocia at delivery and rupture of the tumor are often fatal to the infant. Recently an increasing number of sacrococcygeal teratomas have been detected by antenatal ultrasonographic (US) examination of the fetus.[17] Prenatal US and magnetic resonance imaging (MRI) are useful in making a differential diagnosis between sacrococcygeal teratoma, myelomeningocele, and other tumors. They also provide some information on the extent of the tumor extension into the pelvis and the presence or absence of polyhydramnios, fetal hydrops, and intratumoral hemorrhage. Massive hemorrhage into the tumor may occur spontaneously in utero and cause anemia and hypoproteinemia followed by fetal hydrops. Fetuses with sacrococcygeal teratoma that are mainly solid in appearance and are highly vascularized have a higher risk of developing hydrops, and the presence of a solid tumor is a significant negative prognostic factor.[17,18] High-output cardiac insufficiency as a result of vascular shunting through the tumor is another cause of fetal death. It is generally agreed that a fetus with a large sacrococcygeal teratoma (usually more than 5 cm in diameter) detected prenatally should be delivered by cesarean section to prevent death from tumor rupture or hemorrhage.[16] Since spontaneous intratumoral hemorrhage may result in fetal death, it is recommended that elective cesarean section be performed at a high-risk obstetric center at 32 to 34 weeks of gestation when fetal maturity is deemed adequate for neonatal survival.[16]

Primary treatment of sacrococcygeal teratoma is complete removal of the tumor along with the coccyx. Although emergency removal of the tumor is sometimes performed in a case with prenatally diagnosed sacrococcygeal teratoma, the removal can be delayed until the neonate's general condition becomes stabilized. During that time, the extent of the tumor is examined by MRI or computed tomography (CT), and the serum levels of AFP and beta-human chorionic gonadotropin (β-hCG) should be examined (Fig. 27.1). Tumor resection is usually carried out in the prone position and is performed using a chevron skin incision. When tumors extend extensively into the pelvis, initial dissection through the abdomen is necessary before the presacral dissection. The proximal extent of the tumor can be defined by rectal examination or by barium enema, and the tumor should be dissected close to its capsule so

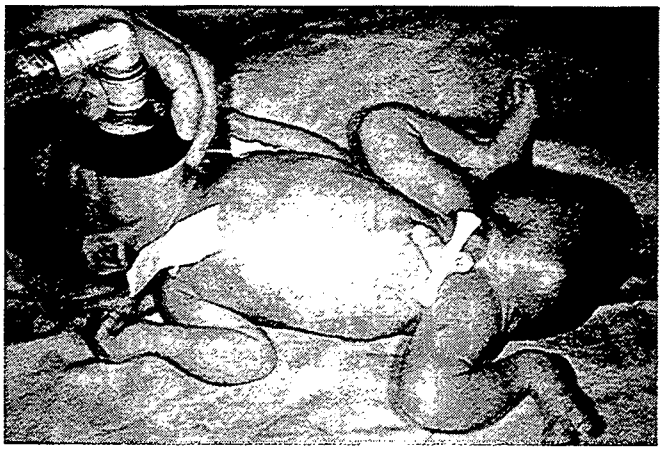


Figure 27.1: Appearance of a sacrococcygeal teratoma

that the retrorectal muscles and nervous network around the rectum can be preserved. Because failure to remove the coccyx is considered to be associated with a high recurrence rate, the coccyx should be removed with the tumor. The dissection between the coccyx and the sacrum makes it easier to dissect the tumor cranially into the retrorectal space. A significant proportion of patients who have undergone resection of sacrococcygeal teratoma are reported to have deficient anorectal function and urinary incontinence (neurogenic bladder).[19,20] These impairments are associated with surgical dissection, and therefore, careful and meticulous surgical techniques are required, especially when the tumor extends deeply into the pelvis and retroperitoneum.

Most tumors in the sacrococcygeal region are mature or immature teratomas, and less commonly yolk sac tumors. A retrospective survey by the Children's Cancer Group showed that 69 percent of sacrococcygeal teratomas were mature teratomas, 20 percent immature teratomas, and 11 percent yolk sac tumors at presentation.[21] The rate of malignancy increases with age at diagnosis, which is presumably caused by a delay in diagnosis of the less apparent lesions. Recurrent disease develops in 7 to 11 percent of sacrococcygeal teratomas within 3 years after resection.[21,22] The recurrent diseases usually are yolk sac tumors and are accompanied by an increase in serum AFP. In these instances in which a mature teratoma recurs as a malignancy, an occult focus of malignancy might have been overlooked when the original tumor was resected.[6] Studies suggest that patients should be carefully followed up for at least 3 years after the resection of benign teratomas. Sacrococcygeal teratomas with malignant components and those with recurrent tumors as malignancy are treated according to the treatment protocol for malignant germ cell tumors. The staging for these malignant sacrococcygeal teratomas is done as for any extragonadal malignant germ cell tumor (Table 27.5).

OVARIAN TERATOMA

The majority of ovarian tumors in children and adolescents occur between ages 10 and 19 years, but they may occur at any age during infancy and childhood (Fig. 27.2). The most common of these tumors is benign cystic teratoma. These tumors usually present as an asymptomatic mass but may cause acute abdomen due to torsion of the ovary and the fallopian tube. Ovary-preserving tumor extirpation is the usual procedure for ovarian teratoma. Maintaining gonadal function and fertility is a concern, in particular when a teratoma develops in the bilateral ovaries, which occurs synchronously or metachronously in 4 to 7 percent of patients.[23,24] Minilaparotomy or laparoscopy-assisted cystectomy as minimally invasive surgery is an alternative to traditional laparotomy in patients with ovarian teratoma.[25] Salpingo-oophorectomy is necessary when ischemia of the ovary due to torsion is irreversible or when foci of malignant tumor are found by microscopic examination of the extirpated specimen.

Ovarian teratomas are sometimes associated with peritoneal implants that contain only mature glial tissues. Since the presence of gliomatosis peritonei does not alter patient prognosis, additional surgical approaches other than sampling or biopsy are usually unnecessary.[26] In patients with ovarian immature teratoma, surgery alone is also curative even if the serum AFP level is elevated.[27] Even when microscopic examination reveals small foci of yolk sac tumor, chemotherapy may be reserved until a recurrent tumor develops.[27] In such cases with relapse salvage is feasible with conventional chemotherapy.

Cystic ovarian tumors that are sometimes seen in newborns are usually not teratomas, but follicular cysts. They most often resolve spontaneously, but sometimes cause torsion, especially when they larger than 6 cm in diameter and 48 cm^3 in volume.[28] We have found that MRI is more useful than US in detecting hemorrhage and for determining indications for surgery in neonatal ovarian cysts.[28]

Table 27.5: Chemotherapy regimens for germ cell tumors in children

1. PVB (Ref.78)

Week	1	2	3
CDDP	↓		
VB	↓		
BL	↓	↓	↓

CDDP (Cisplatin); 100 mg/m^2 on day 1, VB (Vinblastine); 0.15 mg/kg on day 2, BL (Bleomycin); 15 mg/m^2 on days 2, 9 and 16.

2. Modified PVB (Ref.130)

Week	1	2	3
CDDP	↓		
VB	↓		
BL	↓		

CDDP (Cisplatin); 100 mg/m^2 on day 1, VB (Vinblastine); 0.15 mg/kg on day 2, BL (Bleomycin); 15 mg/m^2 on day 2.

3. BEP (Ref.39)

Week	1	2	3
CDDP	↓		
VP16	↓↓↓		
BL	↓		

CDDP (Cisplatin); 100 mg/m^2 on day 1, VP16 (Etoposide); 120 mg/m^2 on days 1, 2 and3, BL (Bleomycin); 15 mg/m^2 on day 2.

4. JEB (Ref.130)

Week	1	2	3
Carbo.	↓		
VP16	↓↓↓		
BL	↓	↓	↓

Carbo. (Carboplatin); dosage calculated from the EDTA glomerular filtration rate (approximately 600 mg/m^2 on day 1, VP16 (Etoposide); 120 mg/m^2 on days 1, 2 and 3, BL (Bleomycin); 15 mg/m^2 on days 2, 9 and 16.

