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Brief review

The importance of immunohistochemistry in the differential diagnosis of molar disease

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Key words

Molar disease • Complete mole • P57 • Twist1

Summary

The differential diagnosis among complete moles, partial moles and hydatidiform abortions may be challenging during routine diagnostic activity. These entities share the histological aspect of enlarged villi, but here we summarize also some peculiar features of all of them. If histology does not clarify this distinction, the immunohistochemistry is the most important tool for pathologists to complete such diagnosis. The correct management of immunohistochemistry and of further possible analysis is also reviewed. Lastly, the most important antibodies, starting from p57, are presented.

The distinction of hydatidiform moles from non-molar abortions, as well as their sub-classification as complete (CM) versus partial (PM), is a very important step in clinical management and accurate risk assessment for persistent gestational trophoblastic disease, and also for the prevention of choriocarcinoma [1 2].

Histologically, the suspicion of a molar disease (MD) arises in presence of enlarged chorionic villi; however, this morphologic feature is not pathognomonic for MD, since also hydropic abortions share this aspect.

In MD, the villi have a typical appearance of the so called "central cisterns", given by a central villous stromal clearing with margining of villous stromal cells around the periphery. Villous capillary outlines can persist in both PM and CM, but only in PM intravascular nucleated red blood cells (NRBCs), reflecting embryo formation, can be detected [3 4]. Indeed, with the exception of two very rare circumstances (twin gestation and placental hematopoiesis), the paradigm complete mole/absent embryo has been always confirmed [5].

The histological distinction between CM and non-molar abortion (NMA) may be very difficult above all in the early stages of gestation. Indeed, early CM share many histological features with the early developmental stage of normal villous stroma, like the basophilic and edematous aspect, as well as a variable degree in the cellularity [6]. Histologically, the most important features to differentiate early CM from a early NMA are represented by the absence of mature stromal blood vessels with well-distinct lumina and by clear signs of stromal karyorrhexis or apoptosis; for early NMA the demonstration of trophoblast proliferation alone is not a reliable way to diagnose a complete mole [5 6]. Indeed, as described in some previous studies, molar disease is not only a disease linked to trophoblastic proliferation, but it is also strongly associated with the abnormal or incomplete maturation of villous stromal components [7-9].

Notably, the immunoistochemistry (IHC) plays a very important role in the differential diagnosis between MD and NMA. First of all, immunohistochemical assessment of the paternally imprinted, maternally expressed p57 gene is widely recognized as the gold standard for CM diagnosis [10 11]. In CM, indeed, villous stromal cells and cytotrophoblast lack nuclear expression of p57; conversely, intermediate trophoblastic cells are positive and serve as an internal positive control (Fig. 1A). Another useful marker that helps in excluding a CM can be the transferrin receptor 1, also known as CD71, an immuno-

