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Prognostic Analysis of Clinicopathologic Factors in 49 Patients With Diffuse Malignant Peritoneal Mesothelioma Treated With Cytoreductive Surgery and Intraperitoneal Hyperthermic Perfusion

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Background: Diffuse malignant peritoneal mesothelioma (DMPM) is a subset of peritoneal mesothelioma with a poor clinical outcome. We performed a prognostic analysis in a cohort of DMPM patients treated homogeneously by cytoreductive surgery and intraperitoneal hyperthermic perfusion (IPHP).

Methods: Forty-nine DMPM patients who underwent 52 consecutive procedures were enrolled onto the study. Cytoreductive surgery was performed according to the peritonectomy technique, and the IPHP was performed with cisplatin plus doxorubicin or cisplatin plus mitomycin C. We assessed the correlation of the clinicopathologic variables (previous surgical score, age, sex, performance status, previous systemic chemotherapy, carcinomatosis extension, completeness of cytoreduction, IPHP drug schedule, mitotic count [MC], nuclear grade, and biological markers [epidermal growth factor receptor, p16, matrix metalloproteinase 2 and matrix metalloproteinase 9]) with overall and progression-free survival.

Results: The mean age was 52 years (range, 22–74 years). The mean follow-up was 20.3 months (range, 1–89 months). Regarding the biological markers, the rates of immunoreactivity of epidermal growth factor receptor, p16, matrix metalloproteinase 2, and matrix metalloproteinase 9 were 94%, 60%, 100%, and 85%, respectively. The strongest factors influencing overall survival were completeness of cytoreduction and MC, whereas those for progression-free survival were performance status and MC. No biological markers were shown to be of prognostic value.

Conclusions: Completeness of cytoreduction, performance status, and MC seem to be the best determinants of outcome. These data warrant confirmation by a further prospective formal trial. No biological markers presented a significant correlation with the outcome. The overexpression of epidermal growth factor receptor, matrix metalloproteinase 2, and matrix metalloproteinase 9 and absent or reduced expression of p16 might be related to the underlining tumor kinetics of DMPM and warrant further investigation with other methods.

Key Words: Peritoneal mesothelioma—Locoregional therapy—Prognosis—Biological markers.

Peritoneal mesothelioma (PM) is a rare tumor, accounting for 10% to 20% of the 2200 cases of malignant mesothelioma registered each year in the United States.[1,2] The prognosis for patients with PM is poor, with a median overall survival (OS) of 12.5 months in the best series.[3] The subset of diffuse

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malignant peritoneal mesothelioma (DMPM) can represent up to 90% of all peritoneal forms of mesothelioma.[4] DMPM is histologically subclassified into the following types: epithelial, sarcomatoid, biphasic (mixed), and undifferentiated (poorly differentiated).[5,6] Seventy-five percent of DMPMs are of the epithelial type, whereas 22% and 3% are of the biphasic and sarcomatoid types, respectively.[7]

A variety of treatment options have been proposed, alone or in combination, but most have failed to palliate symptoms or to change the final outcome. The mechanism of death is related to intraperitoneal progression; the disease remains in the abdominal cavity for most of its natural history.[8] This pattern of spread would seem to indicate the potential usefulness of selectively increasing cytotoxic drug concentrations in the tumor-bearing area by direct intraperitoneal chemotherapy instillation.[9] The advent of locoregional therapy resulting from the combination of cytoreductive surgery (CRS) and intraperitoneal hyperthermic perfusion (IPHP) has dramatically changed the approach to this clinical entity. Phase I/II investigations of CRS plus IPHP have provided promising results,[10–12] because long-term survivors have been reported.The aim of this study was to identify clinicopathologic variables and biological markers with prognostic significance in patients with DMPM treated uniformly by CRS and IPHP.

PATIENTS AND METHODS

After a complete preoperative work-up including clinical examination, chest-abdominal-pelvic computed tomographic scan, ultrasonography, and tumor markers (carcinoembryonic antigen, CA-125, and CA 19-9), patients were considered suitable for the locoregional treatment if they met the following criteria: confirmed histological diagnosis of DMPM; age <75 years; performance status (Eastern Cooperative Oncology Group) ≤ 2; good cardiac, renal, hepatic, and bone marrow functions; no concomitant evidence of pleural extension; no other concomitant neoplasms; and informed written consent. The study was approved by the institutional review board of the National Cancer Institute of Milan, Italy.

