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ORIGINAL ARTICLE – PERITONEAL SURFACE MALIGNANCY

# A Proposal for Modification of the PSOGI Classification According to the Ki-67 Proliferation Index in Pseudomyxoma Peritonei

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## ABSTRACT

**Background.** Pseudomyxoma peritonei (PMP) is a rare malignancy, classified according to the Peritoneal Surface Oncology Group International (PSOGI) classification, whose response to treatment remains highly heterogeneous within the high-grade (HG) category. Molecular profiling of PMP cases might help to better categorize patients and predict treatment responses.

**Methods.** We studied the Ki-67 proliferation rate and P53 overexpression in tissue samples from our historical cohort of HG-PMP patients. We established as cut-off levels the third quartile of each marker to perform univariate and multivariate Cox regression survival analyses. According to these results, the HG-PMP category was divided into subcategories and a new survival analysis was performed.

**Results.** A total of 90/117 patients with PMP undergoing cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC) were selected for secondary analysis. The survival analysis of the HG-PMP category for preoperative variables showed that a proliferation index defined by Ki-67 >15% is a bad prognostic factor, with a hazard ratio (HR) of 3.20 (95% confidence interval [CI] 1.24–8.25). Accordingly, the HG-PMP group was divided using the Ki-67 15% cut-off. The new PSOGI/Ki-67 variable was an independent prognostic factor for overall survival (OS), with an HR of 3.74 (95% CI 1.88–7.47), and disease-free survival (DFS), with an HR of 4.184 (95% CI 1.79–9.75). The estimated 5-year OS rate was 100%, 70% and 24% for the LG-PMP, HG-PMP ≤15% and HG-PMP >15% groups, respectively ($p = 0.0001$), while the 5-year DFS rate was 90%, 44% and 0%, respectively ($p = 0.0001$).

**Conclusion.** Division of the HG-PMP category of the PSOGI classification, according to the Ki-67 proliferation index, provides two well-defined subcategories, with significant differences in terms of OS and DFS, and hence high prognostic value.

Álvaro Arjona-Sánchez and Ana Martínez-López have contributed equally.

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Pseudomyxoma peritonei (PMP) is a rare malignant disease characterized by the progressive and multifocal accumulation of abundant mucinous tumor tissue in the peritoneal cavity. It is generally associated with a perforated epithelial neoplasm of the appendix.[1] The Peritoneal Surface Oncology Group International (PSOGI) has recently published a consensus statement about the diagnosis and treatment of mucinous appendiceal tumors and PMP, recommending, whenever possible, cytoreductive surgery plus hyperthermic intraperitoneal chemotherapy (CRS + HIPEC) in reference centers.[2]

In the past, the histological classification and definition of PMP has been somewhat challenging because of the confusing and overlapping terminology used to refer to it. After building and reaching an extensive consensus, in 2016 the PSOGI used DELPHI methodology to establish a new classification.[3] The PSOGI classification divides PMP into three groups and excludes acellular mucinous-type lesions from its definition. These three groups are defined as PMP with low-grade histological characteristics (LG-PMP), PMP with high-grade histology (HG-PMP), and PMP with the presence of signet ring cells (SC-PMP). Recently, this classification has been validated due to its capacity to predict overall survival (OS).[4]

The molecular profile of PMP could play an important role in classifying the disease more precisely. Multiple mutations, such as *KRAS*, *GNAS*, *FAT4*, *TGFBR1*, *TP53,* and *SMAD3/4*, have been reported in PMP, which are rather similar to those found in colorectal metastasis, but with some differences.[5] Few studies have been published on the molecular features of PMP and their impact on survival. *KRAS* mutations, the deficit of mismatch-repair (MMR) proteins, the overexpression of P53, and the Ki-67 proliferation index have been studied in PMP but no strong conclusions have been reached regarding the prognosis of the histological aggressivity.[6–8] *KRAS* mutations are more frequent than in colorectal cancer and might reach up to 78% in PMP cases;[5] however, their effect on survival is controversial, with positive and negative associations.[6,7] The *TP53* is a tumor-suppressor gene whose mutation is related with HG tumors or worse. In PMP, the overexpression of P53 proteins has been associated with HG-PMP and lower survival rates in univariate analysis; however, these results encourage further studies on the impact of PMP on oncological outcomes.[8]

Ki-67 is a large (395 kD) nuclear protein, present throughout the cell cycle except for the G0 phase, and is commonly used to assess tumor proliferation. The Ki-67 proliferation index has been proven to be a prognostic factor for a large variety of tumors. Numerous reports on the components of cell cycle machinery have shown the presence of Ki-67 in the G1, S, and G2 phases of the cell cycle but not in the quiescent or resting cells at G0, suggestive of its role as a cell proliferation marker in many cancers.[5] Although analysis of the Ki-67 proliferation index is not routinely performed in clinical practice, this index analysis may provide relevant information on the outcome of PMP treatment, since a high Ki-67 index is generally indicative of poor prognosis.[6,9,10]

In this context, we report herein a prospective analysis of a large set of tissue samples collected from a historical cohort to evaluate the impact of the Ki-67 proliferation index and the overexpression of P53 on the survival and disease-free survival (DFS) of PMP patients treated with CRS and HIPEC. According to the findings of this study, we propose inclusion of these markers in the PSOGI classification in order to achieve a more personalized and accurate subcategorization of PMP patients.