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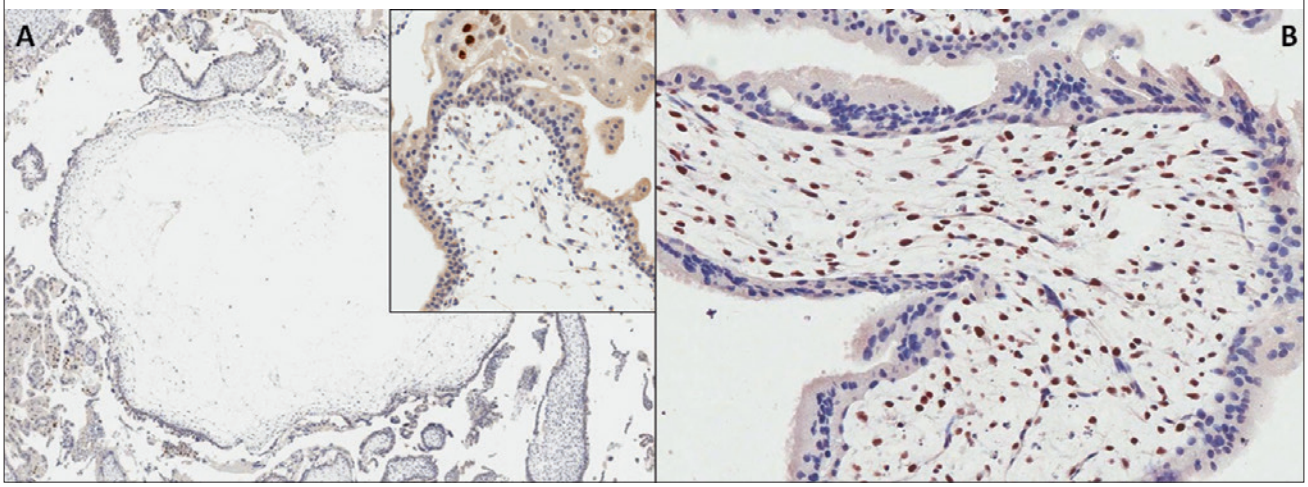
Fig. 1. A. A classical example of complete mole stained for p57 is here shown. Villous and cistern stromal cells and cytotrophoblast lack nuclear expression, but intermediate trophoblastic cells are positive and serve as an internal positive control (original magnification 10X, small box original magnification 20X). **B.** Expression of Twist1 in a case of complete mole: it is highly expressed by the stromal villi cells (original magnification 20X).



histochemical marker that stains immature erythrocytes and in particular NRBCs [4]. Indeed, in CM, due to the lack of embryo formation, NRBCs are not present in the villi vessels, so the CD71 is there totally negative. Notably, a recent study highlights that this kind of marker shows excellent results also in presence of necrotic-hemorrhagic modifications [4]. This is a very important point in gestational pathology, since abortive tissue is often worn out due to delayed fixation, in case of internal abortion, or to necrotic-hemorrhagic modifications related to ischemia.

In the differential diagnosis of gestational pathology, a recent paper shows the utility of another marker in this field: Twist1, also known as Twist [7]. It is a transcription factor that, through the down regulation of the cadherins, promotes epithelial-to-mesenchymal transition (EMT) during embryo development, but also during the carcinogenesis of some tumors [12-14]. In gestational pathology, it appears very robust in distinguishing CM, in which it is highly expressed by the stromal villi cells (Fig. 1B), from PM (only weak and focal positivity) and also from NMA (not expressed) [7]. Another EMT marker, Snai2 (previously known as Slug), can help in distinguishing CM (highly expressed by stromal villi cells) from NMA (generally not expressed), but it is less specific than Twist1, above all in discriminate CM from PM. Notably, as CD71, also these markers show a good reliability in case of necrotic-hemorrhagic modifications.

Other markers, like Ki67 and p63, have been recently proposed in the differential diagnosis between MD and NMA 15; however, immunohistochemically, the use of p57 and of Twist1 as the second choice (e.g.: in case of doubtful result, in case of tissue with necrotic-hemorrhagic changes) appears in our opinion the most reliable. Although p57 immunostaining alone can be very helpful in identifying CM, which lacks p57 expression because of the lack of maternal DNA, this analysis cannot distinguish PM from NMA as both express p57 because of the presence of maternal DNA. Besides the usefulness of IHC for Twist1, it is important to mention the short tandem repeat genotyping, a technique that can determine the parental source of polymorphic alleles, allowing a reliable distinction among all of these entities by discerning androgenetic diploidy, diandric triploidy, and biparental diploidy to rigorously diagnose CM, PM, and NMA, respectively [2].

Concluding, the most reliable approach to the diagnosis of MD starts with morphology, that represents a fundamental step, can continue with immunohistochemistry (p57, CD71 and Twist1 are the most important markers, with Twist1 very reliable even in case of necrotic- hemorrhagic modifications) and, if the diagnosis remains still doubtful, the process can end with ploidy analysis/short tandem repeat genotyping. The latter step is reserved only for doubtful case; the immunohistochemical approach, indeed, is decisive in the vast majority of cases and also appears as the most recommended analysis during routine diagnostic activity because of its low costs.

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