UNIVERSIDAD COMPLUTENSE DE MADRID

FACULTAD DE MEDICINA

TESIS DOCTORAL

Carcinomatosis mucinosa peritoneal de origen apendicular: indicadores biológicos de agresividad.

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Mucinous carcinomatosis peritonei orinating from the appendix: indicators of biological aggressiveness

MEMORIA PARA OPTAR AL GRADO DE DOCTORA

PRESENTADA POR

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UNIVERSIDAD COMPLUTENSE DE MADRID

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TESIS DOCTORAL

CARCINOMATOSIS MUCINOSA PERITONEAL DE ORIGEN APENDICULAR: INDICADORES BIOLÓGICOS DE AGRESIVIDAD.

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“We can do anything we want if we stick to it long enough.”

Helen Keller (1880-1968) writer, disability rights advocate and political activist.

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A mis padres.

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ACRONYM AND ABBREVIATIONS LIST.

AM- Acellular mucin

AUC- Area under the curve

BSA- Body surface area

C-index- Harrell concordance index.

Ca-125- Cancer antigen 125

Ca19-9- Cancer antigen 19-9

CEA- Carcinoembryionic antigen

CRS- Cytoreductive surgery

CT- Computed tomography

DPAM- Disseminated peritoneal adenomucinosis.

ECMO- Extracorporeal membrane oxygenation

ECOG- Eastern cooperative oncology group

GCC- Goblet cell carcinoma

HG-PMP with SRC- High-grade pseudomyxoma peritonei with signet ring cells

HG-PMP-High-grade pseudomyxoma peritonei

HGUGM- Hospital General Universitario Gregorio Marañón, Madrid, Spain

HIPEC- Hyperthermic intraperitoneal chemotherapy

INT- Fondazione IRCCS Istituto dei Tumori, Milan, Italy

LG-PMP- Low-grade pseudomyxoma peritonei.

LN- Lymph node

MACA- Mucinous adenocarcinomas of the appendix

MCP-H- High-grade mucinous carcinoma peritonei

MCP-L- Low-grade mucinous carcinoma peritonei

MDT- Multidisciplinary meeting

MRI- Magnetic resonance imaging

NCDB- National Cancer Database

NEC- Neuroendocrine carcinoma

NET- Neuroendocrine tumours

PCI- Peritoneal carcinomatosis index

PD- Peritoneal dissemination

PMCA- Peritoneal mucinous carcinomatosis.

PMCA-I/D- Peritoneal mucinous carcinomatosis with inconsistent or discordant features

PMI- Peritoneal Malignancy Institute

PMP- Pseudomyxoma peritonei

RCT- Randomized controlled trials

SCT- Systemic chemotherapy

SRC- Signet ring cells

WHO- World Health Organization

LIST OF PAPERS.

This thesis is based on the following papers:

I. Defining stage in mucinous tumours of the appendix with peritoneal dissemination: the importance of grading terminology: systematic review.

Martín-Román L, Lozano P, Vásquez W, Palencia N, Gómez Y, Fernández-Aceñero MJ, González-Bayón L.

BJS Open. 2021 Jul 6;5(4):zrab059. doi: 10.1093/bjsopen/zrab059. PMID: 34355239; PMCID: PMC8342933.

II. Which classification system defines best prognosis of mucinous neoplasms of the appendix with peritoneal dissemination: TNM vs PSOGI?

Martín-Román L, Lozano P, Gómez Y, Fernández-Aceñero MJ, Vasquez W, Palencia N, González-Bayón L.

J Clin Pathol. 2023 Apr;76(4):266-273. doi: 10.1136/jclinpath-2021-207883. Epub 2021 Nov 1. PMID: 34725195

III. Validation of a Nomogram to Predict Recurrence in Patients with Mucinous Neoplasms of the Appendix with Peritoneal Dissemination After Cytoreductive Surgery and HIPEC.

Martín Román L, Lozano P, Baratti D, Kusamura S, Deraco M, Vásquez W, González Bayón L.

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INTRODUCTION

Pseudomyxoma peritonei (PMP) is a rare clinical syndrome characterized by gradual mucin accumulation throughout the peritoneal cavity according to the redistribution phenomenon. Presently, the most frequent origin of PMP is acknowledged to be a perforated mucinous neoplasm of the appendix. Due to its indolent clinical course, it was traditionally diagnosed by the incidental discovery of a "jelly belly" during a laparotomy which already represents an advanced stage of the disease.

