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PATHOLOGY
Neil J. Sebire, Baljeet Kaur, Michael Wells

4.1 INTRODUCTION

Trophoblastic tumors are malignant fetal allografts in maternal tissues and present unique biological, immunological and pathological problems. One of the main functions of trophoblast is to gain access to the maternal circulation, and normal trophoblast infiltrates, invades vessels and can even be transported to the lungs [1-3] in a fashion which is otherwise only seen in malignant neoplasms. Invasion and metastases are criteria used to diagnose malignancy in other neoplasms but are not usually applicable to trophoblast. In addition, trophoblastic tumors are relatively rare and unfamiliar to both physician and pathologist in non-specialized centers and the frequent difficulties and potential dangers of obtaining adequate samples from deeply invasive and hemorrhagic lesions can make pathological diagnosis difficult or impossible. These facts justify the well-established approach of treating patients on the basis of clinical criteria and serological human chorionic gonadotropin (hCG) estimations. It also means that some patients will be treated and cured without our ever knowing whether the disease treated was an invasive hydatidiform mole (IM) or a choriocarcinoma (CC). Terms such as gestational trophoblastic disease (GTD) and gestational trophoblastic neoplasia (GTN) accurately describe this situation [4, 5].

The increasing use of ultrasound in the diagnosis and management of abnormal pregnancies has resulted in earlier evacuation of moles [6, 7] and earlier chemotherapy in GTN [5]. Early evacuation has meant a decrease in the number of molar pregnancies which present with the symptoms and signs of overgrowth of trophoblast and excessive hCG secretion, such as uterine size larger than dates, hyperemesis, toxemia or hyperthyroidism [8,9], and most complete hydatidiform moles (CHMs) now present as miscarriage or terminations of abnormal anembryonic pregnancies towards the end of the first trimester in which a mole may or may not be suspected and when pathological criteria useful to diagnose late second trimester moles are no longer valid [6,7]. The success of chemotherapy has resulted in a marked reduction in the number of pathological specimens available for study and few pathologists can today claim to have seen an IM or a CC in a hysterectomy specimen. However, the aggressive behavior of trophoblastic tumors unchecked by chemotherapy is well known from earlier accounts [10-16]. In recent years, genetic studies have provided us with additional information, which has helped both to understand the nature of these abnormalities and to test and improve the morphological criteria, which remain the basis of pathological diagnosis.
4.2 NORMAL TROPHOBLAST

Trophoblast populations of normal placenta are composed of cytotrophoblast, syncytiotrophoblast and intermediate trophoblast. Villous trophoblast covers chorionic villi (Figure 4.1) and consists predominantly of cytotrophoblast and syncytiotrophoblast. The cytotrophoblast is the trophoblastic stem cell. These rather primitive mononuclear cells have a clear cytoplasm (which reflects their paucity of cellular organelles) and a high mitotic rate. They usually express cytokeratins but produce little or no hormones. Cytotrophoblast shows no Inhibin alpha expression throughout gestation [18]. Syncytiotrophoblast differentiates from fusion of cytotrophoblast and is composed of multinucleated cells with abundant dense cytoplasm. It secretes abundant hCG, some human placental lactogen (hPL) and placental alkaline phosphatase (PLAP) but has little capacity for further proliferation. Inhibin alpha is highly expressed by syncytiotrophoblast in the first trimester but progressively decreases towards term. This links with the observation that serum levels of Inhibin peak at 7-8 weeks, decline in mid trimester and rise again towards term [18]. Production of hCG decreases while hPL and PLAP increase as pregnancy advances [19] and the amounts of villous trophoblast in relation to the amounts of villous stroma (Figure 4.1) gradually decrease with increasing gestational age [20]. Trophoblast also synthesizes estrogen and progesterone [21]. Intermediate trophoblast can be further subdivided into villous intermediate trophoblast located in the villous columns, implantation site intermediate trophoblast located in the placental site and chorionic-type intermediate trophoblast located in the chorionic laeve of the fetal membranes.[17] Cytotrophoblast again acts as the stem cell in the villi which are in contact with the placental bed. Here the cytotrophoblast merges imperceptibly with the trophoblastic columns. The proliferative activity of the villous intermediate trophoblast decreases towards the villous tips. At the base of the trophoblastic columns the trophoblast infiltrates the decidua, spiral arteries and myometrium to become the implantation site intermediate trophoblast. [17]. The implantation site intermediate trophoblast (placental site trophoblast; PST) is composed of mononuclear and multinucleated cells with a dense eosinophilic cytoplasm (Figure 4.2), morphologically different from villous cytotrophoblast and syncytiotrophoblast and defined immunocytochemically by positive staining for hPL and cytokeratins, but containing scanty hCG and PLAP.
Figure 4.1  Villous trophoblast forming polar trophoblastic columns in early pregnancy. It consists of mononuclear cytotrophoblast and multinucleated syncytium as well as villous intermediate trophoblast.

Figure 4.2  Placental site trophoblast infiltrating myometrium. In this area it is predominantly composed of mononuclear intermediate trophoblast. X 180.

Most placental site trophoblast does not express Inhibin.[18] Modern techniques [19,22,23] have therefore resolved the argument about the origin of these cells [24,25] in favour of trophoblast and the term implantation site intermediate trophoblast is now well established. The functions and biological differences between the various types of trophoblast are under study and include the production of proteolytic enzymes which facilitate tissue invasion, thromboplastin and plasminogen activator, which control fibrin formation and may be important in maintaining vascular patency and expression of human leukocyte antigen (HLA) type I, whose role in the success of the fetal allograft is still unknown [26-28].

4.3 CLASSIFICATION OF TROPHOBLASTIC LESIONS (TABLE 4.1)
The common pathology of these lesions is an excessive proliferation of trophoblast. In hydatidiform moles this essentially consists of chorionic villi covered by abnormal villous trophoblast. There are two distinct morphological and genetic types of hydatidiform mole:

Complete mole (CHM), a diploid, hyperdiploid or tetraploid conception in which all of the nuclear genome is derived from the father in the majority of cases [29,30]; and Partial Mole (PHM), a triploid and rarely tetraploid conception with at least two sets of paternal and one set of maternal chromosomes [31-33]. CHM is so named because traditionally, when hydrops is fully developed the majority of villi were involved, whereas in fully developed PHM, hydrops remains characteristically focal. (Such a distinction on the basis of hydrops alone does not hold true in the first trimester, when most HMs are now evacuated and examined). About 85% CHMs and >99% PHMs are associated with no significant consequences following evacuation but a minority progress, manifest as gestational trophoblastic neoplasia (GTN) and require chemotherapy. IM is usually a CHM and rarely a PHM which invades the myometrium, but is rarely encountered in the setting of modern obstetric and gynaecological practice.

Typically, CC is composed predominantly of cells resembling villous cyto and syncytiotrophoblast but, unlike in moles, no chorionic villi are present (except in the rare setting of intraplacental choriocarcinoma, IPCC). The tumour invades vessels and metastasizes aggressively, usually being fatal without treatment. [13]. PSTT can also proliferate excessively. Exaggerated forms of placental site reaction may remit spontaneously or be cured by simple curettage. Placental site trophoblastic nodule (PSN) or plaque has been described (until lately) as benign non-neoplastic lesions with neither local recurrence nor progression to GTN. No specific treatment or follow-up was deemed necessary [34]. Recent advancements in this field are included elsewhere in the chapter (4.17) Rarely, such trophoblastic lesions may progress and invade in a malignant fashion [35]; placental site trophoblastic tumour (PSTT). [36]. Since PSTT is relatively chemoresistant, it is encountered more commonly that typical CC in hysterectomy specimens. Finally, a variant of PSTT has been described, epithelioid trophoblastic tumour (ETT), possibly the malignant counterpart of PSN. [37].

The pathogenesis of trophoblastic tumours is relatively poorly understood because of their rarity and lack of experimental models (38, 39). Studies on gene expression profiles in human trophoblastic subpopulations have elucidated the normal differentiation of trophoblast and relation to intermediate trophoblastic tumours (40). These studies have led to the hypothesis that trophoblastic tumours may recapitulate the development of trophoblast in different locations in the normal early placenta and implantation site (41, 42), such that both ETT and PSN are related to the intermediate trophoblast of chorion leave type, whereas PSTT and EPSR are
related to the intermediate trophoblast of the normal implantation site. Based on morphological and immunohistochemical studies, PSN may represent the benign counterpart of ETT and EPSR the counterpart of PSTT. However, it is more likely that such phenotypes simply represent patterns of trophoblast differentiation. Furthermore, lack of a clear biological link between PSTT and EPSR was demonstrated in a recent study (43).

