

Diffuse Malignant Mesothelioma of the Peritoneum

A Clinicopathologic Study of 35 Patients Treated Locoregionally at a Single Institution

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BACKGROUND. In the current study, the authors report the clinicopathologic features of patients with peritoneal diffuse malignant mesothelioma (DMM) who were treated in a uniform fashion at a single institution to assess prognostic factors.

METHODS. Thirty-five patients were treated with cytoreductive surgery (CRS) and intraperitoneal hyperthermic perfusion (IPHP). The tumors were classified into epithelial, sarcomatoid, and biphasic types. Immunohistochemistry stains were performed for calretinin, WT-1, pCEA, Ber-EP4, epidermal growth factor receptor (EGFR), p16, matrix metalloproteinase-2 (MMP-2), and MMP-9. Statistical correlation was evaluated for age, gender, completeness of cytoreduction (CC), tumor histotype, mitotic count (MC), necrosis, nuclear grade (NG), and biologic markers with regard to overall survival (OS) and progression-free survival (PFS).

RESULTS. The patient group was comprised of 15 men and 20 women with a median age of 52 years (range, 24–73 yrs). Twenty-five patients underwent optimal cytoreduction. There were 32 epithelial tumors and 3 biphasic tumors, and 3 patients had an NG of 1, 19 had an NG of 2, and 13 had an NG of 3. The mean MC was 14.1 (range, 0–160 per 50 high-power fields). Necrosis was present in 11 cases. All the tumors were found to be positive for calretinin and WT-1 and were negative for pCEA and Ber-EP4. The NG and MC were found to be significantly associated with OS ($P = 0.02$ and $P = 0.01$, respectively) whereas CC was found to be associated with both OS ($P = 0.05$) and PFS ($P = 0.03$). No biologic markers were found to be of prognostic significance.

CONCLUSIONS. The results of the current study indicate that NG, MC, and CC may be useful prognostic factors in patients treated with CRS and IPHP. The expression of EGFR, MMP-2, and MMP-9 and absent and/or reduced expression of p16 in DMMs confirms the results of previous studies suggesting their role in tumor pathogenesis and kinetics. *Cancer* 2005;104:2181–8.

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Diffuse malignant mesotheliomas (DMMs) are uncommon tumors, with an annual incidence of approximately 2500 cases in the U.S.¹ A majority of cases occur in the pleura, followed by the peritoneum, pericardium, and tunica vaginalis testis.² DMM is histologically subclassified into the following types: epithelial (tubulopapillary, non-glandular/solid), sarcomatoid, biphasic (mixed), and undifferentiated (poorly differentiated).^{3,4} Approximately 50% of pleural DMMs and 75% of peritoneal DMMs are of the epithelial type, whereas 25% and 15%, respectively, are of the biphasic and sarcomatoid types.⁵ The remaining cases are poorly differentiated and of special subtypes. The latter include desmoplastic, lymphohistiocytoid, small cell, and de-

ciduoid variants.³ A favorable prognosis has been reported with the tubulopapillary variant of the epithelial type, whereas the sarcomatoid type appears to be associated with a poor outcome.⁶

Peritoneal and pleural DMMs are similar in many aspects, including histopathology and immunophenotype.⁷ Differences such as clinical presentation and treatment modality are obviously derived from the anatomic location involved by the tumor. The tumor growth of peritoneal DMMs is characterized by peritoneal seeding and the formation of ascites, eventually leading to the patient's death because of tumor encasement, invasion of the bowel, and intractable ascites. The disease generally is confined to the peritoneal cavity and usually does not infiltrate into the liver parenchyma. In advanced stages, direct extension to the pleural cavity and distant metastases may be noted.⁸ An autopsy study demonstrated that approximately two-thirds of the patients examined had tumor only in the abdominal cavity, and that 78% of patients had died because of complications directly related to intraabdominal disease.⁹ The prognosis of the disease is grim, with survival ranging from 7–13.5 months; to our knowledge, only a few long-term survivors have been reported to date.¹⁰

DMM has been conventionally treated by palliative surgery (for the relief of small bowel obstruction or massive ascites), radiation therapy, or systemic chemotherapy, but neither modality has proved to be satisfactory. However, recent trials of multimodality therapy comprised of cytoreductive surgery (CRS) with peritonectomy procedures and intraperitoneal hyperthermic perfusion (IPHP) have reportedly resulted in the extended survival of selected patients.¹⁰

In the current study, we present the clinical, pathologic, and prognostic features of 35 patients with peritoneal DMM who were uniformly treated with CRS and IPHP at the National Cancer Institute of Milan.

