Contribution of referent pathologists to the quality of trophoblastic diseases diagnosis

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OBJECTIVE: To evaluate the contribution of referent pathologists (RPs) to the quality of diagnosis of trophoblastic diseases and to study the level of diagnostic agreement between the initial pathologists and the RPs.

METHODS: This observational retrospective study was carried between 1 November 1999 and 11 January 2011 using the database of the French Trophoblastic Disease Reference Centre in Lyon. All files for hydatiform moles (HMs), trophoblastic tumours and non-molar pregnancies for which there was an initial suspicion of trophoblastic disease were included, whenever there was rereading of the slides by an RP. A total of 1851 HMs and 150 gestational trophoblastic tumours were analysed.

RESULTS: When the initial pathologist diagnosed a complete mole, the RP confirmed the diagnosis in 96% of cases. When the initial pathologist diagnosed a partial mole, the RP confirmed the diagnosis in only 64% of cases. For trophoblastic tumours, when the initial pathologist diagnosed a choriocarcinoma, the RP confirmed the diagnosis in 86% of cases. When the initial anatomopathology suggested an invasive mole, the diagnosis was confirmed in 96% of cases. Finally, when the initial diagnosis was a placental site trophoblastic tumour or an epithelioid trophoblastic tumour, the RP confirmed the diagnosis in 60 and 100% of cases, respectively.

CONCLUSION: A systematic policy of rereading of slides for all suspicious moles improves the quality of management of trophoblastic diseases at a national level.

Key words: diagnosis / referent pathologist / hydatiform mole / gestational trophoblastic tumour / trophoblastic disease

Introduction

Gestational trophoblastic disease consists of several anatomoclinical entities ranging from benign precancerous lesions [complete (CM) or partial (PM) hydatiform moles (HMs)] to malignant lesions grouped under the term gestational trophoblastic tumours (GTTSs), which includes invasive moles (IMs), choriocarcinomas (CCs), placental site trophoblastic tumours (PSTTs) and epithelioid trophoblastic tumours (ETTs; Mazur and Kurman, 1994; Elston, 1995).

The histological criteria for CM and PM have been described in the literature and have been refined for early forms, which are common (Sulzman and Surti, 1978a,b; Sulzman, 1999; Genest, 2001; Sebire et al., 2003; WHO, 2003; Berkowitz and Goldstein, 2009). In the majority of cases, a CM or a PM can be formally identified by the
application of strict morphological criteria (histology images 1, 2 and 3). However, the diagnosis of HMs and GTTs is sometimes difficult for general pathologists and gynaecopathologists and there are several diagnostic pitfalls (Wells, 2007). Differentiation of CM from PM may be difficult, particularly in the first trimester (Sulzman, 1999; Genest, 2001; Sebire et al., 2003). The differential diagnosis between PM and non-molar abortion may also be difficult (Genest, 2001). In extra-uterine tubal (ectopic) pregnancies, molar pregnancy is overdiagnosed. Concerning GTT, a non-villous trophoblast at the exaggerated placental site may be confused with a PSTT and the atypical non-villous trophoblast of CM may be diagnosed as a CC or a PSTT (Wells, 2007).

The use of referent pathologists (RPs) has been shown to be valuable to improve the diagnosis of trophoblastic diseases. A policy of systematic rereading of slides showed that when a PM was diagnosed the expert pathologist only confirmed the diagnosis in 50% of cases (Paradinas, 1998). This approach has enabled a decrease in the number of abortions carried out wrongly for moles from 16 to 10% (Paradinas et al., 1996). In a study of 132 ectopic pregnancies with an initial diagnosis of tubal HMs, only eight cases of moles (6%) were confirmed after rereading by RPs (Sebire et al., 2005).

However, histological morphological criteria do not always enable a definite diagnosis, and complementary techniques may be necessary to confirm or overturn a possible histological diagnosis. The study of ploidy by flux cytometry in particular and labelling of protein p57 by immunohistochemistry (Castrillon et al., 2001) may be useful diagnostic tools when the morphology is inconclusive (Genest, 2001; Maggiori and Peres, 2007; Kipp et al., 2010).