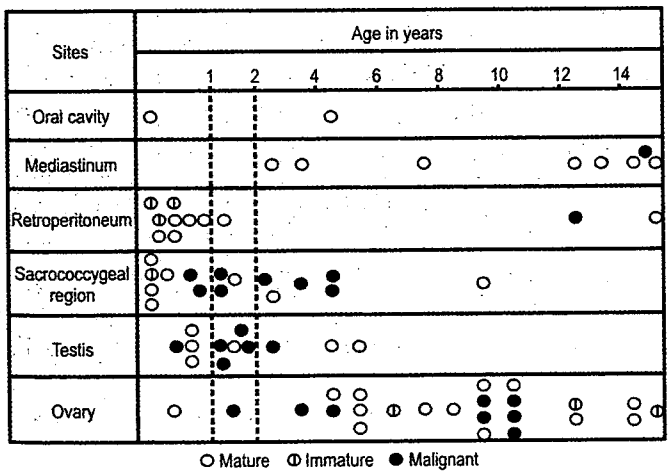


Figure 27.2: Age distribution of teratomas and malignant germ cell tumors according to the site of origin is shown. Cases are from a series at the Department of Pediatric Surgery, University of Tokyo

TESTICULAR TERATOMA

Testicular teratomas present as a painless scrotal mass in infants and children younger than two years of age and are sometimes found at birth.[29,30] Prepubertal teratomas of the testis may be associated with high serum AFP levels, but the clinical course of patients is equally uneventful with radical orchiectomy.[29,30] Because of the favorable characteristics of the disease, testis-sparing surgery or tumor enucleation is attempted in patients whose testicular parenchyme appears to be preserved.[31]

MEDIASTINAL TERATOMA

The mediastinum is the second most frequent site of extragonadal teratomas. Mediastinal teratomas occur in newborns to adolescents, and arise predominantly in the anterior mediastinum, occasionally in the posterior mediastinum, and rarely in the pericardial and

intracardiac region.[32] Mediastinal teratomas typically manifest on CT as a heterogeneous mass containing soft tissue, fluid, fat, or calcification.[33] Some patients are asymptomatic and diagnosis is made incidentally by chest X-ray. However, affected children usually manifest symptoms such as dyspnea, cough, or chest pain. When the tumor causes severe respiratory distress in neonates mimicking congenital diaphragmatic hernia, emergency surgery to relieve lung compression and postoperative care supporting respiration are required.[32,34] Surgical approaches to mediastinal teratomas are either unilateral thoracotomy or median sternotomy, and the latter is necessary in some patients with large, bilaterally invasive lesions.

CERVICOFACIAL TERATOMA

Cervicofacial teratomas, arising in the oral cavity, nasopharynx, orbit, and anterior neck, are rare and usually noted at birth. A form of cervicofacial teratoma that arises from the palate or pharynx in the region of the basisphenoid (Rathke's pouch) is called epignathus.[35] Tumors may be diagnosed prenatally by US that is indicated for maternal polyhydramnios.[36] Polyhydramnios is caused by fetal inability to swallow the amniotic fluid. Life-threatening airway obstruction may occur at birth and require urgent resuscitation including bronchoscopy-assisted intubation or tracheostomy.[36] When an airway-obstructing mass is diagnosed prenatally, delivery by elective cesarean section is recommended, and the airway should be secured while the maternal-fetal placental circulation is maintained. Differential diagnosis includes cervical lymphangioma (hygroma), which should always be kept in mind. Careful examination and, if necessary, CT or MRI enable differential diagnosis between cervical teratoma and lymphangioma. Once the diagnosis of airway-obstructing teratoma is made, early resection after stabilization of the patient is the choice of treatment because it is the most effective method to control the airway.[35,36] The operative mortality rate is low, but significant morbidity such as recurrent nerve injury, hypothyroidism, or hypoparathyroidism may occur. The functional and cosmetic outcome of surgical treatment is generally excellent.

Others

Other sites of teratoma are the retroperitoneum, stomach, and intracranial region. Gastric teratomas account for only 1 percent of all teratomas in children and occur predominantly in boys.[37] A partial gastrectomy is required to remove the tumor.

TUMOR MARKERS FOR GERM CELL TUMORS

The clinical markers are useful in predicting response or indicating the presence of residual or progressive disease.[38] The markers are categorized as follows:

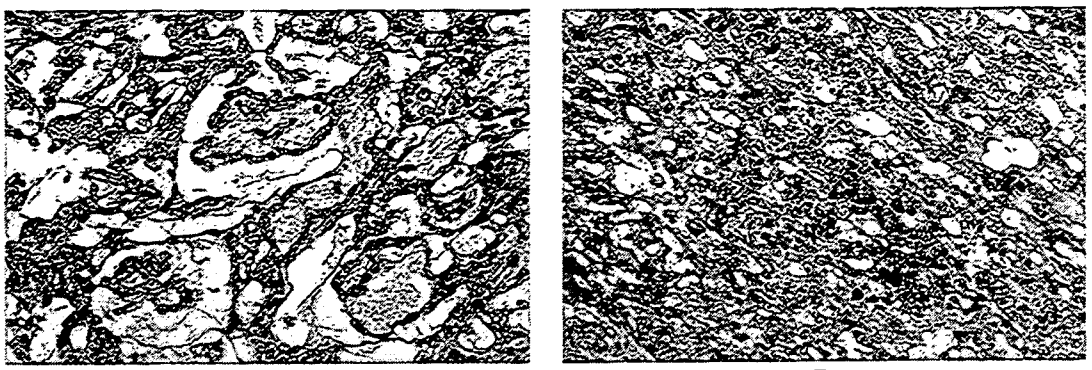
1. Oncofetoproteins – AFP and β-hCG
2. Cellular enzymes – LDH and placental alkaline phosphatase (PLAP).
3. Cytogenetic and molecular markers.

When a tumor of germ cell origin is among differential diagnoses, serum levels of AFP and β-hCG should be measured.

AFP

Serum AFP levels are elevated in patients with yolk sac tumors and correlate well with the clinical course. However, when the patient is younger than 10 months of age, serum AFP is physiologically elevated, and the normal ranges of AFP[39] should always be taken into account. Immunohistochemical staining demonstrates whether tumor cells are positive for AFP[40,41] (Figs 27.3A and B). After surgical excision of a yolk sac tumor, serum AFP levels decrease to normal with a half-life of approximately 5 days. Re-elevation of AFP levels in the serum indicates the presence of metastatic or recurrent disease.

Serum levels of AFP may be increased when there are foci of yolk sac tumors in teratomas. Conversely, when teratomas are associated with increased AFP levels, yolk sac tumor elements occur somewhere in the tumor. A patient diagnosed with immature teratoma and high AFP level at the time of diagnosis is at a higher risk of malignant recurrence.[42] Since malignant foci may not be identified despite extensive histological examination, it is recommended that immature teratomas with high serum AFP levels should be treated similarly to malignant germ cell tumors.[42] Routine postoperative adjuvant chemotherapy, however, is not given because salvage chemotherapy with the conventional dose is effective in eradicating recurrent tumors.[27] In patients with sacrococcygeal teratomas, serum levels of AFP should be measured during the postoperative follow-up, because yolk sac tumors recur in 7 to 11 percent of patients and almost all recurrences are accompanied by an increase in serum AFP level.[21,22] Only in a small number of patients with a huge immature teratoma does