MATERIALS AND METHODS

We studied patients with peritoneal DMM who were diagnosed and treated between 1996–2004 at the National Cancer Institute in Milan, Italy. The 35 patients included in the current study were those that were clinically, histopathologically, and immunohistochemically diagnosed as having DMM and for whom complete clinicopathologic data were available.

Patient Characteristics

Nineteen patients had received preoperative systemic chemotherapy (Table 1). All patients were treated with CRS and IPHP. The eligibility criteria for CRS and IPHP were age < 75 years; a Karnofsky performance status > 70; no severe clinical dysfunction; no concomitant

TABLE 1
Preoperative Systemic Chemotherapy Received by Patients

Previous systemic chemotherapy received	No of patients
Epiadriamycin + ifosfamide	5
Epiadriamycin + ifosfamide/other schedules	1
Cisplatin based therapy	11
Cisplatin based therapy/epiadriamycin + ifosfamide	1
Other schedules	1
Total	19

evidence of pleural extension; no other concomitant neoplasms; normal hematologic function, biochemical liver function, and creatinine clearance tests; and the provision of written informed consent following the guidelines of the Ethical and Scientific Committee of the National Cancer Institute of Milan.

The techniques of CRS have been described previously.¹¹ In brief, CRS is comprised of five different visceral or parietal peritonectomy procedures: omentectomy/splenectomy, left subdiaphragmatic peritonectomy, right subdiaphragmatic peritonectomy, pelvic peritonectomy/sigmoidectomy, and cholecystectomy/lesser omentectomy. The closed abdominal technique was adopted for IPHP techniques. Technical details were described previously.¹² Residual disease after surgery was classified by the completeness of cytoreduction (CC) according to the criteria of Jaquet and Sugarbaker: cc-0: no residual disease; cc-1: minimal residual disease (measuring 0–2.5 mm); cc-2: residual disease measuring 2.5–2.5 cm; and cc-3: residual disease measuring > 2.5 cm.¹³

Pathologic Review

The hematoxylin and eosin-stained slides of all cases were reviewed (mean number, 22 slides; range, 6–39 slides) and the tumors were classified as epithelial, sarcomatoid, and biphasic (mixed epithelial and sarcomatoid) according to the World Health Organization classification.⁴ The epithelial type was subdivided further into predominantly tubulopapillary and predominantly solid groups based on the predominant pattern. Nuclear grade (NG) was assessed according to the following grading system: Grade 1: small nuclei, uniform chromatin pattern, and small pinpoint-sized nucleoli; Grade 2: larger nuclei, some chromatin irregularity, and more prominent nucleoli; and Grade 3: large nuclei, irregular chromatin pattern with clearing, and prominent nucleoli.¹⁴ The mitotic count (MC) per 50 high-power microscopic fields (HPFs) was performed, with the greatest dimension considered to be 0.44 mm and a microscopic field of 0.152 mm². Necrosis also was evaluated microscopically.

A representative paraffin block for each case of peritoneal DMM was selected for immunohistochemical studies using the avidin-biotin-complex immunoperoxidase technique. The following antibodies were used: matrix metalloproteinase-2 (MMP-2) (monoclonal; Novocastra, Newcastle-upon-Tyne, U.K.) at a dilution of 1:40; MMP-9 (monoclonal; Novocastra) at a dilution of 1:40; calretinin (polyclonal; Swant, Bellinzona, Switzerland) at a dilution of 1:3000; WT-1 (C-19) (polyclonal; Santa Cruz Biotechnology Inc., Santa Cruz, CA) at a dilution of 1:400; carcinoembryonic antigen (CEA) (polyclonal; Dakopatts, Glostrup, Denmark) at a dilution of 1:2000; Ber-EP4 (monoclonal; Dakopatts) at a dilution of 1:500; p16 (F-12) (monoclonal; Santa Cruz Biotechnology Inc.) at a dilution of 1:50; and epidermal growth factor receptor (EGFR) (monoclonal; Novocastra) at a dilution of 1:100. The immunohistochemistry stains were scored as 0 (negative), +1 (< 25%), +2 (25–50%), +3 (50–75%), and +4 (75–100%).