Several features make PMP an unique pathology. Firstly, the lack of infiltrative and invasive neoplastic cells is one the main characteristics which would initially surprise and confuse pathologists leading to the vast amount of confusing terminology surrounding this pathological entity. As a matter of fact, it was not recognized to be a malignant entity until recently. However, it was known that if left untreated, the disease would ultimately be fatal due to the accumulation of mucinous tumor total occupation of the peritoneal cavity which led to abdominal distension, intestinal obstruction, malnutrition and cachexia. Secondly, there was a wide range in terms of outcomes of these patients. Once cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (CRS+HIPEC) was established to be the gold standard treatment for these patients, treating physicians observed that, despite optimal treatment, some patients had excellent long term results, while others showed dismal prognosis.

Advances in histopathological evaluation led to the discovery of different histological subgroups. PMP was found to be a disease ruled by heterogeneity both under the

microscope and in terms of survival outcomes. Many study groups then established the well-known relationship between histology and prognosis and several classification systems were proposed; some supporting two prognostic groups, others, three. These derived in the Peritoneal Surface Oncology Group International (PSOGI) consensus in 2016, where experts voted to standardize the pathologic reporting of PMP. In this case, a four-tiered classification system was proposed.

The main aim of this research project was to:

1. Perform a literature review to understand the development of the new classification systems.
2. Investigate the prognostic implications of the recent PSOGI classification and the 8th edition of the AJCC in a cohort of PMP patients treated at the Hospital General Universitario Gregorio Marañón (HGUGM).
3. Develop and externally validate a nomogram to predict the risk of recurrence of PMP patients after CRS+HIPEC treatment.

MATERIALS AND METHODS

This research project has been structured in the form of three papers: the first paper consisted of a systematic literature review which served the purpose of obtaining an historic background into the evolution of the different classification systems; the second paper, evaluated the prognostic impact of the most current classification systems in our cohort; and the third paper aimed to design and externally validate a prognostic model that would predict recurrence at 1, 3 and 5 years after CRS+HIPEC combining factors that were observed to be correlated with DFS.

The literature review was performed following the PRISMA guidelines.

The prognostic implications of the PSOGI and 8th edition of the AJCC classifications were evaluated on the cohort of patients treated for PMP at the HGUGM. Survival analysis evaluated impact of each classification on OS and DFS, and the concordance index was used to evaluate their predictive power.

The nomogram was derived from the cohort of patients from HGUGM. The model was validated on the cohort of patients of Fondazione IRCCS Istituto Nazionale dei Tumori (INT) using the concordance index and calibration plots.

RESULTS

Thirty-eight studies were included in the systematic review in paper I. Ronnett´s classification was the most common classification system (9 studies), followed by the 7th or 8th edition of the AJCC (7 studies) and by the PSOGI classification (6 studies). Results from the systematic literature review conducted in paper one collected enough evidence to favour the four-tiered classification system proposed by the PSOGI classification on the basis of the pathological descriptions provided.

The results from paper II revealed that both the PSOGI and 8th edition of the AJCC classification correlated both with OS (PSOGI: HR 10.2, p=0.039; AJCC: HR 7.7, p=0.002) and DFS (PSOGI: HR 12.7, p=0.001; AJCC: HR 3.7, p<0.001) in our cohort of PMP patients. However, its discriminative capacity did not reach adequate levels with concordance index values lower than 0.7.

In paper III, results from the cox regression model revealed that the PSOGI classification, PCI and tumour marker elevation were all correlated with DFS. These factors were combined into a prediction model and the performance of this model was evaluated on the validation cohort from INT. The prediction capacity of the new model was higher than that of the PSOGI classification alone (concordance indexes of 0.702 vs 0.610, respectively in the validation cohort). Also, the nomogram approximated the perfect model in the calibration plots at 3- and 5-year DFS.

CONCLUSIONS

Overall, the major task accomplished by the PSOGI classification was to establish an universal language to refer to PMP. However, its prognostic implications are still under investigation. Adequate stratification of patients into distinguished prognostic groups is one of the keystones for the development of efficient treatment and surveillance guidelines.

The model developed in paper III proved that the combination of prognostic factors predicted survival outcomes better than histology alone. However, large multicentre studies are required to confirm the model´s validity and to improve its prediction accuracy by including molecular markers that are still being evaluated (i.e., Ki67 proliferation rate).