## METHODS

### *Study Population*

The present prospective study has been conducted using collected tissue samples from a retrospective cohort of patients with PMP treated in our unit with CRS and HIPEC from 1997 to 2020. All patients signed the informed consent and the study was approved by our local Ethics Committee. The entire cohort was classified according to the new PSOGI classification, into LG-PMP, HG-PMP, or SC-PMP groupings and cases with acellular mucin.

The first survival analysis was performed on the entire cohort to define the prognostic factors for survival, validating the prognostic value of the PSOGI classification. A selected secondary cohort was generated by including patients with LG-PMP and HG-PMP (with Ki-67 and P53 histological examination). We excluded those patients with acellular mucin due to the small sample size ($n = 3$), signet ring (also due to the small sample size $n = 2$), and HG-PMP patients who had no Ki-67 or P53 examination (Fig. 1). The origin of the PMP was also studied in our population in order to differentiate between appendicular, ovarian, or pancreatic origins. The description of the different origins has been previously reported.[4]

### *Treatment*

As previously reported by our unit,[4] the volume and extension of the tumor was calculated using the Peritoneal Cancer Index (PCI). The completeness of cytoreduction

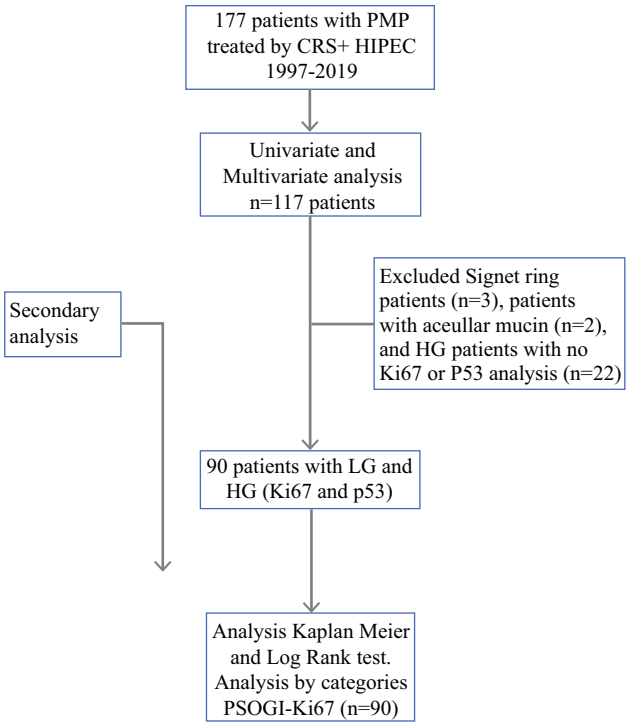



**FIG. 1** Flowchart of the clinical study, with criteria for final secondary cohort selection and analysis applied to the data. *PMP* pseudomyxoma peritonei, *CRS* cytoreductive surgery, *HIPEC* hyperthermic intraperitoneal chemotherapy, *HG* high-grade, *LG* low-grade, *PSOGI* Peritoneal Surface Oncology Group

score (CCS) was quantified (CC0 = no residual tumor; CC1 = residual tumor nodules <0.25 cm; CC2 = residual tumor nodules between 0.25 and 2.5 cm; and CC3 = residual tumor nodules exceeding 2.5 cm). After verifying optimal cytoreduction (CC0–CC1), HIPEC therapy was delivered for 60 min at 41–43°C with one of two chemotherapeutic agents (depending on the origin of the neoplasia) in a 1.5% dextrose solution. Mitomycin C (30 mg/m$^2$) was used for appendicular- and pancreatic-originating cancers, and paclssitaxel (120 mg/m$^2$) was applied for ovarian cancer. We also collected data regarding neoadjuvant and adjuvant chemotherapy. All treatments were decided by consensus of a multidisciplinary team (MDT).

### *Postoperative and Survival Outcomes*

Postoperative morbidity and mortality were quantified up to 30 days after surgery according to the Dindo–Clavien classification,[11] with major complications being defined as third-degree or higher. If a tumor recurrence was suspected, it was confirmed by radiological or surgical image-guided biopsy. After 10 years of follow-up, patient disease status was verified by telephone interview or review of the computerized medical records.

### *Ki-67 and P53 Analysis in High-Grade Pseudomyxoma Peritonei Patients*

HG-PMP patients underwent additional molecular analysis to determine the Ki-67 proliferation rate (%) and the rate of P53 expression (P53%), with the third quartile being established as the cut-off for both (calculating the median and interquartile range [IQR]).

Tissue sections were routinely immunostained using an automated slide processing platform (Leica® BOND™ protocol) on 5 µm sections of the paraffin-embedded blocks. Heat-induced epitope retrieval was performed using BOND Epitope Retrieval Solution 2 for 20 min for all markers. Immunostaining was conducted according to the manufacturer's instructions (Leica Biosystems Newcastle Ltd, Newcastle Upon Tyne, UK). The primary antibodies, Ki-67 (MM1 clone, 1.9 mg/L) and P53 (DO-7 clone, 0.06 mg/L), both mouse anti-human monoclonal antibodies, were used in combination with BOND Polymer Refine Detection. For Ki-67, the labeling index was evaluated by calculating the percentage of positively stained cells in different areas, with a count of at least 500 cells in each section. Positive Ki-67 staining was defined as brownish granular in the nucleus. Staining intensity was not considered relevant. The Ki-67 labeling index was calculated as the number of positive cells/count cells % (Fig. 2).