Antigen p57 is not or is only slightly expressed in CM in contrast to PM or spontaneous abortions (Sebire and Lindsay, 2006). Furthermore, the knowledge of ploidy may help distinguish triploid PM from diploid hydropic abortions. However, ploidy does not distinguish CM of diploid non-molar pregnancies from PM of triploid non-molar pregnancies by digynia (Vassilkos and Kajii, 1976; Gollier et al., 2000), and p57 immunohistochemistry does not differentiate PM from non-molar pregnancies, both are positive. Molecular genotyping, which is more complex to set up, may therefore be necessary to make a definite diagnosis (Bifulco et al., 2008). Genotyping allows the identification of the parental origin of the fetal genome. This is an invaluable aid to distinguish androgenic CM of non-molar diploid pregnancies and PM of non-molar triploid pregnancies.

The expertise of pathologists familiar with the morphological criteria of trophoblastic diseases and with complementary techniques available on demand is therefore invaluable to the quality of diagnosis. The French Trophoblastic Disease Reference Centre (FTDRC) proposed rereading of the slides of all trophoblastic diseases diagnosed since its creation at the end of 1999 until 2007. Two main pathologists gave their expert opinions, exchanging slides if necessary. From 2008 onwards, to make rereading more comprehensive, the centre created a national network of eight expert pathologists who would reread the slides for all moles and GTT sent by initial pathologists at the time of patient registration.

The main objective of this study was to evaluate the contribution of the RPs to the quality of diagnosis of trophoblastic diseases.

Materials and Methods

This observational retrospective study used the database of the FTDRC (Centre Hospitalier Lyon Sud, France).

The study concerned the period between 1 November 1999, date of creation of the FTDRC, and the 11 January 2011. All files for HM, GTT or non-molar pregnancies were included, whenever there was an initial suspicion of trophoblastic disease and there was rereading of the slides by an RP.

Eight RPs are currently involved in rereading slides. Before the creation of the network (1 January 2008), rereading was essentially carried out by two anatomopathologists (A and B) and their activity was not separated in the database. Pathologists C, D and G analysed their reviews of the slides autonomously, while E and F analysed them together. B stopped his activity at the start of 2008 and was replaced in 2010. This new pathologist was not included in the analysis.

Before the creation of the network, the complementary techniques were rarely used and were not entered in the database. Thus, 870 data concerning the use of complementary techniques, especially before 2008, are missing.

A total of 2431 files were considered, including 1762 HMs with a favourable evolution, 608 GTTs and 61 non-molar pregnancies.

Rereading of HMs

A total of 2052 HMs analysed by an RP were registered. A total of 201 cases were referred directly to an RP and were excluded from this analysis since these were not assessed primarily by a general pathologist. The analysis was therefore carried out on those 1851 HMs for which an initial reading was followed by rereading by an RP.

Rereading of GTTs

A total of 177 GTTs underwent rereading by an RP (IM, CC, PSTT, ETT). Twenty-seven slides were excluded as they were referred directly to an RP. The analysis was therefore carried out on 150 GTTs for which the initial and RP diagnosis were available.

Statistical analyses

The data were collected using PARADOX version 9 software. The analysis was carried out using SAS 9.1. The degree of agreement according to the kappa coefficient was classified as described by Landis and Koch (1977): excellent if kappa ≥ 0.81, good if kappa between 0.80 and 0.61 and moderate if kappa between 0.60 and 0.41.

Results

The number of files registered by the FTDRC increased annually, reaching a total 2431 registrations between 1999 and 2010 included (Fig. 1). Two-hundred and twenty-five cases were referred directly to the RPs (9.3%). This increased from 6.6% (74/1116) before the creation of the network (i.e. before 1 January 2008) to 11.5% (151/1315) after the creation of the network.

The mean cost per sample was 73 Euros.

Hydatiform moles

The mean age of the patients at the time of diagnosis was 32 years (15–58 years). The median parity was 1. The mean number of pregnancies before the molar pregnancy was 1.62 (0–13). For 15 registrations, the pregnancy preceding the HM which was the object of registration was also an HM (nine CM and six PM). Nine patients had a family history of molar pregnancies. Twenty patients presented with repeated molar pregnancies: 19 patients had two HMs and one patient had more than three. Eleven patients underwent testing for
mutations in the NLRP7 gene: six patients (54.5%) had no mutation, three (27.3%) had a heterozygous mutation and two (18.2%) had a homozygous or double heterozygous mutation.

The number of slide reviews carried out was 1336 by RPs A and B, 228 by C, 121 by D, 88 by E and F and 74 by G.