A B

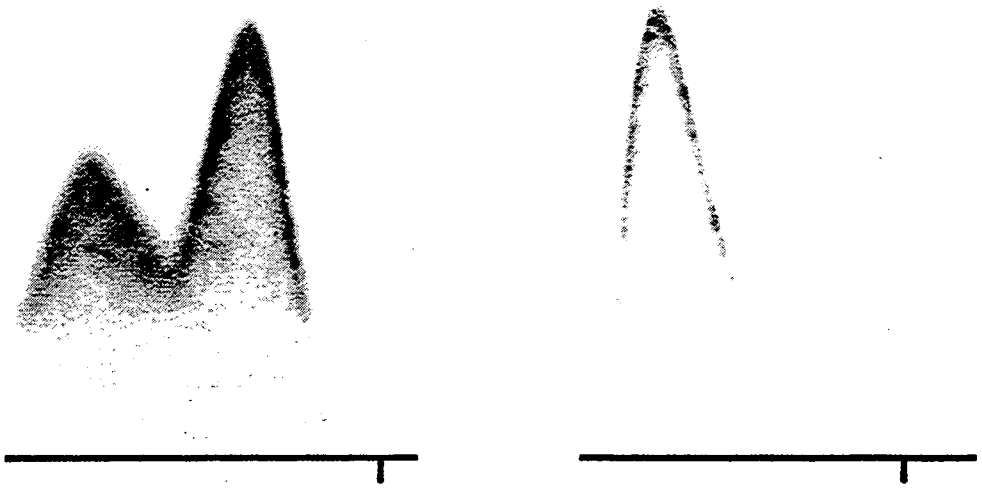
Figures 27.3A and B: (A) Histologically, yolk sac tumor is characterized by the formation of Schiller-Duval bodies, and **(B)** The presence of periodic acid Schiff (PAS)-positive eosinophilic hyaline globules

a moderate increase in serum AFP occur due to the gastrointestinal epithelia involved in the tumor.[43]

Analysis of the AFP subfraction profile is useful to distinguish germ cell tumor derived-AFP and that derived from hepatic tumors.[44-46] In germ cell tumors the fucosylation index, defined as the percentage of AFP of which the sugar chain is fucosylated and specifically binds to *Lens culinaris* agglutinin in total AFP, is 99 percent ±2 percent.[47] The glucosaminylation index, defined as the percentage of concanavalin A-nonreactive AFP in total AFP, is 45 percent ±20 percent. Both indices are significantly higher than those of AFP from hepatic tumors and benign liver disease (Figs 27.4A and B).[44,47] These indices can also be used to differentiate AFP produced in yolk sac tumors from physiologically elevated AFP in early infancy.[39,44] Particularly, determination of the glucosaminylation index is sufficient to differentiate between AFP from yolk sac tumors and that from other sources.[44,45]

β-hCG

Choriocarcinomas secrete hCG, and β-hCG is measured to monitor the serum levels of hCG. Because the β subunit of hCG is identical in amino acid sequence to β subunits of pituitary hormones, radioimmunoassay with antibodies to β-hCG is used to measure serum levels



Figures 27.4A and B: Subfractionation of serum AFP was carried out using concanavalin A (Con A) affinity immuno-electrphoresis. In a patient with malignant yolk sac tumor, two peaks (Con A binding and Con A non-binding) appear **(A)**, whereas only one peak of Con A binding peak will appear in serum AFP from hepatoblastoma **(B)** and benign hepatic conditions such as biliary atresia[34]

of hCG. hCG is produced by one of the two components of choriocarcinoma, syncytiotrophoblasts, and serum β-hCG is a useful marker to monitor the disease course.

In addition to choriocarcinoma, some dysgerminomas contain reduced numbers of syncytiotrophoblasts in tumors, and are associated with slightly elevated serum levels of β-hCG. Elevation of serum β-hCG levels in dysgerminomas is limited compared with that in choriocarcinoma, but RIA for β-hCG is sensitive, and monitoring with β-hCG is sometimes useful in monitoring clinical course of patients with dysgerminoma.

Other Markers for Germ Cell Tumors

CA 125, an antigen defined by a monoclonal antibody, is related to a high molecular-weight glycoprotein that is expressed in coelomic epithelium during embryonic development.[48] In adults the normal values for CA 125 in serum are less than 35 U/ml, and the levels are increased in patients with epithelial ovarian cancer. On the other hand, little is known about the oncologic significance of the marker in children. An increase in serum CA 125 is observed in patients with yolk sac tumor and embryonal carcinoma.[49] Although the significance of CA 125 in childhood germ cell tumors is not yet fully understood, the measurement of serum levels may sometimes be useful in monitoring the disease.

Lactate dehydrogenase isoenzyme-1 (LDH-1) increases in the sera of patients with germ cell tumors including yolk sac tumor, dysgerminoma, and choriocarcinoma. Both total serum LDH activity and LDH-1 activity are increased in patients with yolk sac tumor.[50] The percentages of LDH-1 isoenzyme range from 60 to 88 percent in these patients and are higher than the normal value of 46 percent.

Cytogenetic Markers

Inspite of histologic heterogeneity, adolescent testicular germ cell tumors appear to be relatively genetically homogenous.[51] They have an aneuploid DNA content.[7,52] The characteristic cytogenetic finding is the presence of two copies of 12p of uniparental origin, with retention of 12q heterozygosity, i.e. i(12p).[51] Germ cell tumors associated with more than 3 copies of i(12p) are associated with poor prognosis.[53] The ovarian mature teratomas are cytogenetically normal in 95 percent cases,[54] while 60 percent of the immature teratomas show chromosomal abnormalities although the i(12p) has not been reported in them.[55,56] The histologic grade of immature ovarian teratomas and their DNA content have been reported to be related; grade 1 and 2 tumors are diploid and grade 3 aneuploid.[57] It has also been observed that diploid grade 3 immature teratomas have a better prognosis than aneuploid grade-3 immature teratomas.[56] i(12p) which is absent in pure immature teratomas has been reported in the those immature teratomas containing endodermal sinus tumor component. Most malignant ovarian germ cell tumors are aneuploid and contain the i(12p).[58,59] In children less than 4 years of age, the histology and behavior of GCT derived from the gonadal or extragonadal sites are similar. Majority of these are teratomas, and regardless of the immaturity and site all are diploid tumors of normal karyotype and behave in a benign fashion if they can be resected.[60,61] Malignant GCT in this age group are almost exclusively yolk sac tumors which are often diploid or tetraploid.[57,62] The cytogenetic analyses of these malignant germ cell tumors in children less than 4 years of age has shown recurrent abnormalities involving chromosome 1,3, and 6 but have not shown the presence of i(12p).[62]

MALIGNANT GERM CELL TUMORS

Endodermal Sinus Tumor (Yolk Sac Tumors)

Endodermal sinus tumor (EST) are the commonest pure malignant germ cell tumor in children and the commonest germ cell tumor, benign or malignant, of testes in children.[63] It has been described in the testes, ovary, sacrococcygeal region and less frequently in the vagina, retroperitoneum, mediastinum and the pineal gland. They can metastasize to the lymph nodes, lungs, liver and bone. In the sacrococcygeal teratoma this is the only malignant form described,[64-66] and in most other extragonadal sites it occurs as a component of mixed malignant germ cell tumor.[8,67,68] Grossly they are pale gray to gray yellow tumors that are friable and have varying amounts of mucoid tissue, hemorrhage and necrosis. Microscopically the individual cell may be small, with pale scanty cytoplasm, round to oval nuclei, an inapparent nucleoli, or they may be medium to large sized cells with clear vesicular nuclei and prominent nucleoli.[69,70] Mitosis may be present. There are four basic histologic patterns of yolk sac tumors described, namely pseudopapillary or festoon pattern, microcystic or reticular pattern, solid pattern, and polyvesicular vitelline pattern.

Pseudopapillary or festoon pattern: This form has the classic Schiller-Duval bodies, which are basically

composed of small central blood vessel closely surrounded by two layers of tumor cells, giving the appearance of a primitive glomeruli.