Table 4.1. Abnormal trophoblastic proliferations.

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Embryo</th>
<th>Hydrops</th>
<th>Trophoblast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partial hydatidiform mole (PHM)</td>
<td>Yes</td>
<td>Focal</td>
<td>Some excess</td>
</tr>
<tr>
<td>Complete and invasive hydatidiform mole (CHM)</td>
<td>No</td>
<td>Extensive</td>
<td>Marked excess</td>
</tr>
<tr>
<td>Choriocarcinoma</td>
<td>No</td>
<td>No villi</td>
<td>Neoplastic</td>
</tr>
<tr>
<td>Exaggerated Placental site reaction (EPSR)</td>
<td>No</td>
<td>No villi</td>
<td>Self-limiting</td>
</tr>
<tr>
<td>Placental site trophoblastic tumor (PSTT)</td>
<td>No</td>
<td>No villi</td>
<td>Neoplastic</td>
</tr>
<tr>
<td>Placental site trophoblastic nodule (PSN)</td>
<td>No</td>
<td>No villi</td>
<td>Undetermined</td>
</tr>
<tr>
<td>Epithelioid trophoblastic tumor (ETT)</td>
<td>No</td>
<td>No villi</td>
<td>Neoplastic</td>
</tr>
</tbody>
</table>

4.4 MOLAR PREGNANCY (FIGURES 4.3, 4.4)

Hydatidiform moles are intrinsically abnormal conceptions [44-47] characterized histopathologically by excessive proliferation of trophoblast, and genetically by relative placental over-expression of paternally derived genes. Most complete hydatidiform mole arise when an ovum without maternal chromosomes is fertilised by one sperm that then duplicates its DNA (46XX androgenetic karyotype) and are less frequently from fertilisation by two sperm (46XY androgenetic karyotype). Although nuclear DNA is entirely paternal, mitochondrial DNA remains maternal in origin. However recently biparental CHM’s have been described where patients present with recurrent disease which might be familial or sporadic. Most common mutation in these cases involves leucine-rich region of NLRP7 at chromosome 19q13.3–13.4 (figure 4.3)

Partial hydatidiform moles are almost always triploid (diandric monogynic triploids) and majority result from fertilisation of a seemingly healthy ovum by two sperm (heterozygous PHM). Diploid partial moles probably do not exist, with most reported cases being misdiagnosed complete moles (48). However not all triploides are PHM. A significant proportion (up to one third) of triploid gestation is non-molar digynic monoandric in their genetic composition (49)
4.5 COMPLETE HYDATIDIFORM MOLE

CHM produces voluminous and haemorrhagic evacuation specimen and in advanced gestational age shows classic “bunch of grapes” appearance. No foetal parts are usually seen in CHM.
Figure 4.4 Complete mole presenting as an enlarged uterus with abnormally dilated endometrial cavity in a 55 years old female with uncontrolled vaginal bleeding. Hydropic change is generalized and no foetus is present. (http://www.elearning.virtualpathology.leeds.ac.uk/casesubmission/femalegt/Abnormal %20beta-hCG%20in%20a%2055-year-old%20woman.pdf)

In the classical, fully developed CHM pathological examination shows enlarged, oedematous villi often completely surrounded by excessive circumferential villous trophoblast (Figure 4.5), in which nuclear pleomorphism is usually more intense than in normal pregnancy. Clusters of similar trophoblast fragments are also often seen amongst villi. Fluid tends to collect in ‘cisterns’ in the middle of villi, resulting in localization of other components of villous stroma beneath the cytotrophoblastic layer (Figure 4.6), which shows few or no blood vessels, and this hydropic change tends to be generalized. This description is accurate for CHMs evacuated in the mid-late second trimester but CHMs are now evacuated at much earlier gestations than previously, [7] at an average of 10 weeks’. The most useful features by which to recognize these early CHMs are as follows. [7][50-52]

Figure 4.5 Hydrops and cistern formation in complete mole evacuated at 18 weeks gestation. Hydropic change is generalized and no residual vessels are present. X20.
Figure 4.6 complete moles. Fluid collects in the middle, forming cisterns, and the stroma is compressed against the trophoblastic layers which show circumferential hyperplasia.

(a) Circumferential trophoblast hyperplasia. Abnormal trophoblast hyperplasia is a constant finding in CHM when the sample is adequate and it is recognized by the presence of more than two layers of trophoblast forming sprouts or more extensive circumferential masses (Figure 4.7). Patients who eventually need chemotherapy have, on average, higher serum hCG values than those who do not [7] and, initially a correlation between more abundant and more pleomorphic trophoblast and worse prognosis was reported [12, 53] but this has not been confirmed by further studies [54-57] and is probably explained by the fact that excess trophoblast is only loosely attached to villi and the amounts in the sample may not be representative of the whole, or indeed, of what is left in utero. Serial hCG estimations are a better indication of residual trophoblast activity rather than histological grading systems. In our experience, excess trophoblast is, if anything, usually easier to detect in younger than in older CHMs, since fully developed hydrops in older moles is sometimes associated with attenuation of trophoblastic layers. It should be noted that even for CHM, not all villi will necessarily demonstrate circumferential trophoblast proliferation; in one study this affected 30% of CHM villi (57)

(b) Generalized hydrops may not be fully developed in early gestation CHMs. It is important to stress that complete hydrops, from which CHM derives its name and which results in the characteristic ‘bunch of grapes’ macroscopical appearance of classical mole, (Figure 4.3) requires time to develop. Most CHMs exhibited generalized hydrops when they were evacuated at around 16-20 weeks GA, but with current evacuation at 10-12 weeks’, hydrops in most specimens is only focal. Many CHMs referred to our institution have been erroneously called PHMs initially on this account. (Figure 4.7).

(c) Early gestation CHM may exhibit stromal blood vessels. The presence of vessels in CHM is a major source of diagnostic confusion. It is true to say that when CHMs were evacuated late, most had no vessels.
Several publications have however described the frequent presence of vessels in androgenetic CHMs at early gestational ages [6, 7, 51-52, 59]. In our experience it is rare to see avascular CHMs before the 12th week of gestation [7]; in most cases the vessels are collapsed and empty, although occasionally they are open and rarely may contain nucleated red cells (see below for discussion of this unusual finding). Since villous vascular development is initially independent of a fetal circulation, in our experience whether a mole has vessels or not depends primarily on its GA and, if a fetus is present, on the time lapsed since fetal death occurred. In a large study we found a higher proportion of avascular PHMs than avascular CHMs, reflecting the longer GA of PHMs [7].

(d) **In CM, stromal karyorrhexis is very common even at early gestations.** Karyorrhexis (nuclear debris) in villous stroma of otherwise well-preserved villi is almost always seen in early CHMs and is useful in diagnosis. [4, 28, 50-51]. It should not be confused with cell necrosis in vessels, which is also common in PHMs and HAs after fetal death and it should not be considered in isolation as an absolute diagnostic criterion, since necrosis of stromal cells is possible in other circumstances. However, it is so common and intense in CM and so rare and slight in PHM and HAs that its presence should make one think of the possibility of CM, particularly when the material available is scanty.

(e) **Early gestation CM have characteristic ‘budding’ architecture.** (Figure 4.7) Secondary villi sprout from primary villi in a fashion resembling that of early placentation [50, 52]. Unlike in normal placentation, where villi are regular in size, radiate from a central cavity and have polar trophoblast, the secondary villi of CM are irregular in size and distribution and have circumferential excess trophoblast. These irregular villi are often mistaken for the scalloped villi of a PHM and, although the differences between them are lengthy and difficult to describe, they can be readily appreciated by comparing Figure 4.7 with Figure 4.10 and 4.11.
Figure 4.8 Irregular trophoblastic inclusion in complete mole. Trophoblastic invaginations often result in pseudoinclusions but these are irregular or oval and different from those seen in partial mole (see Figure 4.11). X50.

Figure 4.9 Complete mole in early gestation. Trophoblastic invaginations and budding occasionally result in a characteristic lobulated appearance. X100.

(f) Transverse sectioning of irregular villi in CHM often results in oval or irregular pseudoinclusions of trophoblast (Figure 4.8). These are often cystic and large and appear morphologically different from the more regular small round inclusions seen in PHM (see Figure 4.12).