The histologic diagnosis of DMM was confirmed using the following panel of immunostains: calretinin and WT-1 as positive mesothelial markers, and polyclonal CEA and Ber-EP4 as negative markers.¹⁵

Statistical Analysis

Statistical correlation was evaluated for age, gender, CC, histology subtype, MC, necrosis, NG, and biologic markers with overall survival (OS) and progression-free survival (PFS). OS was calculated from the date of surgery to the date of death or the time of last follow-up and PFS was calculated from the date of surgery to the date of disease progression or death, whichever occurred first. The survival curve distribution was calculated using the Kaplan–Meier method. The log-rank test was used to assess the significance of survival distributions.

RESULTS

Clinical Features

The median age of the patients was 52 years (range, 24–73 yrs). There were 15 male and 20 female patients, with a male-to-female ratio of 0.75:1. There were no significant differences noted with regard to the ages of the male patients (median age of 51 yrs; range, 24–65 yrs) and female patients (median age, 52 yrs; range, 31–73 yrs). There were 11 patients classified with cc-0, 14 patients classified with cc-1, 8 patients classified with cc-2, and 2 patients classified with cc-3. Twenty-five of 35 patients (71%) underwent optimal cytoreduction (cc-0 and cc-1). Follow-up information was available for all 35 patients, with a median follow-up period of 52 months (range, 0.4–86 mos). At the time of last follow-up, 23 patients were alive with no evi-

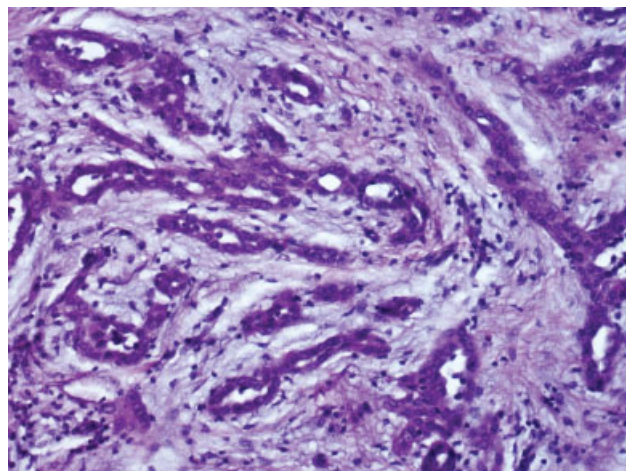


FIGURE 1. Epithelial diffuse malignant mesothelioma of tubular type (original magnification $\times 200$).

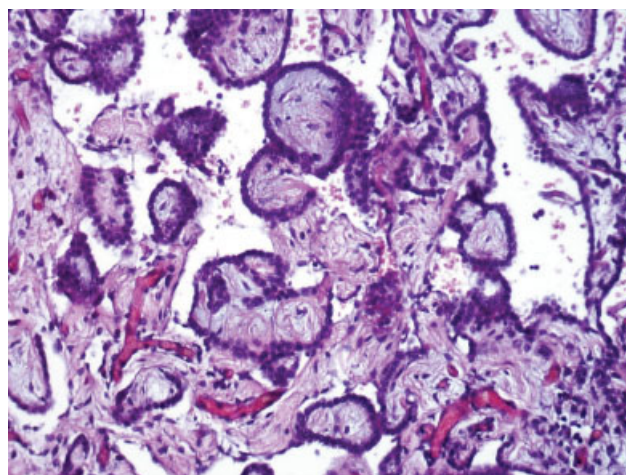


FIGURE 2. Epithelial diffuse malignant mesothelioma of papillary type (original magnification $\times 200$).

dence of disease, 3 patients were alive with disease, and 9 patients were dead of disease. The median PFS was 28 months.