Microcystic or reticular pattern: This in addition to the Schiller-Duval bodies have many eosinophilic, hyalinized intra and extracytoplasmic globules and strands. The globules are positive for periodic acid-Schiff (PAS) and are diastase resistant and occasionally also stain for AFP and α-1 antitrypsin.The strands are also PAS positive and diastase resistant and also stain for laminin.[51]

Solid pattern: This pattern resembles embryonal carcinoma but the cells are smaller and less pleomorphic and have less prominent nucleoli than those of embryonal carcinoma. Hepatoid form, a variant of solid pattern, closely resembles fetal liver and stains for AFP, α-1 antitrypsin, albumin, and third and fourth components of complement.[71]

Polyvesicular vitelline pattern: This is characterized by small empty cystic structures, lined by single layer of malignant cells that are cuboidal to flat, in a loose myxoid stroma. This pattern is said to be associated with a better prognosis.[72]

There are two other distinct patterns described, namely enteric pattern[70] resembling fetal human gut, and the mesenchyme like pattern.[71]

Germinoma

The term germinoma was previously used for extragonadal malignant germ cell tumors which had the same histology as the dysgerminoma of the ovary and the seminoma of the testes.[73] Now the term germinoma is used for all these tumors regardless of the anatomic location.[74] In children germinomas are most commonly found in the ovary, anterior mediastinum and the pineal region. They account for 15 percent of all germ cell tumors.[75] They are the commonest malignant germ cell tumor of the ovary and the central nervous system.[7,66,67] Seminoma, though the commonest malignant germ cell tumor of the adult testis, is almost never found in the testes of infants and young boys, and rarely in adolescent testes.[63] Grossly these are solid, pinkish tumors with a rubbery consistency and have small areas of hemorrhage and necrosis. Microscopically the cells are large round, oval or polygonal, with clear cytoplasm, a distinct cell membrane, large nuclei with one or two prominent nucleoli. They are arranged in nests separated by bands of fibrous tissue with variable amount of lymphocytes. The tumor also has foreign body or Langhans giant cells and granulomas.[51] Antiferritin antibody and placental alkaline phosphatase (PLAP) are positive in germinomas from all sites and are useful markers for this tumor.[76]

Gonadoblastoma

These are neoplasms found in dysgenetic gonads or are sometimes associated with malignant germinomas.[77] They are usually small, soft to firm, gray or brown with a lobulated surface. Multifocal calcification is present making the cut surface gritty. Histologically they are a mixture of immature germ cells and gonadal sex cord cells (granulosa or Sertoli cells).

Embryonal Carcinoma

In the pediatric age group pure embryonal carcinomas are rare, as they are usually a component of mixed malignant germ cell tumors of the testes or mediastinum. Microscopically they are composed of large cells with large overlapping nuclei and prominent nucleoli. There are three main patterns described, namely epithelial, pseudotubular and papillary. Epithelial form is composed of large nests of cells with varying amounts of central necrosis. The pseudotubular and papillary patterns may be mistaken for yolk sac tumor but the cells of this tumor stain negative for AFP and these tumors lack strands and the hyaline globules of yolk sac tumors.

Choriocarcinoma

In the pediatric age group this tumor is also usually a part of the mixed malignant germ cell tumors.[63,64] Choriocarcinoma has two distinct forms, gestational and non-gestational,[78] and these differ in biologic behavior and response to therapy.[74] The gestational form is one which arises from the placenta while the non-gestational form arises from extra-placental tissues in non-gravid individuals. They are very friable, hemorrhagic and necrotic. Microscopically they are composed of two types of cells, namely cytotrophoblasts and syncytiotrophoblasts. The cytotrophoblasts are closely packed nests of uniform, medium sized cells with clear PAS positive cytoplasm and a vesicular nuclei. The syncytiotrophoblasts tend to develop syncytia and cover the nests of cytotrophoblasts and stain positive for β-hCG. The placental form may be seen in very young infancys who present with disseminated metastases and elevated β-hCG. This is actually a metastatic lesion from the placental primary in the mother which has invaded the villous vessels and entered the umbilical vein and fetal blood.

Mixed Germ Cell Tumors

Mixed germ cell tumors are those germ cell tumors that contain multiple histologic types in at least 10 percent of the specimen. The prognosis for mixed germ cell tumors is worse than that for yolk sac tumors.[79]

TREATMENT OF GERM CELL TUMORS

Principles of Surgical Treatment

Surgery is the treatment of choice for all benign tumors like teratomas and also for malignant germ cell tumors, if feasible. In cases of malignant tumors the resection should not involve any vital structures as effective chemotherapy is available which can substantially reduce the bulk of the tumor and make resection less morbid. In such cases initial debulking or biopsy only is advisable followed by chemotherapy and a second look surgery.

Principles of Chemotherapy for Malignant Germ Cell Tumors

Since malignant germ cell tumors are relatively uncommon in the pediatric age group, most of the chemotherapies have been borrowed from the adult experience and they have been found to be equally effective. The common drugs found to be active against malignant germ cell tumors are Dactinomycin (A), vinblastine (V), bleomycin (B), doxorubicin (ADR), cisplatin (P), carboplatin (J) and etoposide (E). As single agents their response rates varies from 28 to 100 percent.[80-85] Synergistically active combinations of drugs have now been increasingly used and have been found to be less toxic and at the same time more effective than single or dual agents. The addition of cisplatinum and carboplatin has further improved the results of these regimens. The commonly used pediatric combination are cisplatinum + vinblastine + bleomycin (PVB),[86] cisplatinum + etoposide + bleomycin (PEB)[87] and carboplatin + etoposide + bleomycin (JEB).[88] Currently PEB is the standard regimen being used in both POG and CCG trials of malignant germ cell tumors. In these trials there is randomization of the treatment of high risk tumors with standard PEB and high-dose cisplatinum + etoposide + bleomycin regimens. For salvage of relapsed or refractory patients, marrow ablative doses of carboplatin and etoposide followed by autologous marrow reinfusion is being tried.[89] Generally the low risk, stage-1 testicular and ovarian malignant germ cell tumors require no chemotherapy. Moderate risk tumors or those with progressive disease or tumor recurrences are managed with 3 to 4 courses of platinum based regimens. For higher risk tumors (higher stage testicular or ovarian tumors or extragonadal tumors) 6 months of platinum based regimen is indicated. Some of the common chemotherapeutic regimen are shown in Table 27.6.

Clinical Presentation, Staging and Treatment of Ovarian Germ Cell Tumors

Ovarian tumors constitute only 1 percent of all childhood malignancies[90] and are more common toward the end of the first decade. Two thirds (67%) of pediatric ovarian tumors are germ cell tumors and the most common of these being benign teratoma, 17 percent

Table 27.6: POG/CCG staging system for ovarian germ cell tumors[98]

Stage	*Extent of disease*
I	• Limited to ovary/ovaries; peritoneal washings negative for malignant cells • No clinical, radiological or histological evidence of disease beyond Ovaries • Tumor markers negative after appropriate postoperative half-life • Gliomatosis peritonei does not upstage the tumor
II	• Microscopic residual or +ve LN (£ 2cm as measured by pathologist) • Peritoneal washings negative for malignant cells • Tumor markers +ve or –ve • Gliomatosis peritonei does not upstage the tumor
III	• LN with malignant metastatic nodule > 2 cm as measured by pathologist • Gross residual or biopsy only • Contiguous visceral involvement (omentum, intestine, bladder) • Peritoneal washings +ve for malignant cells • Tumor markers +ve or –ve.
IV	• Disseminated metastases including liver

are epithelial tumors and 12 percent sex cord-stromal tumors.[91] Two-thirds of the malignant ovarian tumors are germ cell tumors[92] and amongst the malignant germ cell tumors are, in the order of frequency, dysgerminoma, endodermal sinus tumors, immature teratoma, malignant mixed germ cell tumors and embryonal carcinoma.[11,52,90] Among the non-germ cell tumors are the epithelial tumors which are usually mucinous or serous cystadenomas and rarely cystadenocarcinoma.[93] Pseudomyxoma peritonei with tumor implantation on the peritoneal surfaces occurs mostly with mucinous cystadenomas.[94] Sex cord–stromal tumors are usually granulosa cell tumors which present as precocious pseudopuberty. The other sex cord-stromal tumors, namely, thecoma-fibroma and Sertoli-Leydig cell tumors (androblastoma, arrhenoblastoma) are rare. Most of the sex cord-stromal tumors should receive three courses of a cisplatinum based chemotherapy to enhance treatment results.[95]