Overall, p57 labelling was used by the initial pathologist in 10% of cases (94/981) and by the RP in 41% of cases (398/981), whereas determination of ploidy by flux cytometry was carried out in 3.6% (35/981) and 5.9% of cases (58/981), respectively. Other complementary techniques such as fluorescent in situ hybridisation (FISH) and Ki-67 were used in 1.4% of cases by the initial pathologists and by 0.7% of cases by the RPs. The rate of use of at least one complementary technique by the RPs (p57, determination of ploidy by flux cytometry or FISH) increased from 38% in 2008 to 56% in 2010 (P < 0.0001). The evolution in the use of these complementary techniques by the RPs is shown in Table I. For p57, this rate increased from 35% in 2008 (101/291) to 55% in 2010 (164/310). The evolution in the use of p57 by RPs is shown in Table II. The rate of use of flux cytometry by RPs increased from 5% (15/291) to 6% (20/310); two RPs mainly used this technique.

Anatomopathological agreement between the initial pathologist and the RP for the histological diagnosis of HMs was 74% (1366/1851; kappa = 0.52; 95% CI: 0.49–0.55). The degree of agreement according to Landis and Koch was moderate. This rate remained stable since the creation of the FTDRC. The level of agreement between the initial anatomopathology and the RP is illustrated in Table III. When the initial pathologist diagnosed an HM (PM or CM), the rate of agreement with the RP was 84% (1341/1594; kappa = 0.65; 95% CI: 0.62–0.69). This rate was stable over time. Thus, the RPs identified 52 non-molar pregnancies that had initially been wrongly diagnosed as CM or PM and 143 HMs initially considered as non-molar pregnancies.

When the initial pathologist had diagnosed a PM, the rate of agreement with the RP was 64% (380/594). In 28% of cases (168/594), the RP diagnosed a CM and in 7% of cases (40/594) a non-molar pregnancy. Over three-quarters (168/214, 79%) of disagreements were reclassified as CM and 19% (40/214) as non-molar pregnancies.

Figure 1 Annual number of files registered at the FTDRC between 1999 and 2010.
When the initial pathologist had diagnosed a CM, the rate of agreement with the RP was 96% (961/1000). When the initial pathologist was unsure between a CM and a PM, the RP diagnosed a CM in 76% of cases (48/63).

Gestational trophoblastic tumours

The mean age of the patients who presented with a GTT diagnosed histologically was 40 years (15–58 years). The overall anatomopathological agreement between the initial pathologist and the RP for the 150 GTTs was 71% (106/150; kappa = 0.60; 95% CI: 0.51–0.69). The degree of agreement was therefore moderate. The rate of concordance between the initial anatomopathology and the RP is shown in Table IV. When the initial pathologist diagnosed a CC, the rate of agreement was 86% (48/56; kappa = 0.45; 95% CI: 0.20–0.70). When the initial pathologist diagnosed an IM, the rate of agreement was 96% (48/50). Finally, when the initial diagnosis was a PSTT or an ETT, the RP confirmed the diagnosis 6/10 and 2/2 times, respectively.

The discordant diagnoses were classified as major or minor disagreements depending on whether they would have led to significant therapeutic modifications for the patients or not. In 24 cases, a major discordance would have been the cause of over- or under-treatment if the slides had not been reread by an RP. Four supposed CCs were rediagnosed as PSTTs or ETTs. These patients would have been treated wrongly by chemotherapy when hysterectomy is the reference treatment. One supposed PSTT was rediagnosed as a CC: this patient would have wrongly undergone a hysterectomy losing all chance of subsequent pregnancy. Nineteen patients had an initial diagnosis of a benign tumour (14 diagnoses of non-molar pregnancies and 5 HMs), whereas they had a recognised GTT. They would therefore not have benefited from adapted therapeutic management (chemotherapy or hysterectomy) if the slides had not been reread or there would have been a delay in their treatment. Finally, nine minor discordances were found. Two supposed IMs were rediagnosed as CCs and four supposed CCs were rediagnosed as IMs. The reference treatment for both is chemotherapy but the response rates are different for these two histological types. Three supposed PSTTs were rediagnosed as ETTs. The therapeutic impact of this disagreement is low because hysterectomy is the reference treatment for these two histological forms.

### Table III Concordance between the initial pathologists and the RPs for the diagnosis of HMs.

<table>
<thead>
<tr>
<th>RP</th>
<th>Initial pathologist</th>
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<tr>
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<td>PM (n = 594)</td>
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<tr>
<td>PM</td>
<td>380</td>
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<tr>
<td>CM</td>
<td>168</td>
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<td>NMP</td>
<td>40</td>
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<td>Twin pregnancy with CM</td>
<td>4</td>
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<td>Doubt between CM and PM</td>
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PM, partial mole; CM, complete mole; NMP, non-molar pregnancy.