Pathologic Features

There were 32 patients with epithelial-type tumors (Figs. 1-4) and 3 patients with biphasic-type tumors (mixed epithelial and sarcomatoid type) (Fig. 5). No cases of pure sarcomatoid type were present. The epithelial-type tumors demonstrated various combinations of tubular, papillary, and solid patterns. The combination often was variable in different tumors and even within different microscopic fields of the same tumor. Based on the predominant pattern, epithelial DMMs were subdivided further into 16 cases of predominantly tubulopapillary type (Figs. 1, 2) and 16

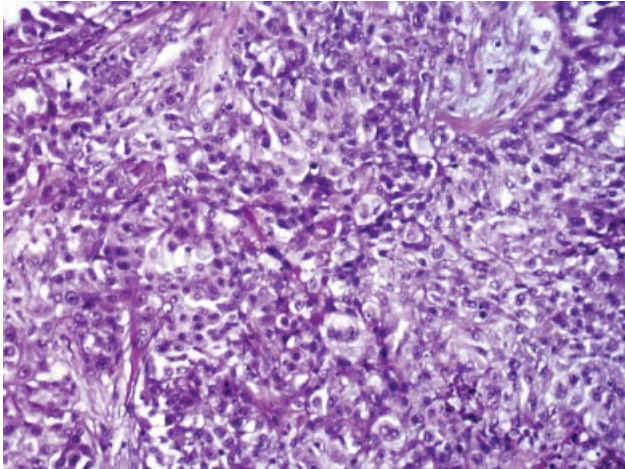


FIGURE 3. Epithelial diffuse malignant mesothelioma of solid type (original magnification $\times 200$).

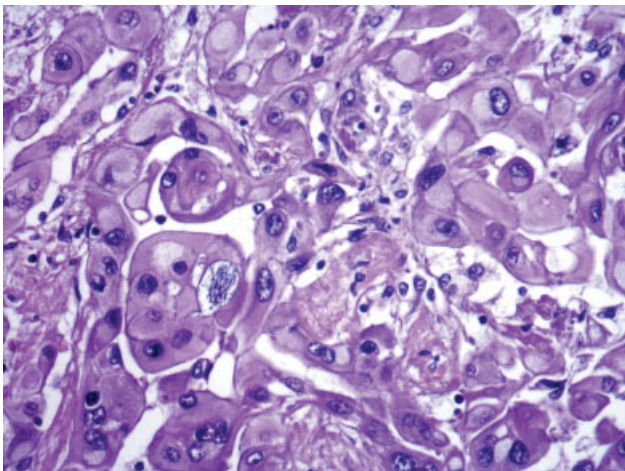


FIGURE 4. Epithelial diffuse malignant mesothelioma of deciduoid variant (original magnification $\times 400$).

cases of predominantly solid type (Fig. 3). One DMM case was qualified as being of the deciduoid variant (Fig. 4). There were 3 cases of NG 1 (9%), 19 cases of NG 2 (54%), and 13 cases of NG 3 (37%). The MC ranged from 0–160 per 50 HPFs (mean of 14.1 per 50 HPFs and median of 5 per 50 HPFs) with 30 cases (86%) demonstrating a MC of < 20 per 50 HPFs. Necrosis was present in 11 cases (31%) and the distribution and extent of necrosis were variable in each case, ranging from a small focus to multiple large geographic areas.

The immunohistochemical results are summarized in Table 2. EGFR was expressed in a membranous pattern in all but 2 cases (94%). The staining intensity generally was diffuse and strong, and was +4 in 22 cases (63%). Conversely, p16 was found to be

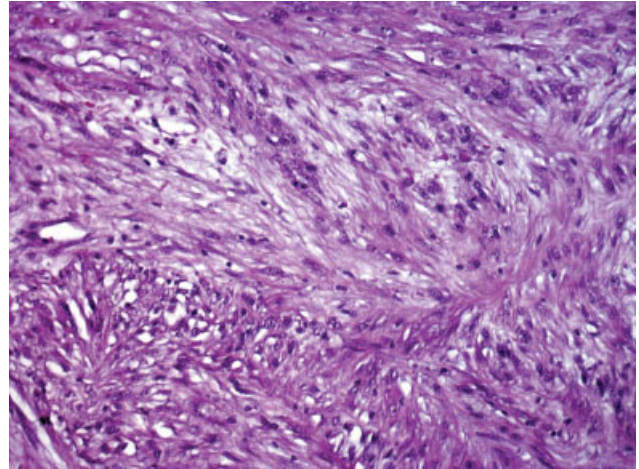


FIGURE 5. Biphasic diffuse malignant mesothelioma present in the sarcomatoid area (original magnification $\times 200$).