Forty-nine patients (21 men and 28 women) were enrolled onto this retrospective study. The study period extended from August 1995 to January 2005. The mean age was 52 years (range, 22–74 years). Three patients were operated on twice because of disease recurrence. Twenty-six (50%) patients had received systemic chemotherapy before the procedure. The drug schedules are outlined in Table 1.

TABLE 1. *Previous systemic chemotherapy regimens*

Chemotherapy regimen	No. of cases
Doxorubicin + ifosfamide	5
Platinum + gemcitabine	1
Other platinum-containing regimens	4
Other platinum-containing regimens and doxorubicin + ifosfamide	4
Other platinum-containing regimens and other regimens	4
Doxorubicin + ifosfamide and other regimens	7
Other regimens	1
Total	26

Cytoreductive Surgery

The techniques of CRS have been described previously.[13] Briefly, the surgical procedure was performed with one or more of the following steps, depending on disease extension: (1) greater omentectomy and right parietal peritonectomy ± right colon resection; (2) pelvic peritonectomy ± sigmoid colon resection ± hysterectomy and bilateral salpingo oophorectomy; (3) lesser omentectomy and dissection of the duodenal-hepatic ligament ± antrectomy ± cholecystectomy; (4) right upper quadrant peritonectomy with Glisson's capsule; (5) left upper quadrant peritonectomy ± splenectomy; and (6) other intestinal resection and/or abdominal mass resection. A ball-tip electrosurgical handpiece was used to dissect the tumor on peritoneal surfaces from normal tissue. The electrosurgery was applied on pure cut at a high voltage. The 2-mm ball-tip electrode was used for dissecting on visceral surfaces, including the stomach, small bowel, and colon. All the patients who underwent intestinal resections received anastomoses just after the completion of CRS, before the initiation of IPHP. No patient underwent diverting ostomies. Cytoreduction was classified into three levels according to the number of procedures performed: level I, one or two procedures; level II, three or four procedures; and level III, five procedures or more.

Peritoneal carcinomatosis was quantified according to the Peritoneal Cancer Index.[14] Accordingly, the mean Peritoneal Cancer Index was 22 (range, 2–39). Residual disease after surgery was classified according to the Sugarbaker criteria[14]: optimal cytoreduction indicated residual disease ≤ 2.5 mm, and suboptimal cytoreduction indicated residual disease >2.5 mm. Details of the surgical procedures performed are listed in Table 2.

Intraperitoneal Hyperthermic Perfusion

The IPHP follows completion of CRS with intestinal anastomoses. The closed-abdomen technique

TABLE 2. *Description of surgical procedures performed during the cytoreductive phase*

Variable	n	%	Mean	Range
Procedure extension (distribution according to the level of extension and the respective duration)				
I	7	13.5	376 min	250–480 min
II	18	34.6	527 min	250–690 min
III	27	51.9	570 min	395–720 min
Total	52	100.0	529 min	250–720 min
Peritonectomy procedures			5.8	1–11
Intraoperative blood units transfused			2 units	0–13 units
Intraoperative fresh frozen plasma units transfused			8 units	0–20 units
Completely cytoreduced cases	43	84		

was used for all our patients.[15] Two inflow catheters were inserted, one in the right subphrenic space and one deep in the pelvic cavity, as well as two outflow catheters, one in the left subphrenic space and the second more superficially in the pelvic cavity. Six thermocouples were used to continuously monitor the inflow, outflow, and intraperitoneal cavity temperatures. The temporary abdominal skin closure was followed with a tight continuous nylon stitch. The catheters were then connected to an extracorporeal perfusion circuit (Performer LRT; RAND, Medolla [MO], Italy). The intraperitoneal temperature was maintained at 42.5°C during the perfusion. Intraperitoneal perfusion regimens were as follows: cisplatin (CDDP; 25 mg/m^2/L) + mitomycin C (3.3 mg/m^2/L) and CDDP (43 mg/L of perfusate) + doxorubicin (15.25 mg/L of perfusate). The volume of perfusate was approximately 3.5 L/m^2 of body-surface area in most cases, resulting in a mean intra-abdominal pressure of 12 to 26 mm Hg.[16] The perfusate was then circulated into the peritoneal cavity at a mean flow of 600 mL/min.