Positive P53 was defined as a brownish granular stain in the nucleus. Overexpressed P53 was defined when the rate was superior to the third quartile after analysis of the IQR (Fig. 3).

### *Statistical Analysis*

Continuous data are reported as medians (IQR) and means (standard deviation), while categorical variables are reported as percentages. Correlations between categorical and continuous variables were tested using the Mann–Whitney U test. Univariate correlations of categorical variables with survival were tested using a Cox regression analysis, with significance set at $p < 0.05$. Multivariate analysis was performed by choosing those variables with a $p$-value <0.25, which would then be included in the major model. For the definitive model only, the variables that achieved a $p$-value <0.05 were included. Data from living patients have been censored.

In a secondary analysis to identify the independent predictors of OS, we conducted the Cox regression model, mentioned previously, in the HG patient group, where Ki-67% and P53% were categorized according to their cut-off level (Q3). In the second selected cohort, the PSOGI classification was divided according to the significant variables in the previous analysis, and a new regression

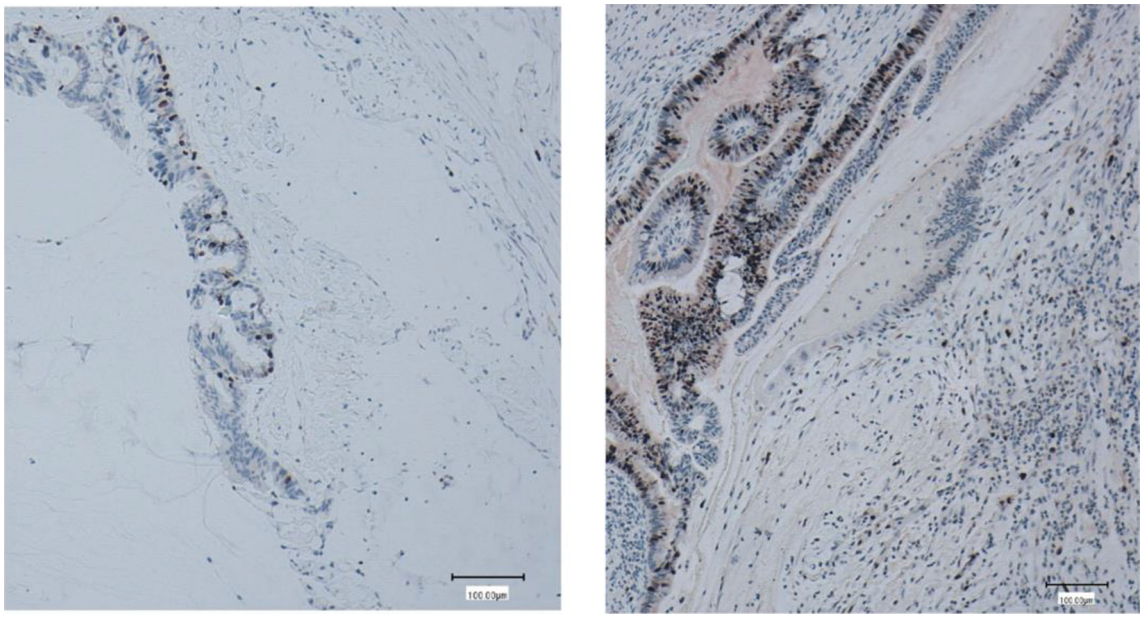


**FIG. 2** Two cases with HG-PMP. Ki-67 (MM1 clone, 1.9 mg/L) antibody was used. Left HG-PMP with Ki-67 ≤15%. Right HG-PMP with Ki-67 >15%. *HG-PMP* high-grade pseudomyxoma peritonei

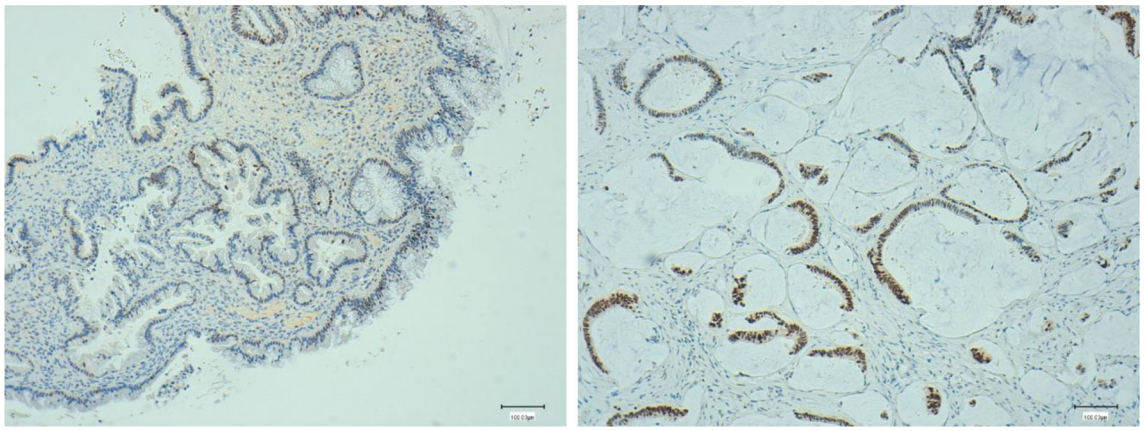


**FIG. 3** Two cases with HG-PMP. Left HG-PMP with P53 <50%. Right HG-PMP with P53 ≥50%. *HG-PMP* high-grade pseudomyxoma peritonei

Cox analysis was then performed for preoperative variables. The survival curves were calculated using the Kaplan–Meier method and the two-tailed log-rank test to analyse the effect of different factors and categories on OS and DFS. OS was calculated from the day of surgery until the death of the patient, regardless of the cause, whereas DFS was calculated from the day of surgery until the diagnosis of a recurrence. Statistical significance was considered when the *p*-value was <0.05. All analyses were performed using the statistical software IBM® SPSS® statistics (version 18.0).