### Table IV Concordance between the initial pathologists and the reference pathologists for the diagnosis of GTTs.

<table>
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<tr>
<th>RP</th>
<th>Initial pathologist</th>
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<tbody>
<tr>
<td></td>
<td>CC (n = 56)</td>
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<tr>
<td>CC</td>
<td>48</td>
</tr>
<tr>
<td>IM</td>
<td>4</td>
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<tr>
<td>PSTT</td>
<td>3</td>
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<td>ETT</td>
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CC, choriocarcinoma; IM, invasive mole; ETT, epithelioid trophoblastic tumour; PSTT, placental site trophoblastic tumour; NMP, non-molar pregnancy; HM, hydatiform mole; GTT, gestational trophoblastic tumour.

### Discussion

To our knowledge, this is the first exhaustive study of the practice carried out by a trophoblastic disease centre, concerned with the level of agreement in the histological diagnosis of HMs and GTTs between initial pathologists and RPs. The total number of files registered at the FTDRC has continued to increase significantly from around 400 between 1999 and 2004 (Golfier et al., 2007a,b) to nearly 2400 at the end of 2010.

In our study, the rate of agreement between the initial pathologists and the RPs for the 1851 HMs was only 74%, which correspond to a moderate level of agreement according to Landis and Koch (1977). This lack of concordance illustrates the difficulty in diagnosing HM and the benefit of using experts in trophoblast pathology. Whereas the RPs confirmed most CM, PM were only confirmed in 64% of cases (n = 594). These result are similar to those published previously in three retrospective studies where the diagnosis of PM was confirmed in only 68% (n = 110), 50% (n = 400) and 31% (n = 67) of cases, respectively (Philippe and Chadli-Debbiche, 1994; Paradinas, 1998; Golfier et al., 2007a,b). In our study, rereading of the slides enabled the correction of 36% of PM diagnoses, eliminating pointless surveillance, with nearly one of five patients being rediagnosed as a
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non-molar pregnancy. Similar proportions (one of three patients) were reported by Paradinas (1998).

The improvement in the quality of diagnosis of trophoblastic diseases brought about by the RPs is due to their knowledge of the histological features of HM, particularly early HM (Sulzman, 1978a,b, 1999; Genest, 2001; Sebire et al., 2003). The poor inter-observer agreement by histology alone (Vassilakos and Kajii, 1976; Bifulco et al., 2008) has prompted the use of complementary techniques such as p57 immunolabelling or the study of ploidy (Zaragoza et al., 2000; Genest, 2001; Maggioni and Peres, 2007; Kipp et al., 2010) in order to improve the diagnostic performance. The combination of ploidy data and histology resulted in a remarkable improvement in agreement, which increased from 60% using histology alone to 78% for histology and ploidy together (Fukunaga et al., 2005).

In our study, p57 labelling was used by 10% of the initial pathologists, while 41% of RPs used it to confirm their diagnosis. Thus, the diagnosis of RPs was more reliable than that of initial pathologists not using complementary techniques. Furthermore, it was interesting to observe that following the official creation of the network of pathologists in 2008 within the FTDRC, the use of at least one complementary technique increased significantly to reach 56% of cases analysed in 2010. All of the pathologists increased their use of these techniques over this period of time. Immunolabelling was the major complementary technique used by the RPs. However, there was heterogeneity of practices between the experts because the rate of use of complementary techniques in 2010 varied between 46 and 81%.

It is important to note that due to the absence of routine molecular genotyping techniques, our study was not aimed at appreciating the accuracy of the diagnoses made by the RPs (Petignat et al., 2003; Bifulco et al., 2008). It was an evaluation of practice allowing us to present the diagnostic modalities perceived necessary by the RPs. The criteria for the use of complementary techniques, namely the type of technique and the frequency of use of these techniques, are not currently the subject of consensus.

In a European and worldwide context, where the possibilities of using expert pathologists within TDRCs are rare, the first step to improve the diagnosis is the diffusion of histological morphological criteria of HM, particularly in their early forms, combined with p57 labelling and/or the determination of ploidy in difficult cases only. The latter stage of diagnosis in structured organisations may be the systematic use of morphology combined with p57 labelling, in order to select cases for molecular genotyping. This approach assures a diagnosis/availability performance ratio of human and financial resources that is probably better than systematic genotyping.

