GYNECOLOGY

Apoptotic index for prediction of postmolar gestational trophoblastic neoplasia

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BACKGROUND: Although 85% of patients with a complete hydatidiform mole achieve spontaneous remission after a few months, 15% of them will experience gestational trophoblastic neoplasia, which requires chemotherapy. To date, there is no biomarker to predict post—molar gestational trophoblastic neoplasia before the initiation of human chorionic gonadotropin surveillance.

OBJECTIVE: The purpose of this study was to assess the relationship between the expression of apoptosis markers in the molar villous trophoblasts and the subsequent development of gestational trophoblastic neoplasia after the evacuation of a complete hydatidiform mole.

STUDY DESIGN: This was a retrospective cohort study of patients with complete hydatidiform mole who were diagnosed, treated, and followed at the Center of Trophoblastic Diseases (Botucatu/São Paulo State and Rio de Janeiro/Rio de Janeiro State, Brazil) from 1995—2014. Patients were divided temporally into derivation (1995—2004) and validation (2005—2014) cohorts. Immunohistochemistry was used to examine tissue expression of the apoptosis inhibitor survivin or the pro—apoptotic enzyme caspase-3. Survivin stains for cytoplasmic and nuclear expression were evaluated independently. Caspase-3 expression was measured as an apoptotic index of positive staining cells over negative staining cells multiplied by 100. Receiver operating characteristic curves were then constructed, and the area under the curve was calculated to test the performance characteristics of the staining to predict the subsequent development of gestational trophoblastic neoplasia.

RESULTS: The final study population comprised 780 patients, with 390 patients in each temporal cohort: 590 patients entered spontaneous remission, and 190 patients experienced post—molar gestational trophoblastic neoplasia. Neither nuclear nor cytoplasmic survivin expression performed well as a predictor of subsequent gestational trophoblastic neoplasia. The caspase-3 apoptotic index was a strong risk factor for subsequent gestational trophoblastic neoplasia development. When the apoptotic index was <4%, the risk of gestational trophoblastic neoplasia had an odds ratio of 35.55 (95% confidence interval, 14.02—90.14; $P < .0001$) in the derivation cohort and an odds ratio of 25.71 (95% confidence interval, 10.13—65.29; $P < .0001$) in the validation cohort. However, in both cohorts, the positive predictive value for gestational trophoblastic neoplasia of an apoptotic index <4.0% was modest (49% in the derivation cohort and 41% in the validation cohort); the negative predictive value for gestational trophoblastic neoplasia of an apoptotic index ≥4.0% was high (97% in both cohorts).

CONCLUSION: The subsequent development of gestational trophoblastic neoplasia after evacuation of complete hydatidiform mole is tied closely to the apoptotic index, which may be a useful biomarker for future prospective studies.

Key words: apoptotic index, Brazil, complete hydatidiform mole, gestational trophoblastic neoplasia, receiver operating characteristic, survivin

Complete hydatidiform mole (CHM) is a reproductive anomaly that occurs because of a lack of maternal chromosome expression and is characterized by diffuse hydropic villi, marked trophoblastic hyperplasia, and the absence of fetal vessels.[1,2] Although 85% of patients with a CHM achieve spontaneous remission after a few months, 15% will experience gestational trophoblastic neoplasia (GTN), which requires chemotherapy.[3] Detection of persistence after evacuation of a CHM relies on strict postmolar follow-up evaluation with human chorionic gonadotropin (hCG) surveillance, which is the only marker capable of detecting GTN at an early stage.[4] Efforts to predict postmolar GTN before the initiation of hCG surveillance, such as using the histologic condition of the trophoblastic tumor,[5] the ploidy assessment of DNA by cytometry,[6] cell proliferation markers,[7] and oncogene expression[8] have been unsuccessful. However, a marker that could prognosticate postmolar GTN would have high clinical utility. Patients with CHM at a high risk of experiencing postmolar GTN could be treated with prophylactic chemotherapy, especially in settings in which it is difficult to maintain a rigorous follow-up regimen.[9] Furthermore, the identification of the patients who are at very low risk of persistence could allow shortened surveillance (especially after hCG reaches normal levels) and the reduction of patient anxiety, work absence, and healthcare costs.[10,11]

Preliminary reports have suggested a relationship between programmed cell death, or apoptosis, and postmolar GTN.[12-16] Depending on the stimulus, trophoblast apoptosis could be initiated by the intrinsic (mitochondria-dependent) or the extrinsic pathway, mediated by death receptors on the surface of the cell membrane. The intrinsic and extrinsic pathways for trophoblast apoptosis are not mutually exclusive, and both could be activated.[17] These pathways culminate in the action of aspartate-specific proteases called caspases (cysteine — aspartic acid — proteases), which are responsible for the mediation of cell death by proteolysis at aspartic acid residues. There are also

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mechanisms for the control of programmed cell death, such as the action of the inhibitor of apoptosis family of proteins, particularly survivin. Survivin is a 16.5 kDA molecule that is expressed during the G2/M phase and is located in the microtubules of mitotic spindles, where it regulates apoptosis by ensuring proper chromosome segregation and cytokinesis.[18,19] The aim of this study was to evaluate the potential for markers of apoptosis to serve as predictive biomarkers for the risk of GTN after evacuation of a CHM.