The ovarian germ cell tumors can present as an asymptomatic abdominal or pelvic mass or with abdominal pain, which can be acute because of torsion of the pedicle. In addition there may be abdominal distension, constipation, enuresis, precocious puberty, vaginal bleeding or amenorrhea depending on the size of the tumor and secretion of various hormones. The initial investigation is an ultrasound of the abdomen and pelvis to localize the mass to the adnexa, to determine whether the mass is solid or cystic and to determine the presence of calcification.[96,97] Abdominal and pelvic CT scan will further help in defining the site of origin, extent and presence of other metastatic lesions. Since secondaries may appear in the thoracic lymph nodes, lung parenchyma or rarely bone,[51] it is essential to include a plain chest X-ray (PA and lateral), CT scan of the chest, skeletal survey and a radionucleide bone scan in the routine work up of these patients. The currently used surgico-pathological staging system for pediatric ovarian germ cell tumors is the one advocated by POG and CCG (Table 27.6). This four stage system is a refinement over the commonly used FIGO system (in adults) by accounting for:

- Higher risk of recurrence in patients with +ve peritoneal fluid washings (which leads to upstaging of the tumors).
- Utility of the tumor markers for prediction of outcome.
- Lack of negative prognostic impact of gliomatosis peritonei if only mature glial tissue is present.

Operative management is the mainstay of treatment of pediatric germ cell tumors but it is imperative to use reproductive sparing techniques. The basic steps[98] are the same as for adult ovarian tumors, namely:

- Collection of ascitic fluid of peritoneal washings for cytology,
- Examination of the entire peritoneal surface and liver and biopsy of any suspicious lesions,
- Unilateral oopherectomy or salpingo-oophrectomy,
- Wedge biopsy of any suspicious lesions of the contralateral ovary. Bilateral involvement is present in 10 to 15 percent of dysgerminoma, but is extremely rare with other histological types of GCT,
- Omentectomy,
- Bilateral retroperitoneal lymph node sampling including the internal iliac, common iliac, low paraaortic and perirenal lymph nodes.

VAC regimes were commonly used for malignant germ cell tumors of adults in the 1960s and 1970s and had led to improvement in survival rates but were associated with high relapse rates of nearly 46 percent.[99] When the same regimens were used in children[100] it that all stage I and II, 86 percent of stage III and 20 percent of stage IV children survived. Einhorn and Donohue in 1977[101] reported increased survival with PVB regimen in testicular malignant germ cell tumors and subsequently the same was reported for malignant ovarian germ cell tumors.[102-104] The currently used PEB regimens are expected to have survival rates of better than 90 percent with localized disease. The PEB regimen and it modification with high-dose cisplatinum has extensively tried by POG and CCG and their results are awaited. The currently used treatment protocol in the POG and CCG trials is shown in Table 27.5.

Endodermal sinus tumors (yolk sac tumors) (EST) of the ovary: EST is among the commoner malignant ovarian germ cell tumors[102] and are invariably associated with an elevated serum AFP level. The degree of elevation of aFP neither correlates with stage nor with the ultimate prognosis.[105] The tumor is so friable that tumor rupture has been reported in up to 33 percent of cases.[106] Though most of these are stage I tumors but surgery alone is inadequate as when surgery is the only modality used the survival rate is only 19 percent.[107] Therefore postoperative treatment with a platinum based regime is a must in all the cases to achieve a disease free survival rate of higher than 89 percent.[51] Also it is well known that EST is a radioresistant tumor[100] and so radiation therapy has no role in the treatment of EST.

Dysgerminoma of the ovary: Dysgerminoma is the commonest malignant germ cell tumor of the ovary in children and adolescents.[7,12] They usually occur in the genotypic females but can also occur in dysgenetic gonads and have an average age of presentation of about 16 years. These are usually large tumors and 10-15 percent of them are known to be bilateral. Majority of patients are developmentally normal but some are known to be associated with precocious sexual development.[100,108] Stage I patients have a high recurrence rate when treated with surgery alone,[109] and so they all must be treated with adjuvant chemotherapy with PEB, postoperatively.[110,111] There are some workers[115] who still recommend that stage I may be treated with oophrectomy or salpingo-oophrectomy alone and the patient followed up without further treatment unless they develop recurrence. These are very radiosensitive tumors and were extensively treated in the past with post-operative radiation therapy with extremely good results. Now, with the availability of effective chemotherapy and also with the awareness about the long-term morbidity following radiation therapy in young girls, specially with respect to reproductive functions, radiation treatment has more or less been given up.

Embryonal carcinoma of the ovary: This is a rare ovarian tumor comprising of only 6 percent of all ovarian malignant neoplasms.[75,112] These are the least differentiated form of germ cell tumors and are usually found admixed with other germ cell components such as mature cystic teratoma, EST or dysgerminoma.[112] By itself the embryonal carcinoma is hormonally inert but the presence of trophoblastic elements within the tumor may be manifested as precocious puberty, vaginal bleeding, amenorrhea, hirsuitism and a positive pregnancy test.[94,113,114] Only 50 percent of patients with stage I embryonal carcinoma of the ovary survive when surgery is the only treatment given and, therefore, postoperative chemotherapy is advocated for all patients and the approach is similar as for ovarian EST.[113-115]

Malignant mixed germ cell tumors of the ovary: These constitute 8 percent of the ovarian germ cell tumors and are usually EST in a dysgerminoma or immature teratoma.[116] Isosexual precocious puberty is seen in 30 percent of the children with this tumor.[116] These are classified and managed according to the predominant histologic type.[116]

Choriocarcinoma of the ovary: Non-gestational pure choriocarcinoma of the ovary is very rare in children and comprises of 0.6 percent of all ovarian germ cell tumors. It is more often a part of a mixed ovarian germ cell tumor and manifests clinically as premature thelarche, pubarche and/or vaginal bleeding. This is a highly malignant tumor with early local, lymphatic and hematogenous spread.[108] Because of its malignant nature adjuvant chemotherapy is recommended for all patients irrespective of the stage of disease.

Polyembryoma of the ovary: There are only 12 reports of this extremely rare tumor in the literature and most of these are in association with other tumors.[117-120] This is again an exteremely malignant neoplasm which is not radiosensitive but responds well to chemotherapy with PEB.

Clinical Presentation, Staging and Treatment of Testicular Germ Cell Tumors

Pediatric testicular tumors are rare in prepubertal boys and account for 2 percent of solid malignant neoplasms in boys.[121,122] Seventy-five percent of these childhood neoplasms are germ cell in origin and two-thirds of the germ cell tumors are endodermal sinus tumors (EST) and lesser numbers are teratomas. The testicular tumors usually present as an asymptomatic scrotal mass. Nearly 85 percent of the testicular EST have elevated levels of AFP at presentation and rarely β-hCG is elevated. Ninety percent of the malignant testicular tumors are localized at presentation and metastatic disease, if present, is typically to the draining lymph nodes or the chest. Preopeartive assessment includes a scrotal ultrasound, ultrasound and CT scan of the pelvis, abdomen and chest, skeletal survey, radionucleide bone scan and evaluation of serum markers, namely, AFP and β-hCG. These are important for proper staging and patient monitoring. The surgical approach mandates an inguinal incision with occlusion of the cord structures by a vascular clamp. The testis is then mobilized into the operative field and a radical orchiectomy performed with ligation of all cord structures at the level of the internal ring (high ligation of the cord). The currently used POG staging system for testicular germ cell tumors is shown in Table 27.7. Boys with stage I or II tumors with normal or unknown markers, persistently elevated markers (after orchiectomy) or microscopic residual tumor in the cord structures should undergo an ipsilateral retroperitoneal lymph node (RPLN) sampling. This approach of RPLN sampling is controversial and some workers[123,124] have questioned its utility in stage I disease.