(g) The stroma of CHM is usually mucoid with little fibrosis. Before the development of hydrops, the villous stroma in CHM contains only a little reticulin around blood vessels [6,7]. With development of hydrops a little collagen may be seen in the condensed stroma at the periphery of cisterns but, unlike in PHM, fibrosis is not usually a feature of villous stroma even with advancing gestational age. In early gestation CHMs the combination of basophilic mucoid stroma and invaginations of trophoblast from secondary villi may result, in cross-sections, in a characteristic lobulated appearance superficially resembling a fragmented intracanalicular breast fibroadenoma.
(h) In most CHMs no evidence of embryonic development is found. CHMs are characterized by excess trophoblast but no embryo. However, we and others [6,7,60, 61] have very rarely found nucleated fetal red cells, amnion or other embryonic tissues in specimens which are morphologically typical CHMs and genetically androgenetic [62]. Some of these may represent examples of CHM with unsuspected twin pregnancies but in others the presence of fetal red cells in molar villi remains unexplained and the possibility that some embryonic development may rarely occur in CHM has not yet been adequately excluded. For practical purposes the presence of embryo, amnion or fetal red cells in mole is strongly suggestive of PHM but CHM is not excluded absolutely and the latter should be considered if other histological features suggest it, or if the mole is diploid on flow cytometry (FC). Furthermore, extremely rare mosaicism for CHM with fetus has now also been reported.

i) Abnormal implantation site fragments are often present. The implantation site in CHM often demonstrates lack of the normal endovascular plugging in association with florid interstitial trophoblast infiltration, with or without pleomorphism. These features, of themselves, have no clinical or prognostic significance when associated with CHM villi but can provide additional diagnostic information.

Ideally, a morphological diagnosis of CHM based on these macroscopical and microscopical features should be confirmed by genetic studies but this is seldom necessary in practice; if required, additional support for diagnosis of CHM rather than PHM can be derived from the finding of a diploid DNA complement by FC [63-67]. This can now be performed from fixed or paraffin-embedded material [68]. Furthermore, confirmation of CHM can simply be achieved using p57KIP2 immunostaining, which is imprinted and expressed in the majority of villous cytotrophoblast and stromal nuclei in all non-CHM conceptions but is absent in CHM. (See later section)

4.6 THE MORPHOLOGY OF GENETICALLY UNUSUAL COMPLETE HYDATIDIFORM MOLE

No morphological differences have been found between XX and XY CHMs [52]. Most CHMs have a diploid karyotype but some are tetraploid [69, 70] and very rarely triploid but androgenetic [66, 71]. Examination by FC and interphase cytogenetics of large numbers of cells from CHMs [7,72-75] has shown hyperdiploid and tetraploid fractions not easily explained by cells in division and which may represent aneuploid cell populations in about 40% of CHMs. This fits well with the common observation of large pleomorphic nuclei in molar trophoblast and has been confirmed by in situ hybridization studies [72, 73] but it does not mean that all the cells in the CHM are tetraploid and does not conflict with earlier cytogenetic studies which showed diploid karyotypes in most CHMs. Very rarely, moles with morphology indistinguishable from that of androgenetic CHM are found to have a normal diploid biparental
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4.7 PARTIAL HYDATIDIFORM MOLE

Traditionally, PHM produces less voluminous specimen than CHM and villous hydrops is focal and noticeable only in advanced cases [79, 80] [31]. Fetal parts or a fetus, often with congenital malformations or growth retardation may be present.

PHM are unequivocally associated with increased risk of GTN, occurring in around 1 in 200 cases and CC derived from PHM is also well described. [81-84] Subsequent studies [33, 51, 85, 86] indicated that PHM was almost always due to triploidy when two chromosome sets came from the father and one from the mother. Triploidy is a commoner genetic abnormality than androgenetic CHM [62] and since about 70-80% of triploids are paternally derived (i.e. PHMs), PHM should be about twice as common as CHM. In specialized centers the association of PHM with triploidy has been well over 90% [7, 60, 65, 66]; tetraploid PHMs are rare [64] but most FC studies include a small proportion of diploid specimens morphologically diagnosed as PHMs.

Confusion between CHM and PHM results from the failure to update diagnostic criteria for the earlier gestational age CHMs encountered in current practice. A diagnosis of PHM based exclusively on the presence of blood vessels or partial hydrops is often wrong and a diagnosis of PHM based on the presence of fetal tissues or amnion is occasionally wrong and due to either twin pregnancy or to the rare...
androgenetic CHMs with fetal red cells, discussed earlier. Confusion between PHM and HA is both conceptual and morphological. HAs include trisomies, monosomies, translocations and maternally derived triploids [87, 88] and some have morphological similarities with PHM. There is no evidence that they present a risk of GTN greater than a normal pregnancy but they may be difficult to differentiate from PHM especially in limited samples [6, 7]. As a group they are more frequent than PHM and at present represent around 10% of cases initially registered as PHMs at a regional centre. [7] Rarely, placental mesenchymal dysplasia can be confused with PHM but in PMD the stem villi are hydropic and there is no excess trophoblast proliferation [89, 90]

The main features useful in diagnosing PHMs are as follows:

(a) Trophoblastic excess is focal and less marked than CHM and may not be detected if the specimen is not extensively sampled. Most classical PHMs were evacuated in the second trimester when excessive trophoblast is usually predominantly syncytiotrophoblast projecting from the surface of villi (Figure 4.11) or invaginating into villous stroma to form pseudoinclusions (Figure 4.12); this excess may be present in both hydropic and non-hydropic villi. Clusters of pleomorphic extravillous trophoblast are not common but may be seen in PHM. Since many PHM are also evacuated in the late first trimester, in many PHMs the abnormal distribution of trophoblast may be more striking than its florid excess, especially if there is limited sampling. In our experience hCG in PHM is usually higher and remains elevated after evacuation for longer than after HA [7], and this is also reflected in a higher tendency for GTN. There is no excess circumferential trophoblast in HA but occasional sprouts of trophoblast around small villi may be seen; however, polar trophoblast is very common in HA, whereas it is less common to find it in PHM. In PHM the amounts of trophoblast, its proliferative activity, its aneuploid fractions on FC and the persistence of elevated hCG after evacuation are significantly less intense than in CHM [7, 67, 91-93]. It should be noted that in PHM, abnormal trophoblast proliferation affects <10% of villi. (57). One recently described case series showed that trophoblastic hyperplasia was present in <20% of their genotyping proved PHM’s and 8% of trisomies showed moderate degree of trophoblast hyperplasia (94)

Figure 4.11 Partial mole. Circumferential hyperplasia is in this case prominent but consists mainly of syncytiotrophoblast. This hyperplasia can be very focal and may not be detected unless sampling is extensive. X50.
(b) In PHM hydrops and cisterns characteristically involve only some of the villi. The diagnostic confusion resulting from interpreting partially developed hydrops in young CHMs as evidence of PHMs has already been discussed. In a small proportion of PHMs hydrops is mild and cisterns may be small or absent in the sample, especially at early gestations; sampling error is a possible explanation for this but in some PHMs early fetal death may result in extensive fibrosis of villi rather than marked hydrops. A common pitfall is to interpret in HA fragments of a gestational sac which has lost its amnion as villi with large cisterns, but as a rule the wall of the gestational sac contains more collagen than surrounding villi and the possibility of HA should always be considered when the suspected cisterns are few and surrounded by smaller villi, particularly when the placental material obtained is scanty. In addition hydropic villi in non-molar miscarriage are usually round in outline and hypocellular in contrast to the irregularly shaped and more cellular villi of PHM (See later).

(c) Vessels, often containing nucleated fetal red cells, are a common finding in PHM. Whereas the vessels of a CHM are often collapsed and inconspicuous, those of a PHM are often open but they gradually decrease in numbers with increasing GA. In some PHMs avascularity of some villi is associated with angiomatoid vessels in others (Figure 4.13). These abnormal vessels, mistaken in early publications for maze-like cisterns [51], are relatively specific for PHM but are identified in only about 20% of cases. [7,51] Usually, angiomatoid vessels are empty, suggesting fetal death long before evacuation. An unusual case of extensive angiomatoid changes resulting in macroscopic grape-like vesicles has been reported in first trimester miscarriage (94)
(d) Karyorrhexis in villous stroma is seldom present or intense in PHM. This excludes nuclear debris found within and in the wall of vessels and villi in which the covering trophoblastic layers are already necrotic. The reluctance of some publications to emphasize this as a useful diagnostic criterion for CHM is understandable, since karyorrhexis is such a non-specific histological abnormality, but its correlation with CHM is very high [7,50,51], although its significance has not yet been explained.