Materials and Methods

Design

This was a retrospective cohort study of patients who had been diagnosed with CHM after uterine evacuation and observed and treated at the Trophoblastic Disease Center of São Paulo State University, Botucatu Medical School, and the Rio de Janeiro Trophoblastic Disease Center (33rd Maternity Ward at Santa Casa da Misericórdia) between 1995 and 2014. The research was approved by the Institutional Review Board of the Botucatu Medical School at São Paulo State University (protocol number 497/2007). All the patients previously had given informed consent for participation. Patients were divided into a derivation cohort (comprised of women treated for CHM between 1995 and 2004) and a validation cohort (comprised of women treated for CHM between 2005 and 2014).

Patients

The patients who participated in this study had been diagnosed with CHM, which was confirmed by histopathologic evaluation, and had their molar tissue embedded in paraffin blocks and stored at the Department of Pathology at Botucatu Clinical Hospital at São Paulo State University and the 33rd Maternity Ward of Santa Casa da Misericórdia do Rio de Janeiro. The patients also attended complete postmolar follow-up evaluation at the reference centers for at least 1 year. Patients were excluded if there was not enough histologic material stored for the immunohistochemical study of the molar tissue or there was inadequate material for this study.

Spontaneous remission was defined as 3 consecutive weekly hCG measurements of <5 IU/L. Progression from CHM to GTN was diagnosed by Fédération Internationale de Gynécologie et d'Obstétrique (FIGO) criteria[20]: rising (>10%) hCG levels for 3 consecutive weeks or plateaued for 4 weeks. Patients with a histologic diagnosis of choriocarcinoma or metastases that was detected during postmolar follow-up evaluation, particularly in the lungs and pelvis, were also classified as GTN cases. Before chemotherapy was started, the patients were evaluated for metastatic disease by gynecologic examination, Doppler ultrasonography of the pelvis, and chest radiograph to check for any pulmonary metastases. GTN was staged according to the FIGO 2000 criteria: I, disease confined to the uterus; II, disease extends to the outside of the uterus but is limited to the genital structures; III, disease extends to the lungs, with or without genital tract involvement; and IV, all other metastatic sites.[20]

Prognostic scoring for resistance to chemotherapy followed the FIGO/World Health Organization Prognostic Scoring System. All patients in the current study with postmolar GTN were classified as low risk according to their FIGO 2000 risk factor score[20] and received single-agent chemotherapy (methotrexate or actinomycin-D). Resistance to primary chemotherapy treatment was defined by rising or plateaued hCG levels for at least 3 consecutive weeks. Patients with resistance to single agent chemotherapy received combination chemotherapy (etoposide, methotrexate, actinomycin D/cyclophosphamide and vincristine or etoposide and cisplatin/etoposide, methotrexate, actinomycin D).[21] After chemotherapy, all the patients underwent follow-up evaluation for at least 1 year with monthly hCG surveillance after the first normal hCG value was obtained.

Pathologic condition

The diagnosis of CHM was confirmed by a histologic review of each case by 1 pathologist at each reference center who was not informed of the clinical progression of the disease, using the criteria described by Szulman and Surti[22]: diffuse swelling of chorionic villi with edema, central cistern formation, absence of embryo, and abnormal trophoblast hyperplasia. From 2010 onwards, $p57^{kip2}$ immunohistochemistry was used routinely in all cases to distinguish complete from partial mole. For this study, all cases before 2010 were also reviewed, and any case in which there was a question of complete or partial mole was stained for $p57^{kip2}$.

Immunohistochemical study

Histologic sections were made from each paraffin block. Slides were deparaffinized with the use of Xylenes and sequential washes with graded ethanols. Slides were then stained according to an avidin-biotin-peroxidase technique. The following primary antibodies were used in each case: rabbit polyclonal cleaved caspase-3 antibody (Asp175; 1:200 dilution; Cell Signaling Technology, Danvers, MA) and mouse monoclonal survivin antibody (clone 5E8; 1:100 dilution; Neomarkers, Fremont, CA). The histologic sections with the primary antibodies were incubated overnight at 4°C.

The sections were interpreted simultaneously by 2 separate observers who were blinded to the clinical outcome using an optical microscope (Olympus, model BX40; Olympus Corporation, Tokyo, Japan) with ×100 and ×400 magnification. The entire length of each section was observed to select the fields with the most villous trophoblast cells. Histologic sections of tonsil tissue for caspase-3 and gastric cancer for the expression of survivin were used as positive controls. Sections that had been incubated without primary antibodies served as negative controls. Only brown staining in the villous trophoblast cells was considered positive. To measure possible interobserver variation in the interpretation of the immunohistochemical sections, a selection of 30 cases were reviewed independently by 2 pathologists and scored for apoptotic index and nuclear and cytoplasmic survivin staining. The Pearson correlation