Immediate Postoperative Surveillance and Follow-Up

In the postoperative period, patients were admitted to the intensive care unit for at least 72 hours and were then discharged to the surgical ward. Analysis of chemotherapy-related toxicity was performed according to the World Health Organization criteria. Grading of complications was performed according to the following criteria: grade 1, no complications; grade 2, minor complications; grade 3, major complications (requiring reoperation, intensive care unit admission, or interventional radiology); and grade 4, in-hospital mortality.[17] We considered only unfavorable events that occurred within 28 days of the procedure. The mean duration of hospitalization was 24 days (range, 8–67 days).

In the first 2 years after the procedure, the patients were followed up with physical examination every 3 months and with tumor marker (CA-125) determination and thoracic and abdominal computed tomographic scans every 6 months. Thereafter, the patients were seen every 6 months up to the fifth year.

Histological Evaluation of Tumors and Study Parameters

Diagnosis of DMPM was confirmed in each patient, including review of pertinent immunohistochemical studies. The panel of immunostains included calretinin and Wilms' tumor as positive mesothelial markers and polyclonal carcinoembryonic antigen and Ber-EP4 as negative markers.[18]

The hematoxylin and eosin slides of all cases were reviewed (the available number of slides ranged from 6 to 39, with an average of 22 per patient), and the tumors were classified as epithelial, sarcomatoid, and biphasic (mixed epithelial and sarcomatoid) according to the World Health Organization classification.[6] Nuclear grade (NG) was assessed according to the following grading system: NG 1, small nuclei, uniform chromatin pattern, and small pinpoint-sized nucleoli; NG 2, larger nuclei, some chromatin irregularity, and more prominent nucleoli; and NG 3, large nuclei, irregular chromatin pattern with clearing, and prominent nucleoli.[14] For the prognostic analysis, we classified the tumors into two groups: NG 1 and 2 and NG 3. The mitotic count (MC) per 50 high-power microscopic fields (HPFs) was determined, with the greatest dimension of .44 mm and a microscopic field of .152 mm^2.

Immunohistochemical stains using the avidin-biotin complex immunoperoxidase technique for matrix metalloproteinase (MMP)-2, MMP-9, p16, and epidermal growth factor receptor (EGFR) were performed on 5-μm sections from representative paraffin blocks of the tumor specimens. Adequate material for the performance of immunostaining was available for 35 (71%) of the 49 patients. The following anti-

TABLE 3. *Intensity of immunostaining of biological markers in a subset of 35 patients with diffuse malignant peritoneal mesothelioma*

	EGFR		p16		MMP-2		MMP-9	
Score[a]	n	%	n	%	n	%	n	%
0	2	6	14	40	0	0	5	14
1+	1	3	11	31	2	6	9	26
2+	3	8	6	17	3	8	8	23
3+	7	20	2	6	7	20	8	23
4+	22	63	2	6	23	66	5	14

EGFR, epidermal growth factor receptor; MMP, matrix metalloproteinase.
[a]0, negative; 1+, <25%; 2+, 25%–50%; 3+, 50%–75%; 4+, 75%–100%.

bodies were used: MMP-2 (monoclonal; Novocastra, Newcastle, UK; 1/40), MMP-9 (monoclonal; Novocastra; 1/40), p16 (F-12, monoclonal; Santa Cruz Biotechnology Inc., Santa Cruz, CA; 1/50), and EGFR (monoclonal; Novocastra; 1/100). The immunohistochemistry stains were scored as 0 (negative), +1 (<25%), +2 (25%–50%), +3 (50%–75%), and +4 (75%–100%).

The following clinicopathologic characteristics and biological markers were evaluated with respect to their correlation with outcome (OS and progression-free survival [PFS]): previous surgical score,[19] age at diagnosis, sex, preoperative performance status, performance of systemic chemotherapy before the locoregional therapy, histological subtype (epithelial vs. nonepithelial), NG, MC, carcinomatosis extension (Peritoneal Cancer Index), completeness of cytoreduction (CC; 0/1 [minimal residual disease or residual tumor <2.5 mm] vs. 2/3 [residual tumor ≥2.5 mm]),[14] IPHP drug schedule (CDDP + mitomycin C vs. CDDP + doxorubicin), MMP-2, MMP-9, p16, and EGFR.

Statistical Analysis

Survival was calculated from the date of operation to the time of death or last follow-up, whichever occurred first. The estimated survival curve distribution was calculated by the Kaplan-Meier method. The log-rank test was used to assess the significance of survival distributions. On the basis of univariate analysis, a subset of variables was chosen (generally, $P < .20$) to include in a Cox proportional hazards analysis to determine which, if any, variables were jointly important in prognosis. All P values are two tailed.