(e) PHMs have abnormally shaped villi often described as scalloped or dentate (Figures 4.11 and 4.12) and invaginations of trophoblast result in rounded inclusions.

Unlike the abnormally shaped ‘budding’ villi of CHM (Figure 4.7) the irregularities in outline in PHM are more angulated and appear in cross-section as rounded inclusions which may be solid or cystic and which contrast with the more oval or irregular inclusions of CHM. Infolding of trophoblastic layers in the hydropic villi of a PHM may also result in pseudo-inclusions similar to those of CM, which are therefore less useful in differential diagnosis. Scalloping and round inclusions can be seen in both hydropic and non-hydropic villi, and although the small villi of PHM are often described as normal some can in fact be very abnormal and may show excess trophoblast (Figure 4.14).

Unfortunately, irregular villi and round inclusions may also occur in non-molar HAs, particularly in trisomies, including the two commonest forms trisomy 16 and 18, and in monosomy 45X0 [95], but in these conditions usually there is no excess trophoblast. (Figures 4.15,4.16)

(f) In PHM villous stroma contains abundant collagen and this increases with GA. In PHMs evacuated at early gestations the stroma may be more immature and mucoid, scalloping less intense and trophoblast more abundant, so that they may resemble CHMs, but as a rule there is a fine mesh of reticulin fibers in non-hydropic villi, with stromal cells often present in this mesh. Fibrosis increases with GA and is present at the periphery of cisterns.

(g) An embryo or fetus, often malformed, frequently dies in early pregnancy and may not be identifiable in the specimen. Fetal tissues were only present in 20% of a
series of PHMs reviewed by our centre [7], but there were fetal red cells in molar villi in nearly 50% and chorioamniotic membranes in 40%. Fetal red cells may persist in vessels long after fetal death and are sometimes seen together with a necrotic embryo. The finding of fetal tissues with a mole is often interpreted as proof of PHM and although in most cases this is true, the possibilities of androgenetic CHM with twin pregnancy and CHM with unexplained fetal red cells in molar villi (see section 4.5) should be considered if the features of the mole are not those of a PHM.

As in CHM, cytogenetic, molecular genetic and interphase cytogenetic studies [73, 96,97] can be helpful for accurate diagnosis. In their absence FC is helpful for differentiating between diploid CHMs and triploid PHMs and between the latter and non-molar diploid HAs, particularly when placental material is scanty.

**Figure 4.14**  Non-hydropic villi in a partial mole are often referred to as normal, but some can be very abnormal and show excess trophoblast and irregular outline. X50.

**Figure 4.15** Irregular villi and rounded trophoblastic inclusions in Patau’s syndrome. The inclusions are few and small. The irregular outline is less angular than in partial mole, there is no excess trophoblast and hydrops is often minimal or absent.
Villous dysmorphism (abnormal villous morphology, AVM) can be seen in specimens from miscarriage or surgical/medical termination of pregnancy (STOP/MTOP) secondary to chromosomal abnormalities. Most commonly encountered chromosomal abnormalities include trisomy 16, 21, 7, 13, 8, 18, 4, 2 and 12. A combination of 2 or more abnormalities may also be acquired. These on histology show markedly irregular villous contour with simple trophoblastic pseudo-inclusions and variable degree of hydropic change. Cisterns are unusual and trophoblastic hyperplasia is very rare but has been reported in approximately 14% and 8% cases respectively in a large series by Buza et al. (94). Trisomies for chromosomes 7, 15, 21 and 22 may also show trophoblastic hyperplasia equivalent in severity to that seen in partial or complete moles (49). No single morphological feature is helpful in differentiating HM from chromosomally abnormal gestation. A constellation of features is helpful. Buza et al have shown that combination of features including 2 villous populations, round or oval trophoblastic pseudoinclusions, and cistern formation increased the specificity to 94% with a positive predictive value (PPV) of 79%. The most specific morphologic features for PHM according to this study were combination of cistern formation and chorionic villous size >2.5 mm, reaching 96% specificity and 90% PPV (94).

Digynic triploid gestations can mimic PHM not only at the morphologic level, but also on DNA ploidy analysis. Dysmorphic villi with irregular contours, trophoblastic pseudo-inclusions and syncytiotrophoblast sprouts are a feature and these are usually non-hydropic. Most villi are vascular and contain nucleated red blood cells. One third of all triploids are digynic, non-molar gestations, without an increased risk of GTN, hence need no follow-up. Genotyping is helpful in excluding PHM in difficult cases but p57 and ploidy analysis play no role (49, 98).

Placental mesenchymal dysplasia, also known as “pseudo-partial mole”, is a form
of androgenetic/biparental mosaicism that mimics PHM in second or third trimester of pregnancy (98, 99, and 100). Villous hydrops of stem villi, often with cistern formation, is seen along with thrombosis and or aneurysmal dilatation of stem villous vessels. Terminal villi appear normal or show chorangiomatoid changes and no abnormal trophoblast proliferation is noted. Villous stroma consists of spindle and stellate mesenchymal cells in a myxoid background. P57 staining may be negative or discordant.

Twin pregnancy with coexisting normal fetus and CHM may mimic the dual villous population of PHM as a result of admixture of large molar villi with smaller, normal-appearing villi. Presence of fetal tissue, nucleated red blood cells or gestational membranes from the normal twin may be taken as evidence of PHM. P57 will show ‘divergent pattern’ of p57 (101,102) with an admixture of p57 negative and positive villi. This is helpful in confirming the suspected morphological diagnosis. Ploidy will show diploid conception and genotyping will be helpful if two population of villi are microdissected.

Mosaic moles are rare and present with apparent diffuse placental CHM change with molar villi intermixed with morphologically normal villi, and a chromosomally and phenotypically normal infant. They appear to represent true mosaicism, with androgenic and biparental cell lines derived from the same sperm. The villi are androgenetic in the molar areas and biparental in the histologically normal areas [98]. Discordant p57 expression characterizes androgenetic/biparental mosaic/chimeric conceptions, with discordant expression in different cell types based on the presence or absence of maternal genetic material in those particular cells. Discordant p57 implies any combination/admixture of negative and positive results for villous stromal cells and cytotrophoblast within individual villi, including positive staining in cytotrophoblast and negative staining in villous stromal cells, or vice versa (101,102).

Figure 4.17 Placental Mesenchymal dysplasia showing hydropic and enlarged stem villi with aneursymal dilatation of vessels. Tertiary villi are normal
Figure 4.18 Twin Pregnancy with large molar villi (right) and normal tertiary villi (left)

4.9 INVASIVE MOLE

The majority of CHMs remit after evacuation and curettage, but the tendency in some instances to invade the myometrium resulting in uterine perforation and extension to adjacent organs is well known and historically approximately 15% of CHMs behaved in this way [12, 15, and 16]. Histologically the appearance of the chorionic villi is similar to that of other CHMs, but swelling of villi is less often seen in deeply implanted invasive moles and histological diagnosis is based on the excessive trophoblastic proliferation, markedly irregular shape of villi and their invasive nature (Figure 4.19). Some degree of myometrial trophoblastic invasion is probably present in most moles; indeed, myometrial invasion is not exclusive to molar pregnancies. Hertig [12], borrowing the terminology used for myometrial placentation, classified invasive moles into accreta, increta and percreta. Since hysterectomy is rarely performed in current practice in the treatment of moles, the few specimens seen are usually in unmonitored and often unsuspected pregnancies and, sometimes, are paradoxically percreta moles (the rarest form) presenting as acute abdomen due to uterine perforation (Figure 4.20) and consisting of a hematoma with molar villi often implanted on a pelvic organ such as the ovary, tube or broad ligament, or on a loop of intestine. IM in association with PHM is very rare. All but one of the IMs in hysterectomy specimens in our archival material are CHMs but rare invasive triploid PHMs requiring hysterectomy have been reported [83,103] and we have seen a PHM which required three curettages, the last under ultrasound control, for removal of deeply implanted myometrial villi.
Figure 4.19 Fundal unsuspected invasive mole.

Figure 4.20 Invasive CHM in myometrial vessels. There is abundant excess trophoblast and hydrops is conspicuous.

