Project: Multicenter validation of a new proposal pseudomyxoma peritonei PSOGI classification including the Ki67 proliferation index.

Coordinator: **Dr. Alvaro Arjona-Sánchez.**

Coordinator Center: Unit of Surgical Oncology, Hospital University Reina Sofia, Cordoba Spain.
Group of research in peritoneal and retroperitoneal oncologic surgery. GE09 Maimonides Biomedical Research Institute, IMIBIC, Cordoba, Spain.

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Collaborator Investigators:

- Dr. Antonio Romero Ruiz (IMIBIC)
- Ana Martínez López (Anatomía Patológica)
- Francisca Valenzuela-Molina (Cirugía General)
- Blanca Rufián-Andújar (Cirugía General)
- Lidia Rodríguez-Ortiz (Cirugía General)
- Melissa Granados-Rodríguez (IMIBIC)

Background:

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 proteins (MMR), the overexpression of P53 and the Ki67 proliferation index have been studied in PMP, but no strong conclusions have been reached regarding the prognosis of the histological aggressivity (6) (7) (8). *KRAS* mutations are more frequent than in colorectal cancer and might reach up to 100% in PMP cases (5). However, their effect on survival is controversial with positive and negative associations (6) (7). The *tP53* is a tumour-suppressor gene whose mutation is related with high-grade tumours or worse. In PMP, the overexpression of P53 proteins has been associated to HG-PMP and lower survival rates in the univariate analysis. However, these results encourage further studies on its impact on oncological outcomes (8).

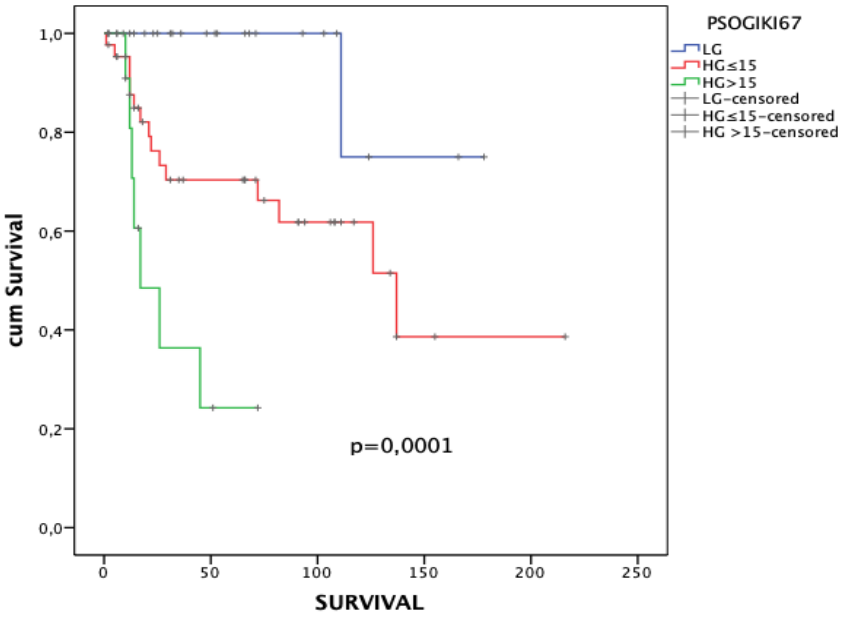
Ki67 is a large (395 KD) nuclear protein, present throughout the cell cycle except for the G0 phase, and is commonly used to assess tumour proliferation. The Ki67 proliferation index has been proven to be a prognostic factor for a large variety of tumours. Numerous reports on the components of cell cycle machinery have shown the presence of Ki-67 in the G1, S and G2 phases of the cell cycle and 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 done 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 (9) (6) (10). In this context, we have performed a prospective analysis over a large set of tissue samples collected from our historical cohort to evaluate the impact of the Ki67 proliferation index and the overexpression of P53 on the survival and disease free survival of PMP patients treated with CRS and HIPEC. According to the findings of this study, we propose inclusion of the Ki67 cut-off in the HG-PMP group creating two subcategories HG-PMP ≤ 15% and HG-PMP > 15%. Both subcategories have obtained significative differences in overall survival and disease free survival, in this local study. Table 1 and 2 and figure 1 and 2. ***(11).***

Variable	**Univariate analysis HR (CI95%)**	**p**	**Multivariate analysis HR (CI95%)**	**p**
PSOGI-KI67	3,588 (1,41-9,08)	0,007	3,74 (1,88-7,47)	0,0001
Neoadjuvant chemo	1,661 (0,59-4,67)	0,498		
Positive lymph nodes	0,527 (0,22-1,25)	0,80		
PCI-med	1,037 (0,48-2,22)	0,40		

Table 1. Overall Survival univariate and multivariate Cox Regression analysis of preoperative prognostic factors including the new classification PSOGI-Ki67. HR: Hazard Ratio.