## RESULTS

### *Patient Characteristics*

As shown in Table 1, a total of 117 patients with PMP underwent CRS and HIPEC; 84 were women (71.8%) and 33 were men (28.2%), and the median age at diagnosis was 56 years (48–66). The histological diagnoses according to the PSOGI classification were acellular mucinous PMP (2.6%), LG-PMP (28.9%), HG-PMP (65.8%), and signet ring cell PMP (2.6%). At least one tumor marker (carcinoembryonic antigen, carcinogenic antigen [CA] 19-9, or CA-125) was elevated in 50% of patients at the time of their diagnosis, although this had no impact on OS.

**TABLE 1** Univariate correlations of categorical variables with survival were carried out using a Cox regression analysis, with significance set at $p < 0.05$

| Variable | $N = 117$ | HR OS (95% CI) | $p$-Value |
|---|---|---|---|
| Sex | Female = 84 (71.8%) | 1.73 (0.76–3.9) | 0.18 |
| | Male = 33 (28.2%) | | |
| Age, years | 56 (48–66) | 1.11 (0.78–1.58) | 0.53 |
| Tumor origin | Appendix = 109 (93.2%) | 0.37 (0.05–2.52)[a] | 0.31 |
| | Ovary = 7 (6%) | | |
| | Pancreas = 1 (0.9%) | | |
| Elevated CEA | 36 (33.3%) | 1.6 (0.67–4.03) | 0.27 |
| Elevated CA19.9 | 22 (20.4%) | 1.8 (0.71–4.02) | 0.20 |
| Elevated CA125 | 28 (25.9%) | 0.79 (0.31–2.01) | 0.62 |
| Peritoneal cancer index | 21 (14–31) | 1.9 (0.84–4.30) | 0.11 |
| Neoadjuvant chemotherapy | 38 (33%) | 1.6 (0.72–3.6) | 0.24 |
| Adjuvant chemotherapy | 44 (41.1%) | 2.1 (0.82–5.38) | 0.11 |
| Residual disease CC0 vs. CC1/CC2 | CC0 = 68.4% (78) | 0.32 (0.11–0.93)[b] | 0.003 |
| | CC1 = 25.4% (29) | | |
| | CC2 = 6.1% (7) | | |
| Morbidity (Clavien–Dindo ≥3) | ≥3 = 16.4% (19) | 1.3 (0.63–2.85) | 0.16 |
| | 3a = 4.3% | | |
| | 3b = 2.6% | | |
| | 4a = 5.2% | | |
| | 4b = 5.7% | | |
| | 5 = 2.6% | | |
| PSOGI classification | Acellular mucin: 2% (3) | 3.54 (1.34–18.65)[c] | $p = 0.01$ |
| | LG: 28% (33) | | |
| | HG: 67.5% (79) | | |
| | Signet ring: 1% (2) | | |

[a]Appendix origin as the reference

[b]CC0 as the reference

[c]LG as the reference

*CEA* carcinogenic embryonic antigen, *CA* carcinogenic antigen, *CC* completeness of cytoreduction, *PSOGI* Peritoneal Surface Oncology Group International, *LG* low-grade, *HG* high-grade, *HR* hazard ratio, *CI* confidence interval

*Treatment*

The median intraoperative PCI was 21 (14–31) and 32% of patients received neoadjuvant treatment administered in a median of four cycles (4–6). CC0 was achieved in 68.4% of patients, CC1 in 25.4%, and CC2 in 6.1%. The highest morbidity rate was 18.3% and the postoperative mortality rate was 3.5%. Adjuvant chemotherapy was administered to 41.1% of patients when an HG cancer histology was reported.

*Survival Analysis*

At the time of this study, 76.2% of patients were living, with a median follow-up time of 47 months (14–108). The estimated 5-year OS was 69.1% and the 10-year OS was 47.6%; the median DFS was 22 months (10–79), the 5-year DFS was 48.3%, and the 10-years DFS was 17.1%. After multivariate analysis, the completeness of cytoreduction, major morbidity, and PSOGI classification were related with OS, with a $p$-value <0.05 (Table 2). The estimated

**TABLE 2** Final Cox regression multivariate model

| Variable | HR (95% CI) | $p$-value |
|---|---|---|
| Major morbidity | 3.19 (1.34–7.61) | 0.009 |
| CC0 | 0.20 (0.06–0.68) | 0.007 |
| PSOGI classification (HG) | 4.83 (1.13–20.54) | 0.03 |

The discarded variables were sex, elevated CA19.9, Peritoneal Cancer Index, neoadjuvant chemotherapy and adjuvant chemotherapy

*HR* hazard ratio, *CI* confidence interval, *CC* completeness of cytoreduction, *PSOGI* Peritoneal Surface Oncology Group International, *HG* high-grade

5-year OS for HG-PMP and LG-PMP was 63% and 100%, respectively ($p = 0.004$) (Fig. 4). For CC0, CC1, and CC2, the estimated 5-year OS was 78.3%, 55.6%, and 25%, respectively ($p = 0.01$). For the presence or lack of major postoperative morbidity, the estimated 5-year OS was 62% and 78%, respectively ($p = 0.02$).