RESULTS

Treatment-Related Complications

Grade 3 complications occurred in eight cases (15%). The most significant morbidities were intestinal fistulas (n = 7), gastric perforation (n = 1), pneumonia (n = 5), fever (n = 3), and sepsis (n = 4). Other complications were pulmonary embolism (n = 1) and pancreatic fistula (n = 1). One patient had an acute hypotensive episode clinically diagnosed as cardiac arrest on the eighth day after the procedure; he was emergently resuscitated, without any short- or long-term sequelae. There were no treatment-related deaths. Nine grade 3 or 4 toxicities occurred in six cases (12%). They were two cases of anemia (grade 3), one leucopenia (grade 3), two gastrointestinal toxicities (grade 3), two acute renal failures (grade 3), and two chronic renal failures (grade 4).

Pathologic Findings

There were 43 cases of the epithelial type and six cases of the biphasic (mixed epithelial and sarcomatoid) type. No pure sarcomatoid case was present. There were 6 cases (11%) of NG 1, 19 cases (37%) of NG 2, and 27 cases (52%) of NG 3. The mean MC was 16 in 50 HPFs (range, 0–160). Immunohistochemical results are listed in Table 3. EGFR was expressed in a membranous pattern in all but two cases (94%).

The intensity was generally diffuse and strong, and in 22 cases (63%) it was 4+. Instead, p16 was only focally positive, with a nuclear staining pattern in 21 cases (60%), and 14 cases (40%) were completely negative for this stain. MMP-2 was expressed in all cases, generally in a diffuse and strong fashion, whereas MMP-9 was expressed in 30 cases in a variable intensity and distribution. No biologic markers were of prognostic value.

Prognosis

At a mean follow-up of 20.3 months (range, 1–89 months), the 5-year OS and PFS were 57% and 31%, respectively. The median PFS was 39.7 months (95% confidence interval, 26.8–52.6 months; Figs. 1 and 2).

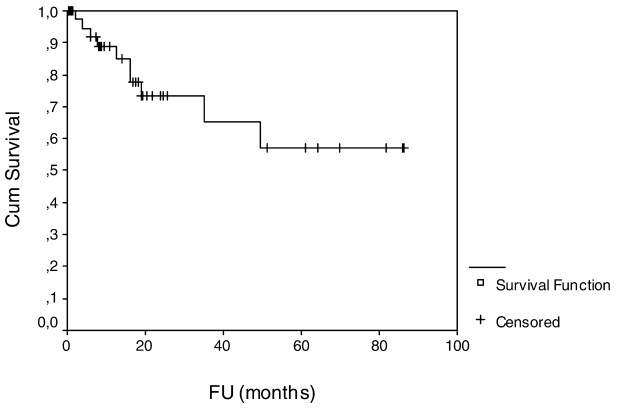

FIG. 1. Overall survival of diffuse malignant peritoneal mesothelioma patients treated with cytoreductive surgery and intraperitoneal hyperthermic perfusion. Cum, cumulative; FU, follow-up.

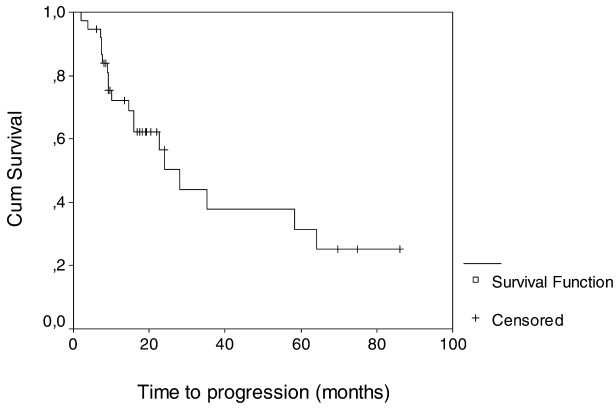

FIG. 2. Progression-free survival in diffuse malignant peritoneal mesothelioma patients treated with cytoreductive surgery and intraperitoneal hyperthermic perfusion. Cum, cumulative.