TABLE 2
Immunohistochemical Results

Score	No. of patients							
	Calretinin	WT-1	pCEA	Ber-Ep4	EGFR	p16	MMP-2	MMP-9
0	0	0	35	35	2	14	0	5
+1	0	5	0	0	1	11	2	9
+2	1	6	0	0	3	6	3	8
+3	6	5	0	0	7	2	7	8
+4	28	19	0	0	22	2	23	5

pCEA: pathologic carcinoembryonic antigen; EGFR: epidermal growth factor receptor; MMP: matrix metalloproteinase.

only focally positive, with a nuclear staining pattern noted in 21 cases (60%); 14 cases (40%) were found to be completely negative for p16.

MMP-2 was expressed in all cases, generally in a diffuse and strong fashion, whereas MMP-9 was expressed in 30 cases but was found to be of variable intensity and distribution.

Calretinin and WT-1 were expressed in all cases to a variable degree. Calretinin was found to be expressed only focally (+1) in the sarcomatoid areas of one tumor and was completely absent in the sarcomatoid areas of an additional two tumors. WT-1 expression was found to be negative in the sarcomatoid areas of all three tumors. Expression of polyclonal CEA and Ber-EP4 also were negative in all cases.

MC (≥ 5 per 50 HPFs vs. < 5 per 50 HPFs) and NG (Grade 1/2 vs. Grade 3) were found to be correlated significantly with OS ($P = 0.02$ and $P = 0.01$, respectively) whereas CC was found to be correlated with both OS ($P = 0.05$) and PFS ($P = 0.03$). No biologic markers were found to be of prognostic value. The OS

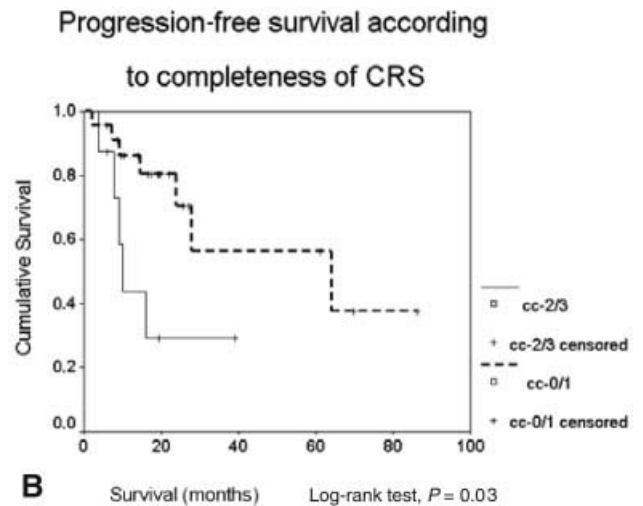
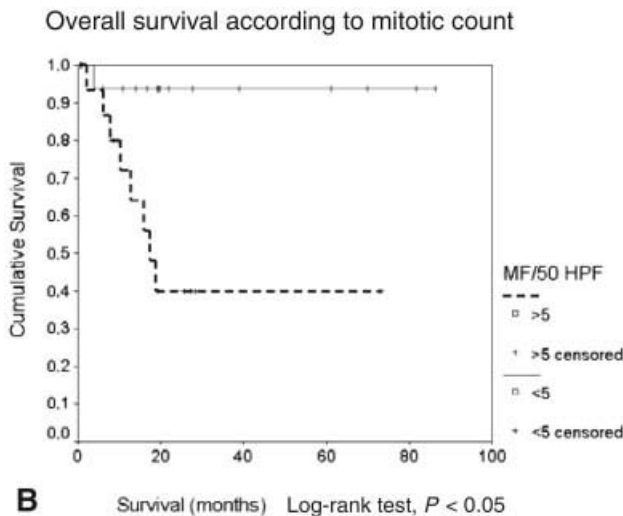
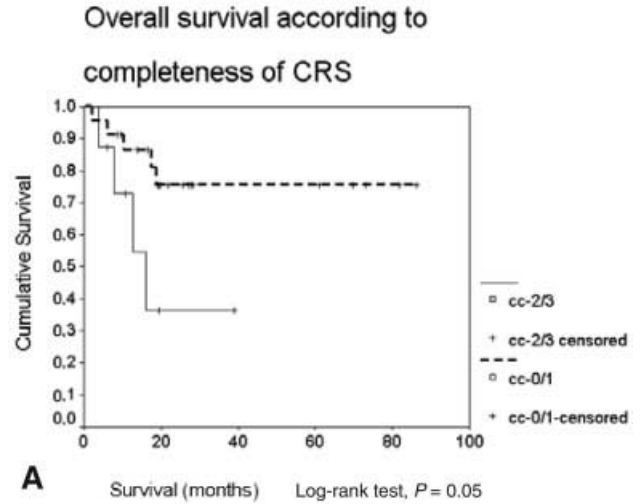
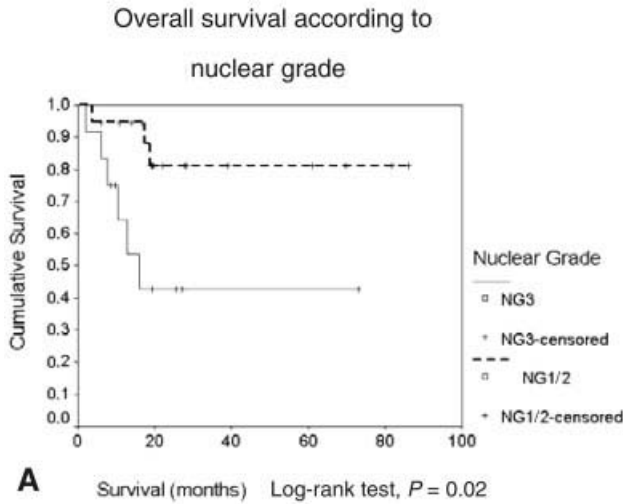