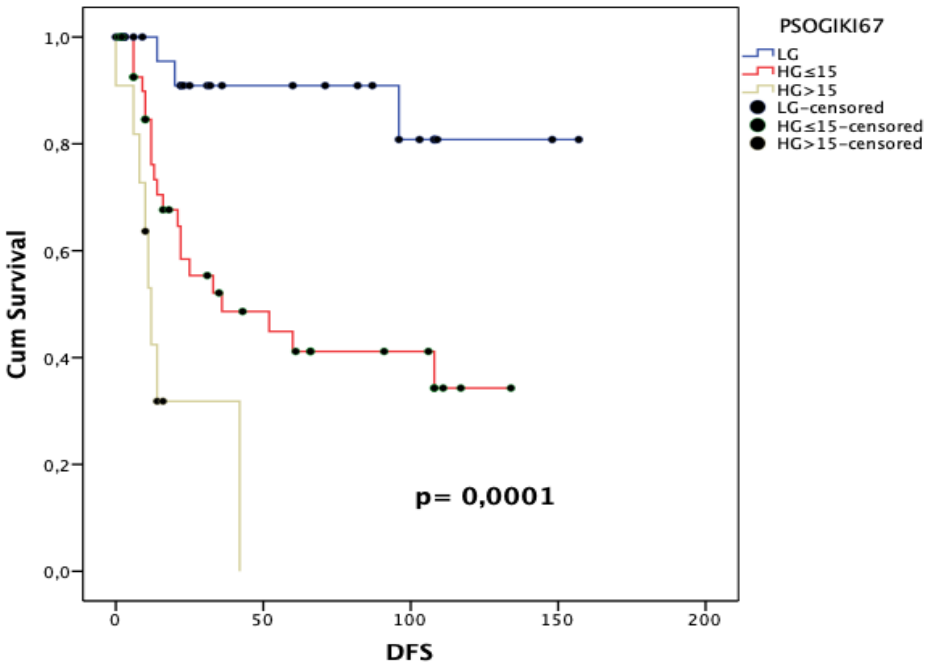
Variable	Univariate analysis HR (CI95%)	p	Multivariate analysis HR (CI95%)	p
PSOGI-KI67	3,588 (1,41-9,08)	0,007	4,184 (1,79-9,75)	0,001
Neoadjuvant chemo	0,527 (0,22-1,25)	0,149	0,506 (0,21-1,17)	0,114
Positive lymph nodes	1,661 (0,59-4,67)	0,336		
PCI-med	1,037 (0,48-2,22)	0,925		

Table 2. Disease Free Survival univariate and multivariate Cox regression analysis of preoperative prognostic factors including the new classification PSOGI-Ki67.



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

Fig 1.Overall Survival Kaplan-Meier curve and Log Rank test between PSOGI-Ki67 categories. P = 0,0001.



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

Fig 2. Disease Free Survival Kaplan-Meier curve and Log Rank test between PSOGI-Ki67 categories, p=0,0001.

Methods:

Study environment:

The present study is included in the PI1901603 study entitled: "Molecular characterization of Pseudomyxoma peritonei and the development of biomarkers and target therapies in a xenograft human model " founding by Carlos III Research Institute 2019. The Regional Ethics Committee code was S1900523, with a favourable dictamen in 26th February 2020.

The start date is estimated in September 2021. The recruitment period will be 12 months.

Study population:

The present prospective study will evaluate the proposed sub-categories of HG-PMP in a multicentre cohort, using a collaborative network of COST action 1701 EuroPMP, and the Spanish Group of Peritoneal Oncologic Surgery (GECOP).

All the patients must have signed the informed consent to analyse the samples of tissue (following the local protocols) and this study is included in the PMP project approved by our local ethics committee.

The number of Hospitals and Centres involved will be defined depending the acceptance to their incorporation in the study. The estimation is about 20 international centres.