For the 150 GTTs, the overall rate of agreement between the initial pathologists and the RPs was only 71%, which was moderate according to Landis and Koch (1977). To our knowledge, only one previous study has investigated the anatomopathological agreement between initial pathologists and RPs for different trophoblastic tumours (Philippe and Chadli-Debbiche, 1994). In this study of 56 GTTs, the diagnosis of IMs was only made in 14% of cases (4/29) in 1994, whereas it was made in 96% of cases (48/50) in our study strictly applying the WHO classification 2003. The diagnosis of CCs was made correctly in 75% of cases (21/28) versus 86% in our study (48/56).

The anatomopathological diagnosis of GTTs is not essential for the initiation of treatment. Thus, in the majority of cases, the diagnosis is made abnormal evolution of hCG according to the FIGO criteria (Ngan et al., 2003).

The precise diagnosis of histological types does not influence treatment, which will be the same for IM and post-molar CC (Vassilakos and Kajii, 1976). However, it is important that the slides are reread by RPs when there is a major disagreement which may have a significant therapeutic impact on the patient. These cases correspond to PSTTs and ETTs diagnosed wrongly as IMs or CCs. They also include IMs and CCs diagnosed as PSTTs or ETTs and GTTs diagnosed wrongly as HMs or non-molar pregnancies. Twenty-four major discordances (16% of GTTs analysed) were thus re-examined by RPs. Furthermore, in our study, rereading by an RP revealed 52 non-molar pregnancies initially diagnosed wrongly as CM or PM. These patients therefore avoided pointless close surveillance and were able to start a new pregnancy without delay if they wanted. In addition, the RPs identified 143 HMs initially considered to be non-molar pregnancies. It is essential to diagnose non-molar pregnancies for two reasons. First, it is necessary to initiate treatment rapidly such as aspiration under echographic control. In women >40 years of age who do not want any more children, a hysterectomy may be proposed, thereby eliminating the risk of abnormal evolution in the uterus (Golffier et al., 2000). The diagnosis of HMs allows the patient to be informed about the risk of GTT, around 15% for CM and <5% for PM (Bagshawe et al., 1986, 1990; Golffier et al., 2007a,b). This risk justifies close monitoring of hCG levels until they become negative.

Prompt management of post-molar GTT affects the prognosis (Golffier et al., 2007a,b, 2010). Rapid diagnosis will allow a decrease in the prognostic FIGO 2000 score (Ngan et al., 2003) and will shorten the delay before initiation of treatment, with lower hCG levels, number of metastases and tumour size. A fall in the FIGO score increases the chances of classifying the GTT as a low-risk form. Monochemotherapy with methotrexate will be proposed, with a final cure rate of nearly 100% and minimal severe toxicity (Chalouhi et al., 2009).

Echography is not reliable for the diagnosis of HMs (Sebire et al., 2001), and histological analysis of products of conception allows the identification of molar pregnancies at best. The systematic histological analysis of a cohort of 1119 women suffering a spontaneous abortion in the first trimester enabled the identification of 33 PM (2.1%) and 7 CM (0.43%; Tasci et al., 2005).

This suggests that the systematic histological analysis of miscarriages may be useful during the first trimester (Fram, 2002; Seckl et al., 2004).

Conclusion

This retrospective observational study, involving 1851 HMs and 150 GTTs, was carried out to evaluate whether systematic rereading of slides by RPs is useful in cases of suspicion of trophoblastic disease. The initial diagnosis was different from the RP diagnosis for 36% of PM and 4% of CM. Concerning GTT, when the initial pathologist diagnosed a CC, the RP confirmed the diagnosis in 86% of cases. When the initial anatomopathology suggested an IM, the diagnosis was confirmed in 96% of cases. Finally, when the initial diagnosis was a PSTT, the RP confirmed the diagnosis in only 60% of cases. The FTDRC with its network of RPs has made a significant contribution to the diagnosis
of HMs and GTTs. Molar pregnancies are rare and complex pathologies. The diagnosis of HMs is difficult and is the source of disagreement. A delay in the diagnosis of malignant forms may be detrimental for the patients. A national policy of systematic review of the histology of all cases where HM and GTT are suspected improves the quality of management of trophoblastic diseases.

**Authors’ roles**