4.9 MOLAR METASTASES

CHMs can spread locally to the cervix, vaginal wall and vulva [58,104,105] but with modern chemotherapy this does not imply a worse prognosis [58]. In the past, up to 10% of patients with CHM had radiological lung opacities which were interpreted as metastases [106] and this was confirmed histologically in some cases [107,108]. With early diagnosis and early chemotherapy, the number of patients with metastases has been reduced and disease is usually limited to the uterus and parametrial tissues. Exceptionally, tissue from CHMs has been found in other sites, including brain, retroperitoneum and inguinal lymph nodes [15]. In pre-chemotherapy days lung metastases sometimes regressed after evacuation or eradication of the mole [109], but the appearance or persistence of metastases long after evacuation of a CHM may indicate GTN or CC. We have not seen tissue from PHMs outside the uterus.
Figure 4.21  Invasive CHM in lung parenchyma presenting as haemothorax (a). Molar villi and trophoblast are seen plugging a bronchial blood vessel (b).

4.10  ECTOPIC MOLES

There is a tendency to overdiagnose tubal moles due to the presence of extensive trophoblast proliferation which may be associated with abnormal implantation at this site. [110, 111]. Burton et al (2001) noted only 3 of 20 cases referred as ectopic moles were confirmed on review and similarly, this is our experience, [112, 113] most referred cases turning out to be non-molar specimens with abundant polar trophoblast in early gestation. It appears that tubal moles are considerably rarer than tubal CC and we have found no reliable reports of tubal mole followed by CC, although GTN after tubal pregnancy does occur [114,115].

4.11  HYDATIDIFORM MOLES WITH TWIN PREGNANCY

The fact that CHMs can coexist with a twin pregnancy has already been mentioned as a possible cause of confusion in the ultrasound and histological diagnosis of PHM and as one of the possible explanations of the finding of fetal red cells in the vessels of a CHM. Twin molar pregnancies are now well-reported. The frequency of dizygotic twin pregnancy in the general population is one in about 110 [116]. CHMs
with evidence of a twin, or a second population of non-molar villi suggestive of a twin, represent about one in 200 histologically confirmed CHMs. Combinations of PHM with a normal twin are considerably rarer and we have only seen a handful of examples: one possible reason is that it may be more difficult to detect morphologically, particularly in early miscarriages.

The outcome of pregnancies with histologically confirmed CM and co-twin has been examined in a series of 77 cases at Charing Cross Hospital. The risk of GTN development was no higher than reported following singleton CM pregnancies, and furthermore, the risk of GTN was not different in those cases managed by early elective termination of pregnancy at diagnosis and those who chose to continue the pregnancy. However, in such cases in which pregnancy continuation is attempted, several complications may develop. Almost half of the pregnancies result in fetal loss before 24 weeks’ gestation, and of the half that reach potential viability, 25% result in intrauterine death and 40% deliver severely preterm at <32 weeks’ gestation. In this series, severe pre-eclampsia was an unusual complication (5%). Therefore, overall, the incidence of GTN in CHM with a twin is similar to that of CHM in a singleton pregnancy and the risk of GTN is unaffected by pregnancy management [117-123].

**Immunohistochemistry in the diagnosis of molar disease**

The basis for the diagnosis of CHM and PHM remains detailed examination of tissue morphology on routine Haematoxylin & Eosin (H&E) stained sections. Additional special stains may be carried out to highlight certain aspects of the microanatomy, such as trichrome stains for collagen, but these have not been shown to provide useful additional diagnostic information. Immunohistochemical methods may be used to identify trophoblast and define the hormonal profile of the tissue but, in contrast to the case with gestational trophoblastic neoplasia, in molar pregnancy, this does not provide clues to the differential diagnosis.[6] Immunomarkers of cell proliferation, such as PCNA and Ki67, have also been examined in this context, and similarly have been reported to be of little practical use in differentiating between HA, CM and PM. [124, 125] Recently, the expression of cell cycle control proteins has been examined and E2F-1 and cyclin E reported to be upregulated in molar tissue [126]. Furthermore, since molar pregnancies are associated with defects in imprinting (see chapter 2), it has been suggested that abnormal expression of imprinted gene products could also provide diagnostic information. Distinguishing between partial moles and complete moles on morphological features is not usually difficult but occasionally, particularly in early gestations, the features indicate a molar pregnancy but determining the type is difficult. The assessment of expression of the p57\(^{KIP2}\) protein, a cell cycle inhibitor, has proved useful in specialized centres. p57\(^{KIP2}\) is a strongly paternally imprinted gene, being expressed predominantly from the maternal allele in most tissues. Hence there is a lack of p57\(^{KIP2}\) expression in cytotrophoblast and villous mesenchyme in CHM compared with strong expression in similar cells in both PHM and HA. [127]. Such immunostaining however, is of no value in distinguishing between PHM and non-molar miscarriage, which is the
most difficult morphological differential diagnosis. p57 immunohistochemical analysis is quite useful for recognition of androgenetic/biparental mosaic/ chimeric conceptions [102]. Pitfalls for p57 staining include occasional p57 positive CHM in cases of retained maternal chromosome 11 and similarly loss of maternal chromosome 11 resulting in p57 positive PHM. We have also encountered cases of non-molar p57-negative biparental diploid, with no morphological features of biparental hydatidiform mole. These have also been reported in a large series by Banet et al [102]. At present therefore, immunohistochemical techniques remain an important but minor adjunct to morphological diagnosis in molar disease.

4.12 PROGNOSIS OF MOLES

No immortal cell lines or long-term growth in nude mice have yet been achieved with trophoblast of moles [128], whereas this can be readily done with CCs. This supports the view that the excessive trophoblastic proliferation in moles intrinsically lacks the features of malignancy in most cases. Nevertheless, molar trophoblast can continue to grow and invade, needing active intervention to preserve the uterus or to avoid a catastrophic hemorrhage. Exceptionally, molar trophoblast can survive for long periods and the immediately preceding pregnancy of a CC may not be the genetically causative one, the tumour genetically deriving from the previous CHM. [129,130]; however, the vast majority (>95%) of complications due to persistent or invasive moles occur in the six months following a molar evacuation [131-132]. The reported incidence of CHMs needing treatment varies according to the criteria for diagnosis of GTN and to whether PHMs and HAs have been reliably excluded in the series. Around 8% of all moles require treatment [86,131,132], including around 15% following CHM and 0.5% following PHM. Figures of up to 30% reported from the USA [134,135] are a consequence of less stringent diagnostic criteria for GTN and many such patients would not have been treated in the UK. A higher incidence of GTN was initially suggested in heterozygous (dispermic) moles [135,136], which comprise about 20% of CHMs [28], but this has not been supported by more recent studies [26,28,137]. The lack of relationship between the amounts of trophoblast in the sample and the incidence of GTN has already been discussed. Although as a group CHMs with higher hyperdiploid fractions may have higher incidences of GTN [67] this does not predict outcome in individual patients. Neoplastic transformation of a mole is recognized when villous stroma is no longer formed but nothing is known of the factors which control this change in behavior. In the past, approximately 3% of CHMs became CCs [11, 12]. Because of early chemotherapy in CHM, the figure now is probably lower, but the real incidence is not known, since histological confirmation in GTN is nowadays seldom available, the patients being treated with chemotherapy on the basis of their hCG levels.

The incidence of GTN after PHM is considerably lower than after CHM. High incidences of 18% and 20% [90, 138] include many diploid PHMs which undoubtedly were misdiagnosed CHMs. Using American
criteria for diagnosis of GTN, 5.5% of patients with PHMs were treated in the New England Trophoblastic Disease Center [139]. Using UK criteria, GTN follows about 0.5% of histologically reviewed PHMs [6] [140]. The incidence of CC after PHM is not known but histologically and genetically confirmed cases are well-reported. We have not observed more trophoblast in PHMs which develop GTN than in PHMs which do not, but trophoblastic excess in PHM can be very focal and the potential for sampling error is great.

Despite numerous small studies suggesting a range of morphological features and immunohistochemical findings being associated with adverse outcome, recent blinded data indicates that there are no features or immunoprofiles, present at diagnosis of CHM that can reliably predict those cases at increased risk of developing GTN and requiring chemotherapy (57)

4.13 CHORIOCARCINOMA

This is a malignant neoplasm of trophoblast with differentiation towards the phenotype of villous cytotrophoblast and syncytiotrophoblast (Figure 4.22). Occurring primarily in patients of reproductive age, rare cases of gestational choriocarcinoma have been reported in post menopausal patients after long latency (141). It can occur after CHM, PHM or non-molar pregnancy but the incidence of CC after CHM (about 1 in 50) is about a thousand times greater than after a normal pregnancy (about 1 in 50,000). The proportion of CCs preceded by moles in reported series varies between 39% and 78%, but this is probably an underestimate due to under-recognition of early complete moles in the historical literature [4]. It is generally assumed that the immediate antecedent pregnancy is responsible for the tumour and although in most cases it may be so, it is not necessarily the case [129,130]. As in the case of hydatidiform mole, there are remarkable differences in the incidence of CC in various parts of the world [4, 28,142]. These are not always explained by an increase in preceding moles but a serious epidemiological study of the problem is hindered by variations in the way in which the information is recorded, bias in the selection of reported cases and the very success of therapy, which all too often nowadays precludes us from knowing the true nature of the trophoblastic disease treated.