TABLE 4. *Clinicopathologic variables with prognostic significance according to univariate (log-rank) and multivariate (Cox proportional hazard model) analysis*

	Overall survival			Progression-free survival		
		Multivariate			Multivariate	
Variable	Univariate *P* value	Hazard ratio (95% CI)	*P* value	Univariate *P* value	Hazard ratio (95% CI)	*P* value
Sex	.22			.27		
Age (< 52 vs. ≥52 y)	.93			.49		
Performance status (0 vs. 1, 2, or 3)	.43			.05	.29 (.10–.83)	.02
Previous surgical score (0 vs. ≥1)	.78			.11		
Previous systemic chemotherapy	.59			.57		
PCI (≥28 vs. < 28)	.12			.10		
Completeness of cytoreduction (0/1 vs. 2/3)[a]	.01	8.62 (2.05–36.24)	.00	.08		
IPHP drug schedule (CDDP + DX vs. CDDP + MMC)	.36			.98		
Histological subtype (epithelioid vs. biphasic)	.09			.07		
Mitotic count/50 HPFs (< 5 vs. ≥5)	.01	10.46 (1.98–55.23)	.01	.19	3.16 (1.13–8.81)	.03
Nuclear grade (high vs. low)	.02			.10		

CI, confidence interval; PCI, Peritoneal Cancer Index; IPHP, intraperitoneal hyperthermic perfusion; CDDP, cisplatin; DX, doxorubicin; MMC, mitomycin C; HPF, high-power field.

[a] 0/1, minimal residual disease or residual tumor < 2.5 mm; 2/3, residual tumor ≥2.5 mm.

At the end of the study period, the final disease status was as follows: 29 patients had no evidence of disease, 10 patients were alive with disease, and 10 had died of disease.

Results of the possible prognostic factors associated with OS and PFS by univariate analysis are shown in Table 4. After the log-rank test, we submitted the variables with *P* values < .20 to multivariate analysis with the Cox proportional hazard model: histological subtype, CC, NG, and MC for OS and performance status, and histological subtype, carcinomatosis extension, CC, and MC for PFS. The backward-elimination method identified the best predictors of the outcome. The strongest predictors of OS were CC and MC > 5 per 50 HPFs (Figs. 3 and 4), whereas those for PFS were performance status and MC (Fig. 5). Table 4 shows the hazard ratios of each variable with their respective 95% confidence intervals and *P* values.

DISCUSSION

DMPM was considered a lethal clinical condition amenable only to palliative treatment options up to the advent of a locoregional therapeutic approach.

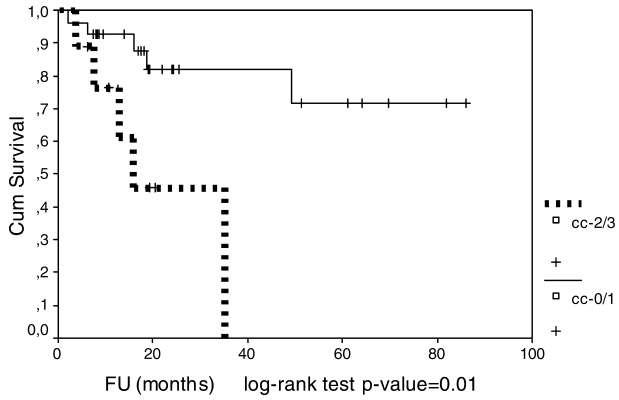

FIG. 3. Overall survival according to completeness of cytoreduction. Cum, cumulative; FU, follow-up; CC-0/1, minimal residual disease or residual tumor <2.5 mm; CC-2/3, residual tumor ≥ 2.5 mm.

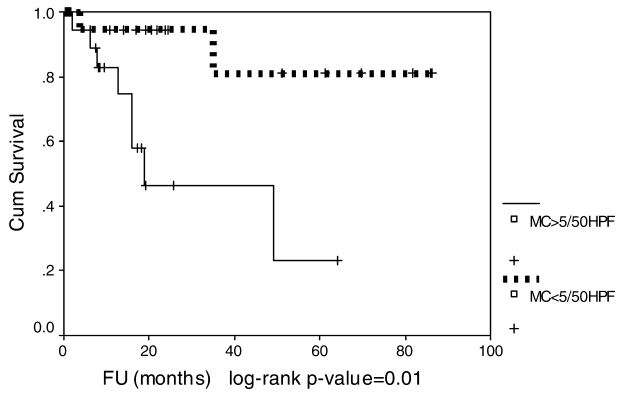

FIG. 4. Overall survival according to mitotic count (MC). HPF, high-power field; Cum, cumulative; FU, follow-up.