FIGURE 6. Overall survival (OS) of all patients in reference to (A) nuclear grade (NG) (NG 1/2 vs. NG 3) and (B) mitotic count (MC) ($MC < 5$ per 50 high-power fields [HPFs] vs. $MC \geq 5$ per 50 HPFs). The NG 1/2 group and $MC < 5$ per 50 HPFs group were found to have a more favorable prognosis compared with the NG 3 group and the $MC \geq 5$ per 50 HPFs group, respectively.

graphs based on NG (Grade 1/2 vs. Grade 3) and $MC (\geq 5$ per 50 HPFs vs. < 5 per 50 HPFs) and the PFS and OS graphs based on CC are depicted in Figures 6 and 7, respectively .

DISCUSSION

The prognosis for patients with untreated DMM is generally considered to be dismal. Survival is reported to range from 9–18 months for patients with peritoneal tumors and from 4–12 months for patients with pleural tumors.^{16–18} Peritoneal DMMs are uncommon

FIGURE 7. (A) Overall survival (OS) and (B) progression-free survival (PFS) in reference to differences in the completeness of the cytoreduction performed (cc) (cc-0/1 vs. cc-2/3). The group with cc-0/1 was found to have a more favorable OS and PFS compared with the cc-2/3 group.

in comparison with pleural DMMs, and to our knowledge only a few series of comprehensive clinicopathologic studies focusing on peritoneal DMMs have been published to date.^{2,14} In the current study, we reported on the clinical, pathologic, and prognostic features of 35 patients with peritoneal DMM who were treated in a consistent fashion.