RESUMEN

RESUMEN

INTRODUCCIÓN

El pseudomixoma peritoneal (PMP) es un síndrome clínico raro caracterizado por la acumulación gradual de mucina en toda la cavidad peritoneal de acuerdo con el fenómeno de redistribución. Actualmente, se reconoce que el origen más frecuente del PMP es una neoplasia mucinosa perforada del apéndice. Debido a su curso clínico indolente, tradicionalmente se diagnosticaba por el descubrimiento incidental de una "jelly belly" durante una laparotomía; esto representa una etapa avanzada de la enfermedad.

Varias características hacen que el PMP sea una patología única. En primer lugar, la falta de células neoplásicas infiltrativas e invasivas es una de las principales características que inicialmente sorprendería y confundiría a los patólogos, lo que lleva a la gran cantidad de terminología confusa que rodea a esta entidad patológica. De hecho, no se reconoció como una entidad maligna hasta hace poco tiempo. Sin embargo, se sabía que si no se trataba, la enfermedad sería finalmente fatal debido a la acumulación de tumor mucinoso con la ocupación total de la cavidad peritoneal que producía distensión abdominal, obstrucción intestinal, desnutrición y caquexia. En segundo lugar, existía gran variación en cuanto a los resultados oncológicos de estos pacientes. Una vez que la cirugía citorreductora y la quimioterapia intraperitoneal hipertérmica (CRS+HIPEC) se establecieron como el tratamiento de referencia para estos pacientes, los médicos tratantes observaron que, a pesar del tratamiento óptimo, algunos pacientes tenían excelentes resultados a largo plazo, mientras que otros mostraban un pronóstico sombrío.

Los avances en la evaluación histopatológica llevaron al descubrimiento de diferentes subgrupos histológicos. Se descubrió que el PMP era una enfermedad regida por la heterogeneidad tanto bajo el microscopio como en términos de resultados de supervivencia. Muchos grupos de estudio establecieron entonces la relación entre histología y pronóstico y se propusieron varios sistemas de clasificación; unos apoyando dos grupos de pronósticos, otros, tres. Estos derivaron en el consenso del Peritoneal Surface Oncology Group International (PSOGI) en 2016, donde los expertos votaron para estandarizar el informe patológico de PMP. En este caso, se propuso un sistema de clasificación de cuatro niveles.

Los objetivos principales de esta tesis doctoral son:

1. Realizar una revisión bibliográfica para comprender el desarrollo de los nuevos sistemas de clasificación.
2. Investigar las implicaciones pronósticas de la reciente clasificación PSOGI y la 8ª edición del American Joint Committee on Cancer (AJCC) en una cohorte de pacientes con PMP atendidos en el Hospital General Universitario Gregorio Marañón (HGUGM).
3. Desarrollar y validar externamente un nomograma para predecir el riesgo de recurrencia de los pacientes con PMP después del tratamiento con CRS+HIPEC.

MATERIAL Y MÉTODOS

La memoria de la tesis se ha estructurado en forma de tres artículos: el primero consistió en una revisión sistemática de la literatura que sirvió para obtener antecedentes históricos sobre la evolución de los diferentes sistemas de clasificación; el segundo trabajo evaluó el impacto pronóstico de los sistemas de clasificación más modernos en nuestra cohorte; y el tercer artículo tuvo como objetivo diseñar y validar externamente un modelo de pronóstico que

predeciría la recurrencia a 1, 3 y 5 años después de CRS+HIPEC combinando factores que se observó que estaban correlacionados con supervivencia libre de enfermedad (SLE).

La revisión de la literatura se realizó siguiendo las guías PRISMA.

Las implicaciones pronósticas del PSOGI y la 8.ª edición de las clasificación del AJCC se evaluaron en la cohorte de pacientes tratados por PMP en el HGUGM. El análisis de supervivencia evaluó el impacto de cada clasificación en supervivencia global (SG) y SLE, y se utilizó el índice de concordancia para evaluar su poder predictivo.

El nomograma se derivó de la cohorte de pacientes del HGUGM. El modelo se validó en la cohorte de pacientes de la Fondazione IRCCS Istituto Nazionale dei Tumori (INT) utilizando el índice de concordancia y gráficos de calibración.