The combination of CRS and IPHP is an innovative treatment strategy that has evolved over the last two decades in the treatment of peritoneal surface malignancies. It has shown good results, in terms of outcome, in malignant PM according to phase II[10–12,20,21] clinical trials. These studies reported 5-year OS rates ranging from 47% to 59%. The rationale concerning the attainment of a synergistic effect between chemotherapies and heat, as well as the pharmacokinetic advantage of locoregional instillation of antiblastic drugs, was outlined elsewhere.[20]

However, this novel combined-treatment approach is expensive and is labor and time consuming. Moreover, it carries a not-negligible morbidity rate ranging from 27% to 35% and a mortality rate ranging from 1.5% to 12%[15,22–25] even with the most experienced teams. Thus, the search for prognostic factors is of the utmost importance to identify the subset of patients that could best benefit from this

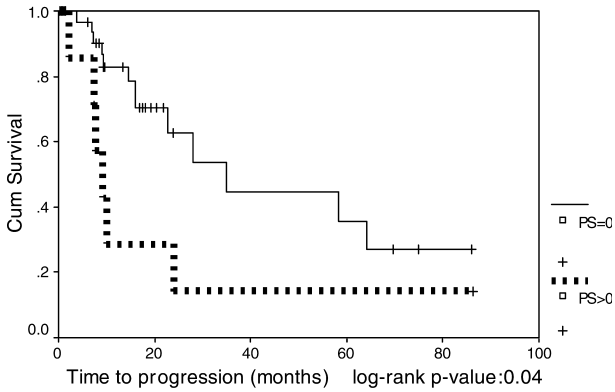

FIG. 5. Progression-free survival according to performance status (PS; Eastern Cooperative Oncology Group). Cum, cumulative.

procedure, thus avoiding unnecessary surgical risk in patients with an unchangeable prognosis.

We observed in our study after performing the multivariate analysis that the CC and MC >5 per 50 HPFs presented the strongest association with OS among the tested clinicopathologic variables. The estimated hazard rate for patients with optimal cytoreduction (residual disease <2.5 mm) was eight times higher than that estimated for patients with suboptimally cytoreduced disease (residual disease >2.5 mm) after adjustment for other variables. This finding is in agreement with experimental evidence that supports one of the eligibility criteria for IPHP. Usually the drugs, even when instilled intra-abdominally, are not able to penetrate tumor tissue deeper than a few cellular layers, so the volume of residual disease remains one the major factors influencing the efficacy of locoregional therapy. Moreover, residual disease has been shown to be of prognostic significance in PM treated by CRS plus IPHP.[11,21,26] However, whether this survival benefit resulted from lower tumor aggressivity or from the surgical effort itself is difficult to ascertain. This series included only the most malignant subtypes of the disease, an aspect that could favor the completeness of the cytoreduction as a primary factor. The eligibility criteria were very restrictive, with exclusion of borderline forms of PM, which present known indolent and less aggressive behavior as compared with their malignant counterparts. A definitive answer to such a question should be provided by another study with a different and well-formulated design.

The second variable that remained in the Cox model as a factor influencing the OS was the MC. Patients with an MC >5 per 50 HPFs presented a hazard rate 10 times higher as compared with those with a lower MC. The data available with respect to

this issue in the literature are conflicting. Ramael et al.[27] and Beer et al.[28] found that patients with a high MC lived for significantly shorter periods than those with a low MC, whereas Kerrigan et al.[29] did not reach the same conclusion. However, both variables (CC and MC) should be taken cautiously as independent surrogate markers for OS because the 95% confidence intervals for their respective hazard rates are fairly wide (2.05–36.24 for CC and 1.98–55.23 for MC).

The prognostic analysis in terms of PFS showed that performance status and MC remained in the model after the backward-elimination method. The preoperative clinical condition has been largely shown to be a prognostic factor in the pleural form of mesothelioma,[30,31] but the same finding has not been demonstrated for the peritoneal counterpart, according to the authors with expertise in locoregional therapy. In this series, it is noteworthy that the performance status did not present a meaningful correlation with OS. This result could be attributed to the facts that the great majority of patients (89%) had a performance status of 0 and that the rate of events (deaths due to disease progression) was not high enough. The independent association between MC and PFS emerged after the multivariate analysis even in the absence of a significant correlation with PFS by univariate analysis. This could have resulted from the presence of a confounding factor among the clinicopathologic variables.