Conventional treatment modalities have reportedly provided little benefit in prolonging survival. Complete surgical resection is not usually considered to be feasible, radiation therapy generally cannot be given in doses sufficient to eradicate the tumor, and systemic chemotherapy is reported to be of limited effectiveness in the majority of patients.¹⁶ Recently

proposed multimodality treatments combining both CRS and intraoperative chemotherapy have resulted in improved survival for many patients.^{16,17} In a series from the Dana Farber Cancer Center, 17 patients received a combination of CRS and 5 cycles of intraperitoneal chemotherapy (doxorubicin and cisplatin).¹⁹ In addition, 11 responders received total abdominal radiation (at a dose of 30 grays), intravenous chemotherapy, or both. The authors of the series reported a median survival of 27.6 months (range, 3.6–66 mos), with 8 patients still alive at a median follow-up of 24 months (range, 3–49 mos). Sebbag et al. reported 33 cases of peritoneal mesothelial tumors, including 30 cases of peritoneal DMMs.¹⁰ The patients were treated uniformly with CRS and perioperative intraperitoneal chemotherapy (cisplatin and doxorubicin). The median survival was 31.0 months. The authors found that clinical factors such as female gender, good health status, and a low CC score (optimal cytoreduction) were associated with improved survival.¹⁰ The current study results also demonstrated a significant correlation between CC and both OS and PFS.

The results of the current study regarding the histopathologic markers showed that MC and NG were statistically correlated with OS. Two other studies have proposed MC as a potentially useful prognostic factor,^{20,21} whereas another study did not reach the same conclusion.² In the two former studies, it was found that patients with a high MC lived for significantly shorter periods than those with a low MC.^{20,21} The inconsistent results in the different studies with regard to this issue could possibly result from the heterogeneity of the treatment modalities.

The division of NG into two groups (Grade 1/2 vs. Grade 3) also was found to be associated significantly with OS in the patients in the current study. A previous study that used the same NG system failed to demonstrate this association.² Again, this could be attributed to the variability of treatment modalities used and the smaller sample size in the previous study. Therefore, it appears that both the MC and NG, which are easily evaluated, could be useful prognostic indicators in patients with peritoneal mesothelioma.

In the current series, 14 tumors (40%) demonstrated an absence of p16 immunoreactivity and 11 tumors (31%) demonstrated reduced expression (+1) of this marker. Overall, p16 was found to be absent or reduced in 25 tumors (71%). This finding is in keeping with that of previous reports.^{22,23} Alterations of the p16INK4 locus in patients with DMM are relatively common. An early immunohistochemistry study by Kratzke et al. demonstrated the uniform absence of p16 in 10 of the 12 cases studied, and focal expression (occurring in < 5% of the specimens) in the remaining

2 cases.²² The recent molecular genetic study of 45 cases of primary DMM revealed alterations of p16 in 31% of cases, promoter methylation in 9%, deletion in 22%, and point mutation in 2%.²³ Similar to many other cancers, DMM exhibits altered cell growth regulation involving the loss of pRb and p53 function.²⁴ Inhibition of the p53-dependent and pRb-dependent growth regulatory pathways may occur through mechanisms involving either homozygous loss of the CDKN2A (p16INK4a / p14ARF) locus at chromosome 9p21 or expression of SV40 Tag.²⁴

In the current study, 22 of 35 cases (63%) demonstrated diffuse and strong immunoreactivity for EGFR, a finding that is consistent with that of a previous report by Trupiano et al.⁷ EGFR, a receptor tyrosine kinase, is reportedly overexpressed in a wide variety of epithelial malignancies as well as DMMs. EGFR signaling leads to an increase in cellular proliferation, an increase in cell motility and angiogenesis, the inhibition of apoptosis, and the expression of extracellular matrix proteins. High levels of EGFR expression are associated with a poor prognosis in some malignancies. An earlier study by Dazzi et al.²⁵ demonstrated EGFR immunoreactivity in 69% of epithelial-type DMMs, 44% of the sarcomatoid type, and 22% of the mixed type. No correlation was noted between EGFR overexpression and prognosis.²⁵ Asbestos (particularly crocidolite), which is associated with the development of DMM, was reported to stimulate the autophosphorylation of EGFR in mesothelial cells, trigger the extracellular-regulated kinase (ERK) cascade, and lead to increases in AP-1 activity and either cell mitosis (if the DNA damage can be repaired) or apoptosis (if it cannot be repaired).^{26,27} In addition, DMM cell lines are reported to express EGFR and transforming growth factor- α (TGF- α), suggesting an autocrine role for EGFR in DMM.²⁸ Several inhibitors of EGFR have been developed to date. In a study using DMM cell lines, EGFR inhibitor (ZD1839) significantly inhibited epidermal growth factor-dependent cell signaling, resulting in a significant dose-dependent reduction in colony formation in a cytostatic manner.²⁸