Vaginal bleeding and marked elevation of serum human chorionic gonadotropin (hCG) are the characteristic clinical findings; however some cases may present with a metastatic disease and subsequent diagnosis of the primary tumour. In rare cases the primary tumour cannot be discovered and is assumed to have undergone spontaneous regression.

Macroscopically uterine CC typically appears as bulky, destructive, single to multiple dark red, shaggy masses with extensive hemorrhage, and variable amount of necrosis. The lesion may line the uterine cavity or is deep within the myometrium (Figure 4.22, 4.23). Microscopically, tumour shows an expansile/ pushing border with characteristic bilaminar pattern reminiscent of pre-villous trophoblast. The neoplastic cells are an admixture of of syncytiotrophoblast, intermediate trophoblast and
cytotrophoblast. The biphasic pattern (Figure 4.24) is easily identifiable but in some cases most of the tumour is either predominantly syncytiotrophoblastic (Figure 4.25) or predominantly cytotrophoblastic (Figure 4.26, 4.27). This monomorphic variant may be difficult to diagnose and requires a careful search for second component. The viable tumour is frequently seen only at the periphery of the nodule and within vascular spaces in adjacent myometrium and as a rule no intrinsic vasculature can be demonstrated. Blood–lakes, pseudovascular channels and geographic necrosis is therefore a frequent accompaniment. A direct relationship between the intensity of the surrounding inflammatory reaction and a better prognosis has been demonstrated [143] and some relationship between the degree of anaplasia and prognosis has been suggested [144]; these studies have less relevance today because of the good therapeutic response seen in most patients.

Some predominantly cytotrophoblastic tumours secrete smaller amounts of hCG [145]. This is not surprising, since synthesis of hCG occurs mainly in syncytiotrophoblast. Some hPL is usually demonstrable in syncytiotrophoblast but, unlike placental site trophoblast, villous cytotrophoblast does not contain significant amounts of hPL. The morphology of CC can be markedly changed after chemotherapy, and when therapy-resistant metastases are removed they are often composed of bizarre mononuclear cells [146] which probably represent therapy-resistant clones. These atypical CCs are sometimes difficult to differentiate from PSTT and ETT but unlike PSTT and ETT, they form hemorrhagic nodules, readily infiltrate vessels, have a high mitotic rate and express more hCG than hPL or PLAP [147].

Figure 4.22 Choriocarcinoma grows by invading maternal vessels and viable tumour is usually only found at the periphery of a hemorrhagic mass. Reduced from natural size.
Figure 4.23  Choriocarcinoma with a circumscribed haemorrhagic and necrotic mass in lower uterine segment.

Figure 4.24  The typical form of choriocarcinoma forms cyto- and syncytiotrophoblast similar to that of villous trophoblast. X180.

Figure 4.25  Predominantly syncytiotrophoblastic choriocarcinoma in endometrial curettings.
Figure 4.26 Some choriocarcinomas are predominantly cytotrophoblastic and secrete less hCG but retain a predominantly intravascular mode of invasion; unlike placental site tumor they contain little hPL, are composed of cells with less dense cytoplasm and have numerous mitoses.

Figure 4.27 Monophasic choriocarcinoma mimicking poorly differentiated choriocarcinoma

CC metastasizes readily and it is not uncommon to find secondary nodules in the cervix or vagina on presentation. The lungs, brain and liver are the distant organs more often involved and sometimes metastases dominate the clinical picture and patients may rarely present with intracerebral hemorrhage or with acute cor pulmonale due to extensive intravascular lung disease [148,149]. Unexplained intracerebral hemorrhage or acute cor pulmonale in a woman of childbearing age should prompt suspicions of CC. Failure to consider this possibility has led in the past to CC developing in immunosuppressed organ recipients [150]. Melena or hematuria from metastases to intestine or kidney are
less common [149]. In contrast, lymph nodes are rarely the site of metastasis and the finding of a tumor with apparent trophoblastic differentiation in lymph nodes without evidence of a gestational primary should raise doubts about the gestational origin of the tumor, which could be of germ cell origin or a carcinoma with trophoblastic metaplasia. CC following a normal term pregnancy is presumably the result of the development of an intraplacental choriocarcinoma (Figure 4.28) but the primary tumor may be microscopical and may not be detected, since pathological examination of apparently normal placentae in normal births is seldom carried out. Origin from specific pregnancies can nevertheless be demonstrated genetically [125]. It should be remembered that placental CC can metastasize to the child as well as the mother [151-153].

A common problem is the differential diagnosis between CC and trophoblast from an early pregnancy or that of a mole. Curettage in an early pregnancy may yield abundant sheets and fragments of pleomorphic trophoblast. As a rule, normal young trophoblast is less pleomorphic than that of CC and its polar arrangement may still be discernible. It is exceptional to find villi when CC develops except in the context of third trimester intraplacental CC. The presence of villi and abundant trophoblast in endometrial curettings is suggestive of a recent miscarriage rather than CC, which has in the past been overdiagnosed by pathologists [154], but in a few cases residual degenerate villi may often be seen [145]. However, the pleomorphic trophoblast from a mole, particularly a CHM, can be morphologically indistinguishable from that of CC, although it has a different biological behavior. Selective sampling of a
second curettage after evacuation of a CHM may yield trophoblast without villous stroma, when a diagnosis of persistent trophoblast should be made and its significance judged together with clinical ultrasound and serological data. As a rule, the longer the period between evacuation of a known pregnancy and the finding of trophoblast in curettage, the more likely its pathological significance provided a new pregnancy can be ruled out [154].

An additional diagnostic difficulty may be differentiating a true gestational choriocarcinoma from non-gestational germ cell tumour or rare choriocarcinomatous differentiation in a somatic malignancy. This can only be achieved with certainty using molecular genotyping of microdissected tumour and non-tumour tissue (see genetics chapter).

4.14 ECTOPIC CHORIOCARCINOMA

Primary CC of the fallopian tube is rare but well-described [110]. (Figure 4.28). About 60% of patients presented with acute symptoms simulating an ectopic pregnancy and the rest had a pelvic mass indistinguishable from an ovarian tumor. In no case was there evidence of a preceding ectopic mole but in two instances there were residual chorionic villi and evidence of a gestational sac, an unusual finding in uterine CC. In all other aspects (distribution of metastases, mortality, response to chemotherapy) the tumors behaved like uterine CCs and only five of 47 patients survived before the advent of chemotherapy; in contrast 15 of 16 patients given chemotherapy survived. Tubal CC may also present with metastases [112] and has been described following fertility treatment [155].

Although cases of gestational CC of the ovary, broad ligament and peritoneal cavity have been reported [15,156,157,158] reliable evidence of their gestational rather than germ cell or metaplastic origin can only be obtained by genetic fingerprinting of the tumor, the patient and the patient’s partner. Trophoblastic metaplasia and hCG production can occur in many carcinomas [159] including ovarian and endometrial carcinomas [160,161]. Histological differentiation from CCs is not always easy, since they can mimic the pattern of vascular invasion and the bilaminar structure of villous trophoblast (Figure 4.24). In some cases there is a second pattern, such as adenocarcinoma, squamous carcinoma or transitional cell carcinoma which gives away the diagnosis, but in others tumors are undifferentiated but for the presence of syncytiotrophoblast or hCG production; suspicions that the tumor may not be gestational should arise if the tumor is producing little hCG for its bulk, if it has a vascular stroma on histology and if it is resistant to chemotherapy. Confirmation
is now possible by comparing genetic polymorphisms of the tumor with those of the patient and her partner, and modern techniques can give a quick answer which can influence clinical decisions [162,163]. The problem is not just academic since in carcinomas with trophoblastic metaplasia surgical eradication offers the only hope of a cure. Carcinomas with trophoblastic metaplasia probably account for the majority of so-called ‘primary choriocarcinomas’, reported in viscera, including lungs, breast, stomach, intestine, liver, adrenal, pancreas, bladder and kidney [159,163-174].(Figure 4.29)

Figure 4.28 Choriocarcinoma in the fallopian tube. Tumor is obliterating the lumen and invading the wall of fallopian tube. Choriocarcinoma does not have intrinsic vasculature.
Figure 4.29  Carcinoma of the colon with trophoblastic metaplasia. The patient was a 64-year-old woman and in places the tumor was an adenocarcinoma, but in places (see intravascular tumor in the lamina propria) was differentiating towards trophoblast with hCG production. X100.