RESULTADOS

Treinta y ocho estudios fueron incluidos en la revisión sistemática en el primer artículo. La clasificación de Ronnett fue el sistema de clasificación más común (9 estudios), seguida por la 7.ª y 8.ª edición del AJCC (7 estudios), y por la clasificación PSOGI (6 estudios). Los resultados de la revisión sistemática de la literatura realizada en el artículo mostraron suficiente evidencia para favorecer el sistema de clasificación de cuatro niveles propuesto por la clasificación PSOGI sobre la base de las descripciones patológicas proporcionadas.
Los resultados del segundo artículo mostraron que tanto el PSOGI como la 8.ª edición de la clasificación AJCC se correlacionan tanto con SG (PSOGI: HR 10,2, p=0,039; AJCC: HR 7,7, p=0,002) como con SLE (PSOGI: HR 12,7, p= 0,001; AJCC: HR 3,7, p<0,001) en

nuestra cohorte de pacientes con PMP. Sin embargo, su capacidad discriminatoria no alcanzó niveles adecuados con valores del índice de concordancia inferiores a 0,7.

En el tercer artículo, los resultados del modelo de regresión de Cox mostraron que la clasificación PSOGI, la PCI y la elevación del marcador tumoral se correlacionaron con la DFS. Estos factores se combinaron en un modelo de predicción y el rendimiento de este modelo se evaluó en la cohorte de validación de INT. La capacidad de predicción del nuevo modelo fue superior a la de la clasificación PSOGI sola (índices de concordancia de 0,702 vs 0,610, respectivamente en la cohorte de validación). Además, el nomograma se aproximó al modelo perfecto en las parcelas de calibración en DFS de 3 y 5 años.

CONCLUSIONES

En general, la principal tarea realizada por la clasificación PSOGI fue establecer un lenguaje universal para referirse a PMP. Sin embargo, sus implicaciones pronósticas aún están bajo investigación. La estratificación adecuada de los pacientes en grupos pronósticos distinguidos es una de las claves para el desarrollo de pautas de tratamiento y vigilancia eficientes.

El modelo desarrollado en el tercer artículo demostró que la combinación de factores pronósticos predijo mejores resultados de supervivencia que la histología sola. Sin embargo, se requieren grandes estudios multicéntricos para confirmar la validez del modelo y mejorar la precisión de predicción mediante la inclusión de marcadores moleculares que aún se están evaluando (por ejemplo, la tasa de proliferación de Ki67).

INTRODUCTION

1. INTRODUCTION.

Pseudomyxoma peritonei (PMP) is rare clinical entity characterized by the progressive accumulation of mucin throughout the peritoneal cavity, most likely, resulting from a perforated mucinous neoplasm of the appendix (1), (2). Classically, it was diagnosed with the finding of a "jelly belly" at laparotomy. The term PMP was initially introduced by Wert (3) in 1884 describing a case of mucinous ascites in the setting of a mucinous ovarian neoplasm. In 1901, Frankel described a similar case arising from a cyst or mucocele from the appendix (4). The term PMP has been historically applied to any condition that caused extensive accumulation of mucin within the peritoneal cavity. Consequently, tumours from various sites with different biologic behaviours were grouped together. This resulted in ongoing confusion surrounding diagnosis and optimal management of patients with PMP, specially in women where differential diagnosis with ovarian mucinous neoplasm is currently still a challenge. Therefore, experts in PMP soon tried to clarify the ambiguity surrounding this entity by stating that PMP should only be applied to describe the "prognostically homogenous group of cases characterized by histologically benign peritoneal tumours that are frequently associated with an appendiceal mucinous adenoma" (5).

From a clinical point of view, there was much debate on debate whether to consider PMP a benign or malignant entity. In most cases, PMP showed an indolent growth pattern without pathological features of invasion and seldomly disseminated via the lymphatic or hematogenous pathways. Consequently, up until recently, PMP was not considered to be a malignant entity, but it was known that if left untreated, the disease would eventually be fatal secondary to the build-up of mucinous tumour within the abdomen leading to emaciation and impossibility of nutrition (2). Surgical treatment was indicated late and

generally consisted of evacuation of mucin with usual macroscopic tumour residue. Some patients had to undergo surgery every 1-2 years to evacuate mucin, until a deadly situation was reached in which the mucin could no longer be evacuated due to the formation of solid tumour nodules over visceral organs.

Nowadays, experts recommend the term of PMP to be used as a clinical, radiologic or syndrome descriptor and not as a histopathologic diagnosis (6). Advances in pathology and molecular biology have allowed a better understanding of this pathology in terms of its biological behaviour and prognosis. This has subsequently resulted in evolution in terminology and new classification systems (6).