Although the sample size in the previous study published by our group has identified significant differences with a sample size of 81 patients. For this multicentre validation the estimation would be N= 86 patients to get a significant differences in the overall survival from 70% (PMP-HG-Ki67≤15%) to 36% (PMP-HG-Ki67 >15%) at 3 years (survivals observed in previous analysis), with a 90% of power and an estimated alpha mistake of 0,05.

The entire cohort will be classified according to the new proposal PSOGI classification in LG-PMP, HG-PMP-Ki67≤15%, HG-PMP-Ki67>15%, or SC-PMP groups.

Treatment

The patients included must be treated in reference Units by cytoreductive surgery and HIPEC. The volume and extension of the tumour was calculated using the Peritoneal Cancer Index (PCI). The completeness of cytoreduction score (CCS) was quantified (CC-0 = no residual tumour; CC-1 = residual tumour nodules less than 0.25 cm; CC-2 = residual tumour nodules between 0.25 cm and 2.5 cm; and CC-3 = residual tumour

nodules exceeding 2.5 cm). After verifying optimal cytoreduction (CC0–CC1), HIPEC therapy was delivered according to local protocols. All treatments must be decided by consensus of a multidisciplinary team (MDT).

Ki-67 analysis in HG-PMP patients

Only the HG-PMP patients must be undergone additional molecular analysis to determine the Ki67 proliferation rate (%). The coordinator centre Ki67 analysis protocol is as follow: *Tissue sections were routinely immunostained using an automated slide processing platform (Leica® BOND™) on 5 µm sections of the paraffin-embedded blocks. Heat induced epitope retrieval was performed with BOND Epitope Retrieval Solution 2 for 20 minutes for all markers.* But the protocol is flexible about The method to calculate the Ki67 proliferation index %, it must be a validated method according with local protocols in each centre included in the study.

For Ki-67, the labelling 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 Ki67 staining was defined as brownish granular in the nucleus. Staining intensity was not considered relevant. The Ki67 labelling index was calculated as the number of positive cells/count cells x 100%. Fig 3.

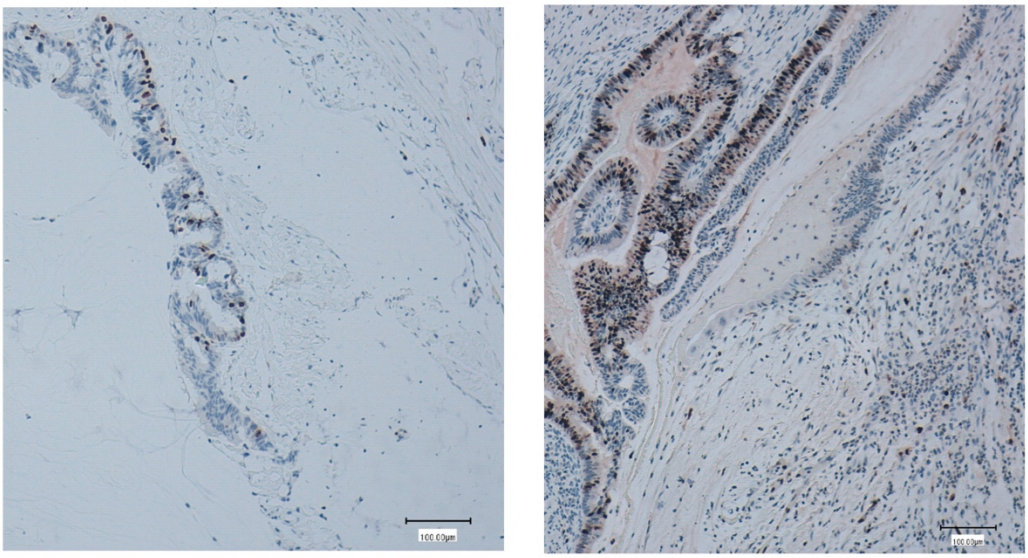


Fig 3. Two cases with HG-PMP. Ki67 (MM1 clone, 1.9 mg/L) antibody was used. Left: HG-PMP with Ki 67 ≤

15%. Right: HG-PMP with Ki67 > 15%.

Statistical analysis

Continuous data are reported as medians (IQR) and means (SD). Categorical variables are reported as percentages. Correlations between categorical and continuous variables were tested with the Mann–Whitney U test. Data from living patients have been censored.

A regression COX analysis was 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 overall survival (OS) and disease-free survival (DFS). OS was calculated from the day of surgery until the death of the patient, regardless of the cause. 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 the analyses were performed using the statistical software IBM® SPSS® statistics (version 18.0).

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