4.15 INTRAPLACENTAL CHORIOCARCINOMA

Gestational choriocarcinoma is a nonvillous trophoblastic neoplasm with the exception of intraplacental choriocarcinoma. This develops near term in an otherwise normal pregnancy. Most reported cases have clinical evidence of a maternal and/or fetal metastatic disease (175). It is extremely important to recognise this condition and once the diagnosis is confirmed both the mother and fetus must be thoroughly evaluated to exclude metastatic disease [151-153].

On gross examination intraplacental CC are non-specific, resembling either placental infarcts or thrombi and may be missed on initial examination.

On histological examination, the lesion shows focal proliferation of atypical abnormal villus cytotrophoblast and syncytiotrophoblast filling the intervillous space with involvement of adjacent villi (Figure 4.30).

Figure 4.30  Choriocarcinoma in third-trimester placenta. The patient developed lung metastases soon after delivery of a stillborn infant. X100.
4.16 PLACENTAL SITE TROPHOBLASTIC TUMOR

This is a rare gestational trophoblastic neoplasm, representing 0·2% of UK gestational trophoblastic disease (48). It is believed to be the malignant counterpart of non-villous implantation site intermediate trophoblast, which infiltrates the placental site in normal pregnancy. Kurman et al [176] described 12 cases in which the lesion remained localized and was sometimes eradicated by simple curettage. They proposed the term ‘trophoblastic pseudotumor’ but within a few years it was clear that, on occasion, this lesion behaved in a locally aggressive way and could metastasize [35,37,177,178] and ‘placental site trophoblastic tumor’ [37], is now the preferred term, which reflects the morphological similarities with placental site trophoblast. Epithelioid trophoblastic tumour (ETT) is probably a variant of PSTT in which trophoblastic differentiation more closely resembles the ‘vacuolated’ trophoblast often seen in the chorion of late pregnancy. In these tumours, PLAP rather than hPL is often the dominant immunoreactant [37,179].

PSTT usually present in the reproductive age group with amenorrhea or irregular vaginal bleeding months or years after a pregnancy, [35,178,180] and origin from both moles (including PHM) and normal pregnancy has been demonstrated genetically [181,182]. Occasionally, the patient may be peri- or post-menopausal [182-184]. The mean interval from the last pregnancy is three years but a range of less than two years to over five years were seen in a review of 34 cases at Charing Cross Hospital [185]. The mean interval tends to be longer, six years, for ETT with intervals of up to 18 years having been documented.

![Image](image.png)

**Figure 4.31** In placental site trophoblastic tumor necrosis is often extensive but unlike choriocarcinoma hemorrhage is less conspicuous. Reduced from natural size.

The uterus in PSTT is usually enlarged and pregnancy proteins such as hCG, hPL and β1-glycoprotein (SP-1) are elevated although hCG is seldom as high as in CC. In rare instances (less than 10% of reported cases), PSTT may be associated with the nephrotic syndrome, which has remitted after eradication of the tumor [35,186]. Fibrin and IgM have been found in glomerular intracapillary deposits. A report of virilization and testosterone production by a PSTT appears exceptional [187]. The disorder should be suspected if hCG concentrations are low for the volume of disease...
present on imaging and free-β-hCG values are high, but these features are not diagnostic. Histological analysis is needed to substantiate the diagnosis.

Curettage may yield decidua or myometrium infiltrated by trophoblastic cells with dense eosinophilic cytoplasm and pleomorphic nuclei (Figure 4.32); they can be mononuclear or multinucleated and they often form clusters or cords which separate myometrial muscle fibers. It may be difficult, or even impossible, to differentiate between an exaggerated placental site reaction and PSTT in endometrial curettings, since there may be scanty material or this may be extensively necrotic [188]; also less confluent PSTT at the periphery of the mass may look identical to placental site reaction [189]. Immunocytochemistry for hCG, hPL, p63, MelCAM and PLAP is helpful in diagnosis. In general, PSTT contains more positive cells for hPL and MelCAM than hCG, p63 and PLAP and finding more hCG than hPL in a tumor may mean that the tumor is not PSTT but predominantly mononuclear CC. These tumors have an intravascular rather than interstitial mode of infiltration. They tend to form hemorrhagic masses and metastasize early [147], so that they behave like CC in all but the fact that they tend to be chemoresistant like PSTT. Sometimes, there are great variations in the amounts of hCG detected serologically during treatment which are not always explained by tumor bulk. Ourselves and others have also noted mixed tumours consisting of morphologic areas representing both PSTT and CC. [17] We have observed tumors with morphology of PSTT in the uterus and morphology of CC in the metastases, as well as tumors with areas indistinguishable from PSTT and areas looking like cytotrophoblastic CC; it is therefore possible that CC and PSTT may not arise from different progenitor cells, and whether differentiation occurs towards villous trophoblast or implantation site intermediate trophoblast could depend on environmental factors, including prior chemotherapy which could select chemoresistant clones [187]. In favor of this is the fact that a common form of presentation of PSTT is as a chemoresistant trophoblastic tumor, but there is no doubt that some PSTTs have their characteristic morphology even when no chemotherapy has been administered.

Figure 4.32  Placental site trophoblastic tumor infiltrating myometrium. Cells are predominantly mononuclear and mitoses are rare. Infiltration is predominantly interstitial and tumors express more hPL than hCG.
The most problematic differential diagnosis for PSTT includes an exaggerated placental site reaction. Confluent sheets of predominantly monomorphic cells, unequivocal mitoses, an elevated Ki67 index, and absence of chorionic villi are all in favour of PSTT. Placental site reaction may have more multinucleate giant cells. The formation of large sheets of trophoblastic cells with some mitotic activity appears to be a reliable criterion of neoplasia but in many instances it may only be possible to say that there is persistent placental site trophoblast and judge whether it is physiological or pathological in the light of clinical, ultrasound and serological observations.

A second area of difficulty arises in the differential diagnosis of PSTT from trophoblastic proliferations like placental site nodules (PSN). In our experience and that of others, regressing lesions often show marked hyalinization or central necrosis and, occasionally, there is difficulty in demonstrating cytoplasmic hPL or hCG, although the epithelial nature of the trophoblastic cells can be demonstrated with immunostains for cytokeratin. It has been proposed that these nodules are derived from chorionic-type intermediate trophoblast and their immunocytochemistry profile supports this hypothesis. Some of these regressing nodules express more p63 and PLAP than hPL but as in PSTT this may be a reflection of their age. As a rule, PSNs are not associated with elevated urinary or serum hCG but we have seen PSNs persist for years after the last known pregnancy. Furthermore, we have recently published the largest study reporting followup and outcome in a series of PSNs with atypia showing that a proportion of APSN are associated with malignant placental site or epithelioid trophoblastic tumours (PSTT/ETT).

Placental site trophoblastic tumours must also be distinguished from ETT, epithelioid leiomyosarcomas and squamous cell carcinomas. Smooth muscle tumours do not stain for Inhibin alpha, HPL or cytokeratin 18. Squamous cell carcinomas will not stain for Inhibin alpha and HPL. ETT usually show stronger staining for PLAP and p63 and weaker staining for HPL.

PSTT forms masses in which necrosis is marked, but hemorrhage is less conspicuous. This reflects a lesser tendency to vascular invasion, infiltration being predominantly interstitial. Necrosis can be very extensive in long-standing tumors and dystrophic calcification can occur in areas of necrosis, tumor surviving...
only around vessels (Figure 4.33). In our experience these long-standing tumors express more PLAP than hPL or hCG [147]. There are good descriptions of the electron microscopy of PSTT and CC [28,198] but this seldom helps in diagnosis.

PSTT can infiltrate extensively and extend into adjacent organs such as the ovary, parametrium, rectum or bladder. Distant metastases can occur in lungs, peritoneum, liver, pancreas and brain, and we have observed retroperitoneal and supraclavicular lymph node metastases in a tumor with long evolution. It appears that mitotic counts (more than five mitoses per 10 high-power fields) may predict tumors with metastasizing potential [147,178,199,200,206]; even this may not prove reliable in some cases and we have seen a tumor with 11 mitoses per 10 high-power fields in curettings which apparently regressed, the patient refusing hysterectomy and being delivered of a normal infant two years later [147].