Table 27.7: POG/CCG staging of testicular tumors[98]

Stage	*Extent of disease*
I	• Limited to testis, completely resected by high inguinal orchiectomy or transscrotal orchiectomy with no spill
	• No clinical, radiographic, or evidence of disease beyond testes
	• Tumor markers normal after appropriate post-operative half life; patients with normal or unknown markers at diagnosis must have negative ipsilateral retroperitoneal node sampling to confirm stage I
II	• Transscrotal orchiectomy with gross spill of tumor
	• Microscopic residue present in scrotum or in spermatic cord (< 5 cm from proximal end)
	• Retroperitoneal LN involved (< 2 cm)
III	• Retroperitoneal LN involved (> 2 cm)
	• No visceral or extraabdominal involvement
IV	• Distant metastases, including liver

Known RPLN enlargement (stage II to IV disease) must be confirmed histologically by sampling.

In children 80 to 85 percent of the testicular germ cell tumors present as stage I disease.[98] As an adjuvant therapy most centers use PEB regimen for all testicular germ cell tumors more than stage I at presentation. For stage I disease surgery alone results in survival rates of 85 to 100 percent[125-128] and most of the others who relapse can be effectively treated with salvage chemotherapy with PEB.[109] The currently used protocol (POG and CCG) is shown in Table 27.5.

Testicular endodermal sinus tumors: These tumors are localized (stage I) in up to 85 percent of the cases and has an overall survival rate of about 70 percent.[79,126] For stage I tumors only surgery is recommended with a prolonged follow-up. While for stage II or III disease surgery should include high ligation and orchiectomy + RPLN sampling followed by chemotherapy with platinum based regime, namely PEB or JEB.

Clinical Presentation and Treatment of Extragonadal Malignant Germ cell tumors

Extragonadal germ cell tumors account for nearly two-thirds of all pediatric germ cell tumors.[62] The most common sites of extragonadal germ cell tumors in children are sacrococcygeal region, anterior mediastinum, pineal, and rarely the retroperitoneum, neck, stomach and vagina. Most of the extragonadal germ cell tumors are benign but when malignant these are very aggressive tumors and therefore adjuvant chemotherapy with PEB or JEB is recommended for all malignant extragonadal germ cell tumors, irrespective of the stage. The POG/CCG staging of extragonadal germ cell tumors is depicted in Table 27.8.

Table 27.8: Staging of malignant extragonadal germ cell tumors (POG/CCG)[98]

Stage I	:	Complete resection at any site; coccygectomy for sacrococcygeal site; negative tumor margins; tumor markers positive or negative
Stage II	:	Microscopic residual; lymph nodes negative; tumor markers positive or negative.
Stage III	:	Gross residual or biopsy only; retroperitoneal nodes negative or positive; tumor markers positive or negative.
Stage IV	:	Distant metastases including liver

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CHAPTER 16

Yoshiaki Tsuchida
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Neuroblastoma

In remembrance

Prof. Yoshiaki Tsuchida (25-10-36 to 28-06-05) was a renowned pediatric surgeon, who devoted most of his life to basic and clinical research. He has contributed to the International Society of Pediatric Oncologists with various academic achievements in the field of pediatric oncology. This is probably the last chapter by him on his favourite subject.

Neuroblastoma is the second most common solid tumor in infancy and childhood after brain tumors. It accounts for 7 to 10 percent of cancers of childhood, and the annual incidence is 1 per 100,000 children under the age of 15 years in the United States[1] and 1 per 76,000 in Japan where mass screening is carried out.[2]

The history of neuroblastoma dates to 1864 when Virchow first described its typical histological features.[3] In 1901, Pepper reported autopsy findings of 6 infants with suprarenal and liver tumors, which were probably the first patients with the disease type now called stage IV-S neuroblastoma.[4] In 1907, Huntington recorded older patients with "sarcoma" of the adrenal gland and metastases to the skull.[5] In 1910, Wright first used the term neuroblastoma by likening the rosettes and neural fibrils of such tumors to the developing adrenals,[6] and since then the term neuroblastoma has been widely used.

The biology of neuroblastoma is enigmatic and it is important to understand it to the extent possible in order to improve its treatment and clinical results further. It is widely accepted that neuroblastoma is a tumor of the sympathetic nervous system. While neuroblastoma may produce catecholamines, this is not always true in advanced neuroblastoma. Le Douarin and her associates have shown that the fate of neural crest-derived cells is highly dependent upon environmental causes.[7] That is, neural crest cells that are supposed to produce catecholamines may begin to synthesize acetylcholine under specific conditions[8] and vice versa.[9] Cloned adrenergic neuroblastoma cells may even become cholinergic after serial transplantation.[10] In addition, we have confirmed that only 75 percent of clinically detected neuroblastomas have the key enzyme, i.e. tyrosine hydroxylase, required to produce catecholamines.[11]

Cytogenetically, chromosome 1p deletions, extrachromosomal double minutes, and homogeneously staining regions (HSRs) are commonly observed in neuroblastoma cell lines and advanced-stage neuroblastoma tumors. It was also recently found that

an HSR represents genomic amplification of *MYCN*, which plays a key role in determining the aggressiveness of neuroblastoma.[12,13] However, stage IV neuroblastomas or cell lines that lack *MYCN* amplification are also progressive, and some of them show evidence of *MYCN* expression in terms of mRNA and/or *MYCN* oncoprotein.[14,15] It was also recently shown that a small proximal locus mapped between 1p35-36.1 and 1p36.23 may function as a suppressor gene of *MYCN* amplification.[16] However, the relation is not simple,[17] because it has been reported that chromosome 17q gain may also be associated with *MYCN* amplification.[18] In addition, loss of heterozygosity on chromosomes 2q, 9p, 11q, 14q, and 18q has been reported in some patients with advanced neuroblastoma.[19,20]

Cellular DNA content or ploidy is relevant to clinical outcome.[21] Hyperploidy is closely associated with a favorable patient outcome, while diploidy usually predicts poor patient prognosis. Ploidy analysis using *in situ* hybridization showed that numeric chromosome aberrations are found in neuroblastic/ganglionic cells, but not in the Schwann cells which have long been thought to be neoplastic in origin.[22] This led to the hypothesis that Schwann cells in neuroblastoma are infiltrating normal cells that are responsible for the differentiation of neuroblastoma cells.[22]

In sympathoadrenal lineage cells in the later stages of neural crest development, the *Trk-A* tyrosine kinase receptor for which nerve growth factor (NGF) is a ligand is expressed.[23] While NGF binding to the receptor transmits a signal that leads immature sympathetic neurons to differentiate into mature ganglion cells, deprivation of NGF results in apoptosis, or programmed cell death, of the neurons. In neuroblastoma, *Trk-A* is expressed in tumors with a favorable prognosis, and tumor cells expressing high levels of *Trk-A* differentiate in response to NGF.[23,24] The NGF that is produced by schwannian stromal cells may regulate the differentiation and survival of neuroblastoma cells.[25] *MYCN* amplification downregulates *Trk-A* expression and low or absent expression of *Trk-A* is associated with an unfavorable prognosis.[23,24] *Trk-B*, a high-affinity receptor for brain-derived neurotrophic factor (BDNF), also is expressed in aggressive neuroblastoma.[26]

The presence of cells with characteristic features of apoptosis (e.g. condensed nuclear fragments and eosinophilic cytoplasm) and the demonstration of a ladder of DNA fragments indicate that apoptosis is involved in the process of neuroblastoma cell death.[27] A number of studies showed that neuroblastoma expresses Bcl-2 which inhibits apoptosis, but there is no definitive evidence regarding the relationship between Bcl-2 and other prognostic factors.[28] Expression of proteases involved in the process of apoptosis, caspase-1 and caspase-3, is high in the nuclei of neuroblastoma with favorable prognostic characteristics.[29,30]