Proteolytic degradation of the extracellular matrix and basement membranes by proteases is a key component of disease progression in patients with malignant neoplasms (e.g., tumor cell invasion and metastasis). To date, proteases, including MMPs, have been investigated only rarely using surgical specimens of peritoneal DMM.^{29,30} The results of the current study demonstrated the constant expression of MMP-2 and, to a lesser degree, of MMP-9. All the patients expressed MMP-2 to some extent, with 23 demonstrating a staining intensity of +4 in DMM cells. MMPs are a family of zinc-dependent enzymes that currently is

comprised of at least 20 members. 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 malignancies, including gastric, colon, and pulmonary carcinomas.^{29–31} MMP-2 and MMP-9 are key enzymes for degrading Type IV collagen, a major component of basement membranes, and are particularly expressed in mesenchymal-derived tumor cells.³² In a study of patients with pleural DMMs in which semiquantitative gelatin zymography was used, increases in MMP-2 and pro-MMP-2 activity were found to be independently associated with a poor prognosis, but MMP-9 activity had no prognostic significance.³³ MMP expression is regulated by various factors, including growth factors, cytokines, and cell contact with the extracellular matrix. A study using reverse transcriptase–polymerase chain reaction (RT-PCR) demonstrated that various growth factors such as epidermal growth factor and TGF- α increased the secretion of MMP-9 and MMP-3, but production of MMP-2 did not appear to be affected.³⁴ DMM cell lines expressed EGFR and *c-erb* B4, which can recognize various growth factors. To our knowledge, only a few small studies to date have investigated MMP immunohistochemically on surgical specimens of DMM, with varying results that were not always consistent with those found with RT-PCR, Western blot analysis, and gelatin zymography on DMM cell lines as well as fresh tissue from DMM specimens.^{33,35–37}

We believe the current series is unique in that all the patients were treated uniformly by CRS with peritonectomy and IPHP at a single institution. A study regarding the possible correlation of various clinical and pathologic factors with prognosis in such a series could be valuable and less biased than previous studies. However, it is hard to assess to what extent a selection bias could have occurred in the current study. The subset of patients who fit the eligibility criteria for CRS is highly selected; in that sense, this group may not be representative of the DMM population as a whole. Feldman et al. studied 49 patients with peritoneal DMM who were treated with CRS and IPHP and evaluated various clinicopathologic factors in association with outcome.³⁸ They found that a history of previous debulking surgery and the absence of deep tissue invasion were statistically significantly associated with favorable PFS and OS, whereas minimal residual disease after surgical resection and patient age younger than 60 years were associated with favorable OS only. Feldman et al. also demonstrated statistically strong trends toward a correlation between high-grade histology (solid epithelial and sarcomatoid types) and unfavorable outcome compared with low-

grade histology (adenomatoid and tubulopapillary types).³⁸ They did not evaluate NG and MC, which we found to be statistically significant. Their finding of an association between histologic grade and survival is noteworthy³⁸ because solid epithelial-type and sarcomatoid-type tumors, which they designated as high grade, tend to be accompanied by high NG and high MC. However, DMM, whether peritoneal, pleural, or in another location, generally demonstrates a wide spectrum of architectural patterns from one microscopic field to another, which makes it difficult to classify each case as one definitive histotype on many occasions. Therefore, an evaluation of NG and MC might be easier and more reliable with less interobserver bias, as demonstrated in the current study.

In summary, the results of the current study suggest a significant correlation between NG, MC, and CC and survival. Therefore, these three indicators might be useful prognostic factors in those patients with epithelial and biphasic types of peritoneal DMM who are treated with CRS and IPHP. With regard to sarcomatoid-type DMM, we were unable to demonstrate any correlation because of a lack of this subtype in the current series. Overexpression of EGFR, MMP-2, and MMP-9 and absent/reduced p16 expression in peritoneal DMMs might be involved in tumor pathogenesis and kinetics. These biologic markers need to be assessed further with other methods of detection and, ideally, in a prospective fashion. More solid conclusions could then be drawn, possibly leading to the use of these markers as potential therapeutic targets in DMM.

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