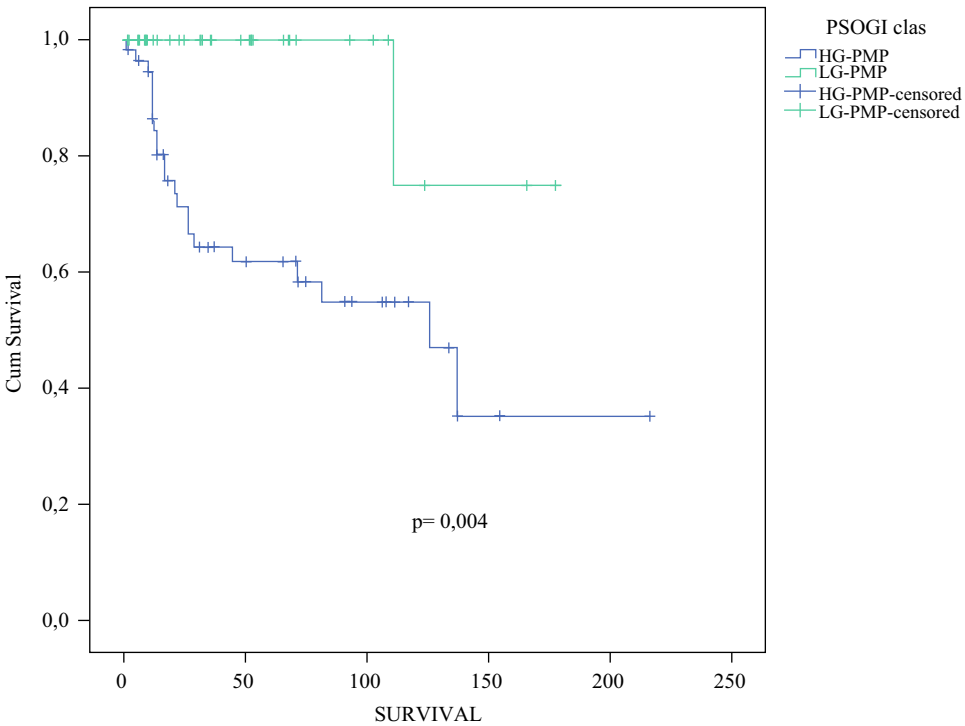
The survival Cox regression analysis of the HG-PMP group for preoperative variables only showed as a significant prognostic factor when the Ki-67 proliferation index was >15%; the P53 (50% cut-off) did not show as a significant prognostic factor for survival (Table 3). The mean OS was 128 ± 17 months for a Ki-67 proliferation index ≤15% and 31 ± 8 months when >15%. The 5 year OS was 72% and 22%, respectively ($p = 0.016$) (Fig. 5).

### *Peritoneal Surface Oncology Group International/Ki-67 Categories Survival Analysis*

After the above analysis, the HG-PMP group was divided into two new subcategories using the Ki-67 15% cut-off (PSOGI/Ki-67). Multivariate analysis showed the new PSOGI/Ki-67 variable as the sole prognostic factor for OS and DFS (Tables 3 and 4). The comparison between the different categories in the PSOGI/Ki-67 classification revealed that the mean OS was 161 ± 14, 128 ± 17, and 31 ± 8 months for the LG-PMP, HG-PMP/Ki-67 ≤15% and HG-PMP/Ki-67 >15% groups, respectively, while the mean DFS was 138 ± 10, 66 ± 9 and 19 ± 5 months, respectively. The Kaplan–Meier and log-rank test showed that the estimated 5-year OS rate was 100%, 70%, and 24% for the LG-PMP, HG-PMP ≤15%, and HG-PMP >15% groups, respectively ($p = 0.0001$) (Fig. 6), while the 5-year DFS rate was 90%, 44%, and 0%, respectively ($p = 0.0001$) (Fig. 7).

## DISCUSSION

PMP is a rare disease that is treated in referral centers with CRS in association with HIPEC. Given its particular biological and histological characteristics, the nomenclature used to describe PMP has not yet been standardized, but the latest classification from a PSOGI consensus[3] has been validated to predict the survival of patients who have undergone CRS + HIPEC.[4] The PSOGI PMP classification defines the HG-PMP group G2 as including one or more of these features: destructive invasion, high cytologic




| Time (months) | 1 | 50 | 100 | 150 | 200 |
|---|---|---|---|---|---|
| Patients at risk HG-PMP | 72 | 37 | 21 | 4 | 1 |
| Patients at risk LG-PMP | 31 | 13 | 8 | 2 | 1 |

**FIG. 4** Overall survival Kaplan–Meier curve and log-rank test between PSOGI categories ($p = 0.004$). *PSOGI* Peritoneal Surface Oncology Group, *Cum* cumulative, *HG* high-grade, *LG* low-grade, *PMP* pseudomyxoma peritonei

**TABLE 3** Univariate Cox regression analysis of preoperative prognostic factors stratified to HG-PMP

| Variable | Univariate analysis [HR (95% CI)] | *p*-value |
|---|---|---|
| Positive lymph nodes | 0.91 (0.28–2.98) | 0.88 |
| **Ki-67 >15%** | **3.20 (1.24–8.25)** | **0.016** |
| P53 >50% | 0.79 (0.28–2.21) | 0.66 |
| Neoadjuvant chemotherapy | 0.85 (0.30–2.45) | 0.77 |
| Elevated CA19.9 | 1.79 (0.47–6.85) | 0.39 |
| Elevated CA125 | 1.02 (0.31–3.34) | 0.96 |
| Elevated CEA | 1.30 (0.35–4.84) | 0.69 |