Telomerases are DNA-protein structures at the ends of eukaryotic chromosomes and are thought to be important in the positioning, protection, and replication of chromosomes.[31] Telomerase, an RNA-dependent DNA polymerase, stabilizes telomeres and the telomere maintenance is essential for attainment of immortality in tumor cells. Several studies have shown that telomerase activity is detectable in neuroblastomas, except for stage IV-S tumors.[31-33] High telomerase activity is associated with advanced stage or *MYCN* amplification, while neuroblastomas with low or undetectable telomerase activity are usually tumors that are diagnosed in infants and have favorable prognostic characteristics.[31]

Ha-*ras* p21, a product of the Ha-*ras* gene, is expressed in normal neuronal cells and participates in the signal transduction pathway relating to NGF.[34] Expression of Ha-*ras* p21 is significantly associated with patient prognosis and higher expression predicts a favorable patient outcome.[35] Prognostic discrimination based on Ha-*ras* and *Trk-A* gene expression in patients with stage III and IV diseases therefore appears useful, and the survival rate of patients with neuroblastoma highly expressing both genes is significantly better than that of patients with tumors with low expression of the genes.[36]

P-glycoprotein, a plasma membrane efflux pump, plays a role in drug resistance and is responsible for multidrug resistance against the vinca alkaloids, anthracyclines, and epipodophyllotoxins. However, the role of P-glycoprotein in predicting prognosis in neuroblastoma is controversial.[37,38] Expression of the *MDR1* gene that encodes P-glycoprotein is correlated with *MYCN* gene expression in neuroblastoma without *MYCN* amplification, and high expression of *MDR1* is significantly associated with poor outcome.[39] High expression of the *MRP* gene that encodes multidrug resistance-associated protein (MRP) is also associated with poor survival in patients with neuroblastoma and in subgroups of patients without *MYCN* gene amplification and those with localized disease.[40]

The genetic and molecular analyses described above not only have revealed biological differences between neuroblastomas with favorable prognosis and those with aggressive biological behaviors, but also have provided insight into both tumorigenesis and spontaneous

regression of neuroblastoma. It is generally accepted that there are at least two types of neuroblastoma: one seen mainly in infancy and associated with particularly good prognosis; and the other usually encountered in older children with an extremely poor prognosis, often associated with *MYCN* amplification. Brodeur and others place an intermediate group[41] between these two groups, but identification of the intermediate group is equivocal because the prognosis of patients with neuroblastoma is not determined by *MYCN* amplification alone. Furthermore, the prognosis of stage IV neuroblastoma patients older than 12 months of age does not differ greatly whether *MYCN* is amplified or not.[42]

CLINICAL SYMPTOMS

Neuroblastoma may diagnosed prenatally. Seventeen patients with prenatally diagnosed neuroblastoma were identified in a cohort of 591 patients in the Italian Neuroblastoma Registry.[43] It was reported that the tumor was solid in 13 patients (76.5%) and cystic in 4 (23.5%). The tumor occurred in the adrenal gland in 16 and in the retroperitoneal sympathetic ganglion in one. Fifteen patients were in stage I or II, and the remaining 2 patients had stage IV-S disease.[43] The treatment strategy is controversial, but it is generally considered that it should not differ much from that in infantile neuroblasoma identified by mass screening.[43]

Clinical symptoms of postnatally diagnosed neuroblastoma differ based on the mode of diagnosis and the stage/age of the disease. Infants with neuroblastoma identified by mass screening usually present with a small tumor mass in the adrenal glands or paravertebral sympathetic ganglia, or less frequently with multiple hepatic metastases together with a small primary tumor mass. The distribution of tumor masses diagnosed in mass screening is thus similar to that of neuroblastomas identified by antenatal diagnosis. The primary tumors in the adrenal glands or paravertebral sympathetic ganglia are usually not palpable in patients diagnosed by mass screening. On the other hand, multiple hepatic metastases cause significant liver enlargement. Infantile neuroblastoma may also metastasize to the skin and bone marrow.

Some tumors can develop intra- and extraspinally, and can be of the dumbbell or hourglass shape. The site of origin of neuroblastoma varies with age, since adrenal tumors are more common in children than in infants.[44] In children, a fixed, hard, irregular mass is frequently palpable. Tumors originating in the pelvis may cause mechanical obstruction and result in difficulties in defecation or urination. A dumbbell-type tumor can cause paraplegia or fecal/urinary incontinence. Larger thoracic tumors may cause dyspnea or dysphagia, and may also cause superior vena cava syndrome.[44] Upper thoracic and cervical neuroblastomas are sometimes associated with Horner's syndrome. It has been presumed that neuroblastoma of adrenal origin may leads to renovascular hypertension, but the serum renin level is usually normal, and if elevated serum active or inactive renin levels are associated with neuroblastoma, anomalies of the vascular system such as middle aortic syndrome should be considered, as observed in one of the authors' patients or reported by others.[45]

Neuroblastomas in children older than 12 months of age often metastasize to the lymph nodes, bone, and bone marrow. Neuroblastomas have a predilection for the bones of the skull, orbit, jaw, and long bones, and metastases to the orbit produce the characteristic unilateral or bilateral periorbital ecchymosis and exophthalmos. Metastases to the bone marrow are so common that routine examination of bone marrow is essential. Lung and brain metastases are rare at diagnosis.

Hypertension, diarrhea, and opsoclonus-myoclonus syndrome are important paraneoplastic syndromes of neuroblastoma. Excretion of catecholamines and stretching (constriction) of the renal arteries are possible causes of hypertension in neuroblastoma, but the latter is less plausible because serum total rennin is usually within the normal ranges. Neuroblastomas are known to produce vasoactive intestinal peptide (VIP), which causes intractable diarrhea. Interestingly, the VIP-producing tumors are mature ganglioneuroblastomas or ganglioneuromas.[46] Opsoclonus-myoclonus syndrome has been observed in up to 4 percent of neuroblastoma patients.[47] This syndrome is neither due to direct involvement of the brain by tumor, nor to the production of catecholamines. While the mechanism is unclear, this syndrome may respond to high doses of corticosteroids.[44,47]

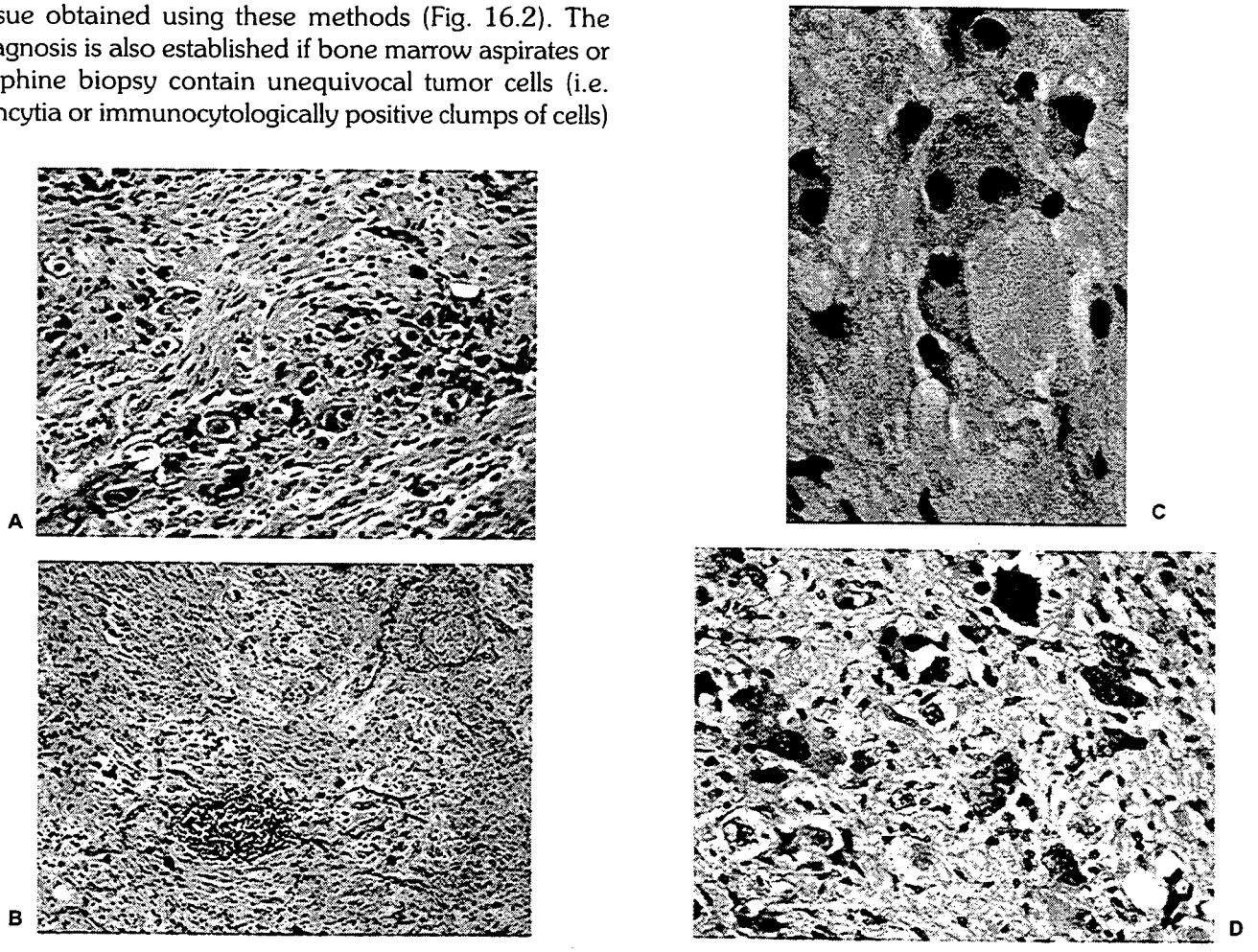
ASSOCIATED ANOMALIES AND FAMILIAL OCCURRENCE