Other factors possibly related to prognosis according to the literature, such as age at diagnosis, sex, previous debulking in PM treated by CRS, and IPHP,[11,21] were not shown to be predictive of outcome in our series. This lack of correlation is not surprising. Although our series is one of the largest published in the field of locoregional therapy, the sample size cannot be considered big enough to extrapolate reliable results.

p16, also known as INK4a, is a tumor-suppressor gene located on human chromosome 9 in the region 9p21. Two alternatively spliced gene products are encoded by p16: the proteins P16 and p14ARF. The p16(INK4a) protein, by inhibiting cyclin-dependent kinase, downregulates Rb-E2F and leads to cell-cycle arrest in the G_1 phase. The p14(ARF) protein interacts with the MDM2 protein and neutralizes MDM2-mediated degradation of p53. Because p53/Rb genes are not altered in malignant mesothelioma, additional components of these pathways, such as p16(INK4a) and p14(ARF), are candidates for inactivation. The recent molecular genetic study on 45 primary malignant mesothelioma specimens revealed alterations of p16 in 31% of cases, promoter methylation in 9%, deletion in 22%, and point mutation in 2%.[32] In our series, the immunoreaction of p16 was absent or reduced in 25 cases (71%), and this finding is in agreement with previous reports.[32,33]

EGFR is a cell-surface receptor involved in the regulation of cell growth and differentiation. The binding of the ligand to the receptor causes activation of its intrinsic tyrosine kinase activity and rapid internalization of the receptor-ligand complex into the cell; this leads to an increase in cellular proliferation, an increase in angiogenesis, inhibition of apoptosis, and expression of extracellular matrix proteins. The overexpression of EGFR is associated with a poor prognosis in some cancers. An earlier study showed EGFR immunoexpression in 69% of the epithelial type of diffuse malignant pleural mesothelioma, 44% of the sarcomatoid type, and 22% of the mixed type. No correlation between EGFR overexpression and prognosis was identified.[34] Twenty-two (63%) of 35 cases showed diffuse and strong immunoreactivity for EGFR, a finding consistent with a previous study.[35]

The pattern of DMPM progression within the abdominal cavity suggests an important role of proteases, including the MMPs, in the evolution of the disease. Our study demonstrated the constant expression of MMP-2 and, to a lesser degree, of MMP-9. All the cases expressed MMP-2 to some extent, and 23 patients showed a 4+ staining intensity in DMPM cells. Overexpression of MMPs, particularly MMP-2 (gelatinase A), MMP-9 (gelatinase B), and MMP-11 (stromelysin 3), is related to tumor progression and metastasis in various carcinomas, including gastric, colonic, and pulmonary carcinomas.[36–38] In a study of pleural diffuse malignant mesotheliomas (DMMs) using semiquantitative gelatin zymography, increasing MMP-2 and pro–MMP-2 activity were independently associated with a poor prognosis, but MMP-9 activity had no prognostic significance.[39] Only a few small studies have investigated MMP immunohistochemically on surgical specimens of DMM. The results were variable and not always consistent with those found by reverse transcriptase-polymerase chain reaction, Western blot, and gelatin zymography on DMM cell lines, as well as fresh tissue of DMM.[39–43]

A study on the possible correlation of various clinical and pathologic factors with the prognosis in a series of DMPMs uniformly treated by CRS and IPHP, like ours, could be less biased than previous studies that addressed the same issue and included

patients with different histological subtypes and different treatment modalities. The lack of correlation between the biological markers and the outcome in this study should be taken with caution because of the inherent limitations of the immunohistochemistry method in assessing these markers and because of the limited sample size of our series. Moreover, it is difficult to assess to what extent a selection bias could have occurred in this study. The subset of patients who fit the eligibility criteria for locoregional therapy is highly selected, and in that sense, this group might not be representative of the entire DMPM population. In addition, the retrospective nature of our series constitutes another methodological limitation of our study.

We conclude that CC, performance status, and MC seem to be the best determinants of outcome. These data need confirmation by a prospective formal trial. No biological markers presented a significant correlation with the outcome. However, their pattern of immunostaining, which suggests overexpression of EGFR, MMP-2, and MMP-9 and absent or reduced expression of p16, might be related to the underlining tumor kinetics of DMPM and warrants further investigation with other methods.

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