Multivariate analysis was not performed since only one variable achieved a level of significance <0.25

*HG-PMG* high-grade pseudomyxoma peritonei, *HR* hazard ratio, *CI* confidence interval, *CA* carcinogenic antigen, *CEA* carcinoembryonic antigen

Bold values represent statistically significant variables

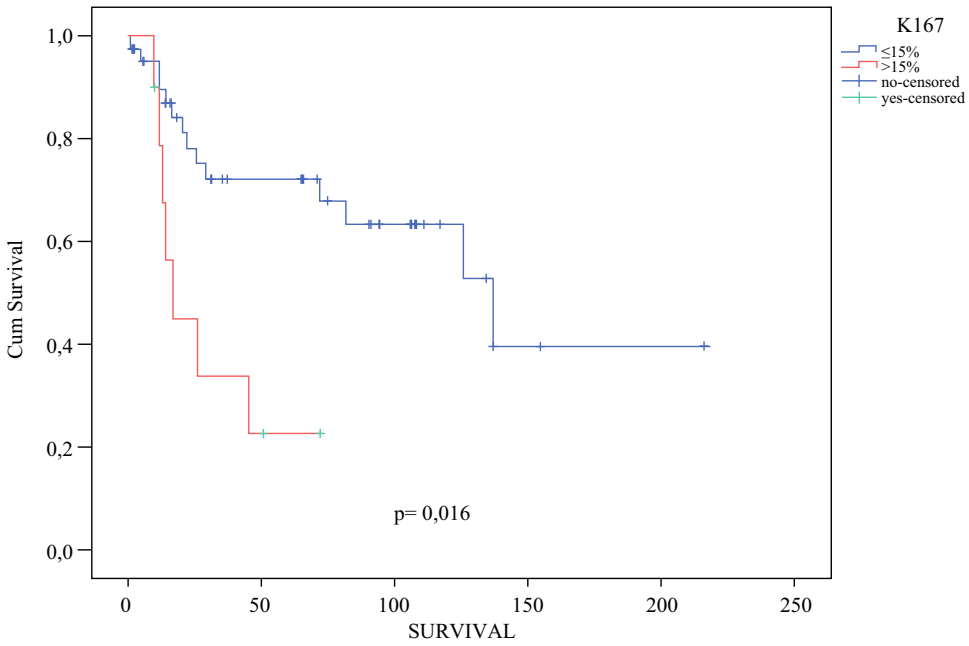



**FIG. 5** Overall survival Kaplan–Meier curve and log-rank test according to percentage of Ki-67 ($p = 0.016$). *Cum* cumulative

| Time (months) | | 1 | 50 | 100 | 150 | 200 |
|---|---|---|---|---|---|---|
| Patients at risk | Ki67≤15% | 39 | 21 | 11 | 2 | 1 |
| Patients at risk | Ki67>15% | 10 | 2 | 1 | 0 | 0 |

grade, high tumor cellularity, angiolymphatic invasion, or perineural invasion.[3] This category includes a large number of patients who might present different outcomes after CRS + HIPEC. This heterogeneity in therapeutic responses urges for a more precise stratification of cases, which remains unmet. On this issue, the characterization of the molecular profile of the tumor might help to allocate patients more accurately.[5] Our study has conclusively shown significantly different outcomes within the HG-PMP category when divided according to the Ki-67 proliferation index (cut-off level of 15%). Thus, a new subcategorization in the HG-PMP group is proposed (Table 5).

The treatment of PMP in recent years is based on radical CRS + HIPEC, obtaining long-term survival when the patient shows an LG-PMP, and complete cytoreduction is achieved.[2,12,13] The PSOGI guidelines for the management

**TABLE 4** Overall survival univariate and multivariate Cox regression analysis of preoperative prognostic factors, including the new classification PSOGI-Ki-67

| Variable | Univariate analysis [HR (95% CI)] | *p*-value | Multivariate analysis [HR (95% CI)] | *p*-value |
|---|---|---|---|---|
| PSOGI-Ki-67 | 3.588 (1.41–9.08) | 0.007 | 3.74 (1.88–7.47) | 0.0001 |
| Neoadjuvant chemotherapy | 1.661 (0.59–4.67) | 0.498 | | |
| Positive lymph nodes | 0.527 (0.22–1.25) | 0.80 | | |
| PCI – median | 1.037 (0.48–2.22) | 0.40 | | |

*PSOGI* Peritoneal Surface Oncology Group International, *HR* hazard ratio, *CI* confidence interval, *PCI* Peritoneal Cancer Index