PSTT grows more slowly, metastasise later, and more commonly involve lymph-nodes, and produce less hCG than do choriocarcinomas (48). The importance of recognizing PSTT lies on the one hand in its lesser metastasizing potential, which makes it amenable to surgery, and on the other in its greater resistance to chemotherapy, which accounts for therapeutic failures when surgery is no longer able to arrest its progress. Hysterectomy with sampling of pelvic lymph nodes is the treatment of choice. Ovaries are conserved unless the patient has a family history of ovarian cancer or is postmenopausal. Chemotherapy is indicated for patients with metastases or poor risk factors such as disease presentation beyond 4 years from the antecedent pregnancy. However, young nulliparous women may strongly desire to preserve fertility, especially when there seems to be a focal abnormality in the uterus. Although uterine-sparing surgery is possible, multifocal microscopic uterine disease can happen and could compromise survival (201-204). Appropriate counselling is needed.

Higher mortality, 20% overall in our experience, appears to be linked with lung metastases and presentation greater than four years from the previous pregnancy. [185]

**Figure 4.34** Regressing placental site nodule. There is often central hyalinisation around individual cells, and cells may not express any hormones, although they are usually positive for cytokeratin structural antigens.

### 4.16.1 EPITHELIOID TROPHOBlastic TUMOUR
This is the most recently described and rarest trophoblastic tumour that simulates a carcinoma. It is composed of malignant trophoblast with differentiation towards intermediate trophoblast phenotype, and is distinct from both CC and PSTT although mixed tumours with two or three of these phenotypes together have been noted. In one of the original papers of 14 cases, 5 cases had a focal mixed pattern [37]. It has been suggested that PSN may be the benign counterpart of ETT and possibly it is this lesion which progresses to ETT. A rare group of larger ‘atypical’ PSNs, measuring over 5 mm with more cellularity and a ki67 index between small PSN and ETT exists [190] and PSN has been reported adjacent to ETT. [196].

ETT, like other GTN, presents in reproductive age females ranging from 15 to 48 (mean, 36) years. Most patients present with abnormal vaginal bleeding occurring 1 to 18 (mean 6) years after a prior gestation, which is usually a full-term delivery (205)

This tumour presents as a discrete uterine mass. Microscopically, there is a characteristic nodular, expansive growth pattern rather than the more diffuse, infiltrative pattern seen with PSTT. The tumor is composed of sheets and nests of mononuclear trophoblast with clear, eosinophilic and vacuolated cytoplasm resembling ‘chorionic’ type intermediate trophoblast [17, 37, 207]. There may be areas of necrosis and dystrophic calcification. The most characteristic distinctions however, from monomorph CC or PSTT, are the nodular architecture and the immunohistochemical profile. ETT usually stain diffusely positive with p63, PLAP and cytokeratin, whereas hCG and hPL positivity is weak and scattered [37,181,207,208]. It would seem from the recent literature that ETT is truly a rare but distinct trophoblastic tumor rather than just a morphological variant of longstanding PSTT, although the biological behaviour appears similar.
The tumour is confined to uterus when diagnosed, in majority of these patients. Hysterectomy is the treatment of choice for these patients. Metastases and death occur in about 25% and 10% of patients, respectively. Lung resection of metastatic ETT has occasionally been used successfully. ETTs are not as responsive to the chemotherapeutic agents used in treating other GTN types; therefore, drug combinations of platinum with etoposide and paclitaxel are used in managing metastatic ETT (205).

4.16.2 DIFFERENTIAL DIAGNOSIS OF TROPHOBLASTIC TUMOURS

In cases with morphological findings of a predominantly monomorphic, apparently trophoblastic tumour the differential diagnoses include monomorphic (or post-chemotherapy) CC, PSTT, ETT and non-gestational malignancy with trophoblastic differentiation. The important features of each lesion are previously described but it should be emphasised that on small biopsy material it may not be possible to establish the diagnosis with certainty on morphological or immunohistochemical grounds alone. The most important distinction is between non-gestational carcinoma with trophoblastic differentiation and GTN, since the response to appropriate chemotherapy is markedly different and in such cases DNA analysis of the tumor may provide valuable information in determining with certainty the gestational or non-gestational nature of the lesion. [129]

4.17 Placental Site Nodule (PSN) and Atypical Placental Site Nodule (APSN)

PSNs occur in the reproductive age group and represent the remnants of a previous, sometimes unrecognized, pregnancy and its extravillous trophoblast. The antecedent pregnancy has been reported to have occurred as many as 108 months before the diagnosis (209-212). Many patients have a history of therapeutic abortion and cesarean section, and a significant number have a history of a tubal ligation (210).

These lesions are usually incidental findings at hysterectomy or in biopsy specimens of endometrium, lower uterine segment, and cervix (210-214), with rare cases occurring in Fallopian tube, broad ligament and ovary (209, 215-217). Less commonly, patients may be symptomatic, presenting with abnormal uterine bleeding, recurrent pregnancy loss, abnormal cervical smear results or infertility (17, 209, 212).
Macroscopically, PSN appear as single or occasionally multiple, yellow to tan nodules or plaque ranging from 1 to 14 mm (average 2.1). Histological examination reveals small, well-circumscribed nodules composed of intermediate trophoblast of chorionic type with central hyalinisation (Figure 4.37). The trophoblastic cells mainly singly dispersed or arranged in small clusters and cords and rarely as diffuse sheets. They have abundant and eosinophilic to clear cytoplasm, relatively small uniform nuclei and a few having large, irregular, and hyperchromatic nuclei. Multinucleated cells are occasionally present. Mitotic figures are rare or absent.

It is now recognised that rare lesions may be encountered with histopathological features intermediate between typical PSN and PSTT/ETT, represented by the term Atypical Placental Site Nodule (APSN).

There are no well-established objective histological criteria to definitively distinguish APSN as an entity, other than general agreement that they represent lesions which appear ‘atypical’ to histopathologists experienced in this area. The lesions are generally larger in size than typical PSN (which rarely exceed 4mm), with higher cellularity, more cohesive nests and cords of viable trophoblastic cells and increased proliferation compared to typical PSNs but exact criteria for distinguishing typical from atypical for these variables have not been established (17, 217-218).

Despite this proposed description, the clinical significance of APSN remained uncertain, since only rare cases had been reported, and they were thought to behave in a benign fashion (42, 219). However, recent data demonstrates the apparent transformation of PSN, through APSN to malignant ETT and PSTT (220) and ETT (221). The largest series on APSN (197) reported that 15% of histologically confirmed APSN were associated with malignant PSTT and ETT, either concurrently with the APSN diagnosis or subsequently.
Figure 4.38. Atypical placental site nodule—(A). Circumscribed nodule with higher cellularity (B). Cells with cytologic and nuclear atypia and necrosis within center of nodule (inset with higher Ki-67 labeling index)

Cells in APSN usually exhibit positivity with anti-cytokeratin antibodies and may be variably positive for inhibin, bHCG, human placental lactogen, mel-Cam (CD146), epithelial membrane antigen and placental alkaline phosphatase (18, 210, and 212). The trophoblastic cells in PSNs exhibit low proliferative activity with a Ki-67 labeling index of about 5%, similar to that in the intermediate trophoblastic cells in the chorion laeve (210).

The diagnosis of APSN should therefore prompt thorough radiologic investigations to exclude an underlying mass lesion, and these patients should be clinically followed up in view of the association with malignant GTD (PSTT/ETT).

4.18 PATHOLOGICAL LESIONS ASSOCIATED WITH TROPHOBLASTIC TUMORS

The increased pelvic and uterine vascularity associated with moles and CC is well known and sometimes persists even after hCG has returned to normal. It may be a source of life-threatening hemorrhage, which may need either hysterectomy or uterine artery embolization when the uterus needs to be preserved. Luteal ovarian cysts used to accompany hydatidiform mole and CC in a variable number of patients and an incidence of 94% in one report is quoted by Park [15], although they are rarely seen in current practice. Luteinization of granulosa and theca cells may occur throughout both ovaries but its intensity varies in different patients and does not appear related to hCG levels in serum. Rupture can occur [222] and needle aspiration of the cysts has been recommended to prevent this. It is important not to confuse these ovarian lesions with metastatic CC

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