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Mesothelin and osteopontin as circulating markers of diffuse malignant peritoneal mesothelioma: A preliminary study

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ABSTRACT

Background: The differential diagnosis between diffuse malignant peritoneal mesothelioma (DMPM) and other peritoneal surface malignancies (PSM) is still challenging. Serum mesothelin and osteopontin are increasingly used as markers of pleural mesothelioma, but their role in DMPM is unclear. We assessed the diagnostic and prognostic values of mesothelin, osteopontin, CEA, CA19.9, CA125, and CA15.3 in DMPM patients.

Methods: Markers were dosed before cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (HIPEC) by enzyme-linked immunosorbent assay (ELISA) in 30 DMPM patients and 14 controls with other PSMs. Receiver-operating characteristics (ROC) curve were plotted. The performance of each marker was assessed by the area under the ROC curve (AUC-ROC).

Results: Mean mesothelin levels were 7.84 ng/dl (SD = 5.14) in DMPM group and 3.00 ng/dl (SD = 1.25) in controls (P = 0.001). Mean CEA levels were 5.3 ng/dl (SD = 4.7), and 61.96 ng/dl (SD = 112.5) in the two groups (P = 0.008). No statistical difference was seen for osteopontin (P = 0.738), CA19.9 (P = 0.081), CA125 (P = 0.600), and CA15.3 (P = 0.365). AUC-ROC was 0.836 for CA19.9, 0.812 for mesothelin, 0.793 for CEA, and lower for CA125 (0.652), osteopontin (0.531), and CA15.3 (0.481). Using diagnostic cut-offs selected by ROC methodology, sensitivity, specificity, positive and negative predictive values were 70.0%, 100.0%, 100.0%, and 60.9% for mesothelin >5.21 ng/dl, and 90.0%, 85.7%, 93.1%, and 80.0% for CA19.9 < 8.8 U/dl. At multivariate analysis, osteopontin correlated with survival (hazard rate 6.46; 95%CI 1.81–23.05; P = 0.004).

Conclusion: When assessing PSMs of unknown origin, elevated mesothelin with low CA19.9 may increase the suspicion index for DMPM. Ospeopontin warrants further investigations as a prognostic marker for DMPM.

Introduction

Diffuse malignant peritoneal mesothelioma (DMPM) is a rare and locally aggressive neoplasm that has been traditionally regarded to as a rapidly lethal malignancy. When treated with palliative surgery and systemic or intraperitoneal chemotherapy, survival did not exceed one year [1,2]. More recently, median survival has been 13 months in an Italian registry study collecting 81 cases [3], and 8.7–26.7 months in patients receiving systemic pemetrexed with platinum compounds or gemcitabine, that are currently the most active combinations for DMPM [4,5].

During the last years, a comprehensive treatment approach has reportedly resulted in survival improvements over historical and contemporary non-randomized controls [1]. Median survival up to 40–92 months has been reported in selected DMPM patients treated by aggressive cytoreductive surgery (CRS) combined with

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hyperthermic intraperitoneal chemotherapy (HIPEC), to control the microscopic residual disease [6–11].

DMPM diagnosis is difficult, due to its rarity and unspecific clinical presentation [1–3]. Nevertheless, the recent development of a potentially effective treatment underlines the need for a prompt diagnosis, as delay or failure to start appropriate therapies may negatively affect the prognosis of these patients. Circulating tumor markers that could be used as an adjunct to clinical and radiological assessment would be valuable tools in the initial evaluation of peritoneal disseminations of unknown origin.

We have previously reported that circulating CA125 and CA15.3 are useful in monitoring response to treatment in DMPM patients undergoing CRS/HIPEC, but the role of markers in the initial diagnosis of DMPM is still limited [12]. Serum mesothelin and osteopontin have been extensively studied in pleural mesothelioma, but their value in DMPM remains unclear [13,14]. The primary aim of the present study was to assess the diagnostic significance of serum mesothelin and osteopontin, together with a set of markers of common use in clinical oncology. Secondary aim was to test the prognostic value of markers.

Patients and methods

The present study was part of a clinical-translational research project supported by a grant from the Italian Health Minister (Ricerca Finalizzata RF-2009-1546664), aimed at rationalizing the comprehensive treatment of DMPM. All patients were treated according to a protocol approved by the Institutional Ethics Committee and gave written informed consent.

Study population

The present study population includes thirty patients with DMPM who were prospectively selected to undergo CRS/HIPEC in our institution from March 2009 to October 2015, and prospectively followed. Patients underwent an intensive preoperative work-up including clinical history, physical examination, gastroscopy, colonoscopy, thoracic, abdominal, and pelvic computed tomography (CT)-scan with venous and oral contrast medium.