In contrast to Wilms' tumor and acute leukemia in childhood, neuroblastomas are associated less frequently with combined congenital abnormalities. Nishi and coworkers found no Down's syndrome and no undescended testicle but 5 cases of mental retardation in 288 patients with neuroblastoma.[48] Familial occurrence of neuroblastoma is rare.[49]

DIAGNOSIS, STAGING, AND HISTOLOGICAL CLASSIFICATION

The diagnosis of neuroblastoma should be established histologically.[50,51] Characteristic histological features of neuroblastoma are described below. In most cases, a tissue diagnosis of neuroblastoma based upon hematoxylin and eosin staining is not difficult, especially if features suggestive of neuronal differentiation are present (Figs 16.1A to D). However, in some cases neuroblastomas are characterized by densely packed, small blue cells with little differentiation. Electron microscopy and implementation of immunohistochemical methods are recommended to confirm the diagnosis.[50] Core-needle biopsy or fine-needle aspiration is recommended to make the diagnosis by some but discouraged by others because of the small amounts of tissue obtained using these methods (Fig. 16.2). The diagnosis is also established if bone marrow aspirates or trephine biopsy contain unequivocal tumor cells (i.e. syncytia or immunocytologically positive clumps of cells) and increased urine or serum levels of catecholamines or metabolites >3.0 SD above the mean are seen.[50] Serum neuron-specific enolase (NSE) is not decisive of diagnosis, but is of value in monitoring the clinical course.[52]

Evans' staging system[53] has long been used for neuroblastoma. However, the International Neuroblastoma Staging System (INSS) was first proposed in 1988, and after revisions in 1993[50] is currently used worldwide (Table 16.1). Before the INNS, the staging system of the Children's Cancer Group of the United States, its modification by the Japanese Society of Pediatric Surgeons, and that of the Pediatric Oncology Group of the United States were used. Each system had its strengths, but the differences made it difficult to compare the results of clinical trials and biologic studies



Figures 16.1A to D: Histopathological features of neuroblastoma are shown: **(A)** Small uniform cells with dense darkly staining nuclei and scant cytoplasm. **(B)** Rosette formation is seen. **(C)** Photomicrograph depicting a histopathological diagnosis of ganglioneuroma. **(D)** Photomicrograph depicting a histopathological diagnosis of ganglioneuroblastoma with islands of neuroblastoma cells and fibrous stroma

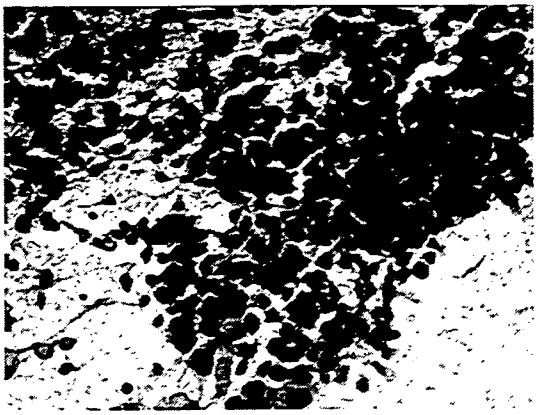


Figure 16.2: Photomicrograph showing the round cell appearance on fine-needle aspiration cytology in a case of neuroblastoma

performed by different groups and in different countries. The tests recommended for the assessment of extent of disease are well outlined in the report by Brodeur and his associates.[50]

It is tempting to consider that the histopathology of neuroblastomas, ganglioneuroblastomas, and ganglioneuromas parallels the pattern of differentiation expressed by the developing sympathetic nervous system.[54] Typical neuroblasts are small uniform cells with dense, hyperchromatic nuclei and a paucity of cytoplasm. The neuritic process or neuropil is noted, and pseudorosettes consisting of neuroblasts surrounding areas of eosinophilic neuropil are seen in a majority of cases. Tumors such as primitive neuroectodermal tumors, undifferentiated soft tissue sarcoma, Ewing's sarcoma, and non-Hodgkin's lymphoma should be carefully differentiated from neuroblastoma. The International Neuroblastoma Pathology Classification (INPC) was recently established.[51] Originally, Shimada and colleagues[55] established a classification in which the presence of Schwann's cells, degree of cellular differentiation, and mitosis-karyorrhexis index are determined to define favorable or unfavorable histologic types. The original Shimada classification was reviewed by an international panel of six member pathologists, and the INPC was approved.[51] In both the Shimada classification and INPC, the age of the patient at diagnosis is one of the factors predicting favorable or unfavorable prognosis.

IMAGING OF NEUROBLASTOMA

As neuroblastoma originates from the sympathetic ganglia and adrenal medulla, imaging investigation must focus on the pertinent area. However, it should be remembered that it is not uncommon for neuroblastoma to present first with the symptoms produced by metastases or even with peculiar clinical manifestations before the actual tumor is detected (Fig. 16.3). Although the imaging evaluation of a child with a presumed neuroblastoma varies from institution to institution, our routine procedures are as follows: plain radiograph of the chest and abdomen; abdominal sonography (US); magnetic resonance (MR) imaging; and bone scintigraphy with ^{99m}Tc MDP (methylendiphosphonate)

Table 16.1: International Neuroblastoma Staging System[50]

Stage	*Definition*
I.	Localized tumor with complete gross excision, with or without microscopic residual disease; representative ipsilateral lymph nodes negative for tumor microscopically (nodes attached to and removed with the primary tumor may be positive)
IIA.	Localized tumor with incomplete gross excision; representative ipsilateral nonadherent lymph nodes negative for tumor microscopically
IIB.	Localized tumor with or without complete gross excision, with ipsilateral nonadherent lymph nodes positive for tumor; enlarged contralateral lymph nodes must be negative microscopically
III.	Unresectable unilateral tumor infiltrating across the midline, with or without regional lymph node involvement; or localized unilateral tumor with contralateral regional lymph node involvement; or midline tumor with bilateral extension by infiltration (unresectable) or by lymph node involvement
IV.	Any primary tumor with dissemination to distant lymph nodes, bone, bone marrow, liver, skin and/or other organs (except as defined for stage IV-S)
IV-S.	Localized primary tumor (as defined for stage I, IIA or IIB), with dissemination limited to skin, liver, and/or bone marrow (limited to infants < 1 year of age)