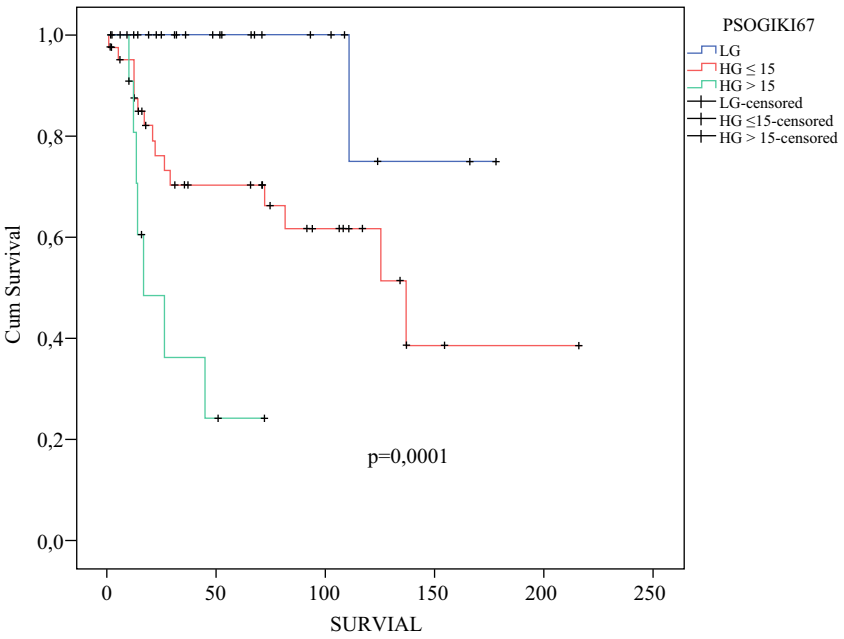



**FIG. 6** Overall survival Kaplan–Meier curve and log-rank test between PSOGI/Ki-67 categories ($p = 0.0001$). *PSOGI* Peritoneal Surface Oncology Group, *Cum* cumulative, *HG* high-grade, *LG* low-grade

| Time (months) | | 1 | 50 | 100 | 150 | 200 |
|---|---|---|---|---|---|---|
| Patients at risk | LG | 26 | 12 | 6 | 2 | 1 |
| Patients at risk | HG ≤ 15 | 42 | 21 | 11 | 2 | 1 |
| Patients at risk | HG >15 | 10 | 2 | 1 | 0 | 0 |

of these patients include a detailed list of recommendations on diagnosis and treatment, but no molecular features have been considered.[2] The poor prognostic factors described in this guideline include age over 75 years, aggressive histology, elevated serum tumor markers, PCI >20, and the involvement of the different abdominal structures. None of these have been identified as independent prognostic factors in our study with the exception of aggressive histology. In that sense, the PMP molecular profile could provide several new prognostic factors that may help to individualize and personalize the treatment of these patients.

Several molecular markers, such as *RAS* mutation status, mutational *TP53*, Ki-67 proliferation index, and the MMR proteins have been studied, albeit scarcely, in patients with PMP.[5,6,8] Ras proteins play a causal role in human cancer. The three *RAS* genes (*HRAS, NRAS,* and *KRAS*) are collectively mutated in one-third of human cancers, where they act as prototypic oncogenes. *KRAS* is mutated in nearly one-quarter of a wide spectrum of cancers, predominantly adenocarcinomas. *NRAS* is mutated in <10% of all tumors and *HRAS* is mutated at a relatively low frequency overall and primarily in squamous epithelial carcinomas.[14] *KRAS* mutations confer resistance to epidermal growth factor receptor inhibitors (cetuximab and panitumumab) in colorectal metastasis.[15] The role of *KRAS* mutations in colorectal peritoneal metastasis has been addressed by two international collaborations, which documented an impairment on survival.[16,17] The only study on

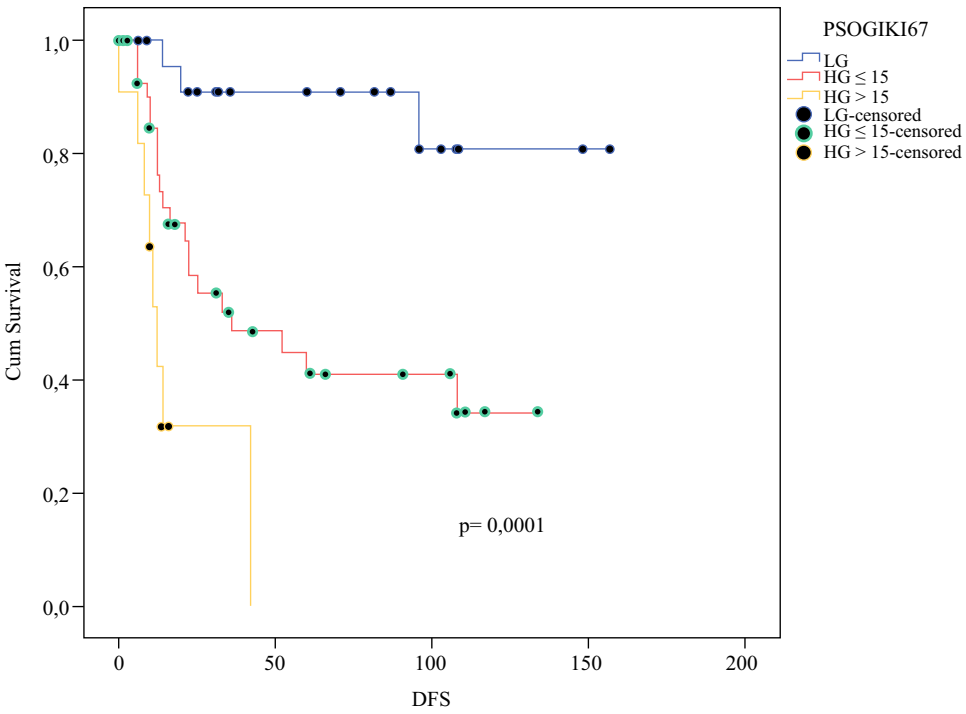



**FIG. 7** Disease-free survival Kaplan–Meier curve and log-rank test between PSOGI/Ki-67 categories ($p = 0.0001$). *PSOGI* Peritoneal Surface Oncology Group, *Cum* cumulative, *HG* high-grade, *LG* low-grade, *DFS* disease-free survival

| Time (months) | 1 | 50 | 100 | 150 | 200 |
|---|---|---|---|---|---|
| Patients at risk LG | 30 | 13 | 7 | 1 | 1 |
| Patients at risk HG ≤ 15 | 41 | 13 | 7 | 0 | 0 |
| Patients at risk HG >15 | 10 | 0 | 0 | 0 | 0 |