Eligibility criteria for CRS/HIPEC included: histological diagnosis of DMPM confirmed in our Pathology Department according to a standardized protocol including haematoxylin-eosin-stained sections and immunohistochemical studies [15]; age <75; World Health Organization (WHO) performance score ≤2; no significant comorbidities; preoperative CT-scan showing peritoneal disease amenable to potentially complete cytoreduction, and no extraperitoneal metastases.

Our operative technique has been described elsewhere [16]. Briefly, the goal of the surgical cytoreduction was to remove all visible tumor by means of peritonectomy procedures and visceral resections, as needed. Closed-abdomen HIPEC was performed for 90 min, at 42.5 °C, with cisplatin (45 mg/l of perfusate) plus doxorubicin (15 mg/l of perfusate). Perfusate volume was 4–6 l. The extracorporeal circulation device Performer HRT (RAND, Medolla, Italy) was used.

Control group

Fourteen patients affected by different peritoneal surface malignancies (PSM) constituted the control group. This included serous-papillary peritoneal carcinoma (SPPC) (n = 1), desmoplastic small round cell tumor (DSRCT) (n = 1), pseudomyxoma peritonei originating from low-grade appendiceal mucinous neoplasms (LAMN) (n = 4), multicystic mesothelioma (n = 2), and peritoneal metastases from colorectal cancer (n = 2), appendiceal mucinous carcinoma (n = 3), and appendiceal mixed adeno neuro-endocrine carcinoma (MANEC) (n = 1). These patients were selected for CRS/HIPEC according to the same inclusion criteria, and operated on in our institution during the same period. The cytoreductive surgical technique was the same as in DMPM group. HIPEC was performed with cisplatin (25 ml/m^2/l) plus mitomycin-C (3.3 mg/m^2/l) for 60 min (n = 5), mitomycin-C alone (35 mg/m^2) for 60 min (n = 3), or cisplatin (45 mg/l) plus doxorubicin (15 mg/l) for 90 min (n = 2), according to the different PSM [16]. HIPEC was not performed in two patients who had grossly incomplete cytoreduction.

Both DMPM and control group underwent regular follow-up. Clinical evaluation, thoracic/abdominal CT-scan, and marker determinations were performed three-monthly during the first 2 years, and six-monthly afterward.

Serum marker determinations

Mesothelin, osteopontin, carcino-embryonic antigen (CEA), carbohydrate antigen 19.9 (CA19.9), carbohydrate antigen 125 (CA125), and carbohydrate antigen 15.3 (CA15.3) were dosed preoperatively. Peripheral blood samples were collected at a median of 2 days (range 1–3) before CRS/HIPEC. Serum separation was achieved by centrifugation of the blood sample (room temperature, 3200 rpm for 15 min); finally, supernatants were divided into aliquots and stored at −80 °C until the day of the assay.

The Quantikine® ELISA human osteopontin and the Quantikine® ELISA human mesothelin immunoassay R&D Systems Europe Ltd were used to measure osteopontin and Mesothelin serum levels according to the manufacturer's instructions. Briefly, these assays employs the quantitative sandwich enzyme immune assay technique. A monoclonal antibody specific for human osteopontin/mesothelin has been pre-coated onto a microplate. Standards and samples was pipetted into the wells and any osteopontin/mesothelin present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human osteopontin/mesothelin was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and color develops in proportion to the amount of osteopontin/mesothelin bound in the initial step. The color development is stopped and the intensity of the color was measured with a microplate reader.

Each assay was validated using a recovery test by spiking and dilution. The accuracy of these assays was tested against serum samples using known concentrations of the tested analytes. The assays were performed in duplicate. The assays had intra-assay coefficients of variation that were <5%, and interassay coefficients of variation <6%.

Serum concentration of CEA, CA19.9, CA125, and CA15.3. were measured by an electrochemiluminescence immunoassay using a Roche C6000 Automated Analyzer (Roche Diagnostics GmbH), Mannheim, Germany. Normal values were ≤5 ng/ml for CEA, ≤37 U/ml for CA19.9, ≤35 U/ml for CA125, and ≤30 U/ml for CA15.3.

Definitions

In this study, sensitivity was the percentage of patients with DMPM who had elevated marker levels; specificity was the percentage of patients without DMPM who had normal markers levels. Positive predictive value (PPV) was the percentage of patients with elevated marker levels who were affected by DMPM; negative predictive value (NPV) was the percentage of patients with normal marker levels who were not affected by DMPM.

Peritoneal involvement before and after the cytoreduction was intraoperatively assessed and prospectively recorded. Peritoneal

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