**TABLE 5** Disease-free survival univariate and multivariate Cox regression analysis of preoperative prognostic factors, including the new classification PSOGI-Ki-67

| Variable | Univariate analysis [HR (95% CI)] | *p*-value | Multivariate analysis [HR (95% CI)] | *p*-value |
|---|---|---|---|---|
| PSOGI-Ki-67 | 3.588 (1.41–9.08) | 0.007 | 4.184 (1.79–9.75) | 0.001 |
| Neoadjuvant chemotherapy | 0.527 (0.22–1.25) | 0.149 | 0.506 (0.21–1.17) | 0.114 |
| Positive lymph nodes | 1.661 (0.59–4.67) | 0.336 | | |
| PCI – median | 1.037 (0.48–2.22) | 0.925 | | |

*PSOGI* Peritoneal Surface Oncology Group International, *HR* hazard ratio, *CI* confidence interval, *PCI* Peritoneal Cancer Index

*KRAS* mutational status in PMP has shown a higher rate of this mutation than the colorectal carcinomas, reaching up to 100% in some studies.[6] Recently, a molecular review in PMP established a rate of mutation in *KRAS* of around 78% in 19 published studies.[5] No significant differences were identified between HG and LG PMP disease.[18] A recently reported study suggested that *KRAS* mutations were associated with inferior progression-free survival (PFS), but no strong conclusions have been reached for OS.[7]

The deficiency of MMR (dMMR) has been studied in PMP, showing a low incidence of 6.3%, with an increasing risk of death by 9.8-fold;[8] however, other studies have not shown conclusive results, possibly due to their smaller sample sizes.[19,20]

P53 is normally involved in negative regulation of G0 to G1 transition, inhibiting proliferation and inducing programmed cell death. Mutational P53 loses its function of initiating apoptosis, resulting in out-of-control cell growth. The mutation of *TP53* and an overexpressed P53 has been

related with HG-PMP in previous studies showing a strong positivity (more than 50%) in 36% of patients,[6,8,21] or 44% when the cut-off used was 10%.[6] In our study, like Kabbani et al., we used a cut-off of 50%, resulting in 19% of patients with overexpressed P53 in the HG-PMP group.[20] Although in the latter studies the overexpression of P53 has been related with aggressive histology and lower OS,[6,8] in our study the accumulation of P53 was not related with changes in survival rates when the analysis was performed in the HG-PMP group.

The Ki-67 protein is highly expressed in the cell cycle during the G1, S, and G2 phases, but not in the quiescent or resting cell G0 phase, which is suggestive of its role as a cell proliferation marker in many cancers.[9] The Ki-67 labeling index has been included as the main prognostic factor and decision making process for several cancers. This is the case for the gastrointestinal and pancreatic neuroendocrine tumor classifications, which are based on the Ki-67 labeling index.[22] Other tumors, such as breast, prostate, or renal cancer, also use this index to establish the prognosis and help in decision making for the treatment and surveillance of these patients.[9] Recently, one study in diffuse malignant peritoneal mesothelioma has established a cut-off of 9% for the Ki-67 index, which implies an important effect on the prognosis of these patients.[10]

To incorporate the Ki-67 index to the PMP work-up, including not only histological features but also molecular biomarkers to the classification, means a step forward in improving the management of these patients. In PMP, only one study has related the Ki-67 index to lower survival, using a high cut-off of 50%; however, this finding was not significant in multivariate analysis.[8] In our study, the Ki-67 index was an independent prognostic factor for survival, using a cut-off of 15% (based on the Q3 quartile) in the HG-PMP category. This allowed the HG-PMP category to be split into two subcategories: $\leq$15% and >15%. This new subclassification, PSOGI/Ki-67, resulted in an independent factor for OS and DFS, with significant differences between the LG-PMP, HG-PMP/Ki-67 $\leq$15%, and HG-PMP/Ki-67 >15% groups.

Admittedly, our study has some limitations, such as the small sample size, which nonetheless is in accordance with a rare disease such as PMP, and the retrospective collection of the tissue samples. However, the sample ($n = 117$) was large enough to demonstrate significant differences, and the analyses of the Ki-67 proliferation index and P53 overexpression were performed prospectively, using a cohort of preserved tissue samples recently re-evaluated by our pathologists, ranging from the Ronnett classification to the PSOGI classification.[4] Our results set the basis for a larger validation in an international collaborative cohort.

## CONCLUSION

Division of the HG-PMP category, from the PSOGI PMP classification, according to the Ki-67 proliferation index, provides two well-defined subcategories, with significant differences in terms of OS and DFS. This new proposal, and the promising use of molecular markers, should be considered for validation in an international collaborative study.

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