1.1 INCIDENCE

1.1.1 Incidence of mucinous appendiceal neoplasms.

Appendiceal neoplasms can be broadly categorized into non-epithelial and epithelial. The 2019 World Health Organization (WHO) classification included neuroendocrine neoplasms (neuroendocrine tumours (NET) and neuroendocrine carcinomas (NEC)), colonic-type adenocarcinoma, mucinous neoplasm and goblet cell adenocarcinoma (GCC) into the epithelial group and sarcomas and lymphomas into the non-epithelial group (7). NET represent the most frequent primary tumour originating from the appendix (8). However, several population based studies have identified mucinous neoplasms to be the most frequent epithelial pathological subtype representing from 23 to 55% of all appendiceal carcinomas (9), (10), (11). Two population based studies found the incidence of appendiceal mucinous neoplasms to be from 0.4 to 2.8 cases per 1,000,000 persons-year (12), (13).

Special consideration is warranted in patients with one episode of complicated appendicitis managed conservatively. The risk of an underlying malignant neoplasm of the appendix found during interval appendectomies was approximately around 0.7-3% (14), (15), (16). Alarming rates have been described in a recent randomized clinical trial performed in the Netherlands where an appendiceal mucinous neoplasm was found in 20% of cases above the age of 40 with a previous periappendicular abscess (17). A meta-analysis found a pooled prevalence of appendiceal neoplasms at interval appendicectomy after an episode of complicated appendicitis of 11% (95% CI 7-15%)(18). In this setting, the most frequent type of appendiceal primary tumour found was mucinous neoplasm (43%). A recent population study based on the SEER database identified that the risk of appendiceal adenocarcinoma or PMP was significantly higher in patients with periappendicular abscess

(OR 15.05, p<0.0001, perforated appendicitis (OR 4.09, p=0.0018) and patients above the age of 40 (OR 26.46, p<0.0001) (19). This population based study confirmed once again, that the most frequent histological subtype found at interval appendicectomy after an episode of complicated appendicitis was a mucinous neoplasm, whereas NET were most frequently found after an episode of uncomplicated appendicitis.

1.1.2 Incidence of pseudomyxoma peritonei.

PMP is a rare peritoneal malignancy with an estimated around 1-3 per million population per year (20). A large population based study carried out in the Netherlands based on nationwide pathology database (Pathologic Anatomic National Automatic Archive or PALGA) found the incidence of PMP to be estimated at 1 per million inhabitants per year, with a certain predominance for women (14). The observed incidence of primary appendiceal neoplasms was 0.9 cases per 1,000,000 inhabitants per year with a 20% progression rate to PMP in mucinous subtype (14). On the other hand, a large population based study performed in the United States from 1973 to 1998 found lower incidence rates of primary appendiceal neoplasms. The age-adjusted incidence of primary appendiceal lesions according to the Surveillance, Epidemiology and End-Results (SEER) program of the National Cancer Institute was found to be 0.12 cases per 1,000,000 people per year (9). An updated study including patients up until 2007 as published by Turaga and colleagues revealed an increase in incidence over the years possibly secondary to improvement in detection and recognition of this entity (10) with an overall age-adjusted incidence of 6 cases/1,000,000 persons-year.

1.2 PATHOPHYSIOLOGY OF PERITONEAL DISSEMINATION.

The peritoneum is considered to be a large serous organ, macroscopically formed by a thin elastic structure covering the interior surface of the abdominal wall (parietal peritoneum) and the surface of the intraperitoneal organs (visceral peritoneum) (21). Its functions include maintenance of the homeostasis of peritoneal fluid, regulation of inflammatory response and fibrin formation (21).

The exact mechanisms through which cancer cells attach to the peritoneum causing peritoneal carcinomatosis remain unclear. Current models indicate that it is a stepwise approach starting with the seeding of cells from the primary tumour once there is serosal invasion or perforation (primarily or iatrogenic). Consequently, transcoelomic spread occurs mainly in a clockwise direction following the flow of the peritoneal fluid dictated by the effects of gravity, the peristaltic movement of the gastrointestinal tract and the negative pressure produced by the diaphragmatic muscles (22). The second step involves the invasion of the submesothelial tissue to develop a tumour growth colony or peritoneal implant. Submesothelial tissue invasion is mediated by the interaction of free cancer cells with mesothelial cells via adhesion receptors (CD44, integrins, selectins…) and the production of cytokines (interleukins, epithelial growth factor…) which trigger mesothelial cells to contract exposing the submesothelial basement membrane. Free cancer cells are then able to adhere and invade the submesothelial layer facilitated by the action of metalloproteases (transmesothelial route) (22), (23).

In the context of PMP, the most widespread model is that an initial mucinous neoplasm of the appendix causes a continuous mucin production. Mucin build-up within the appendiceal lumen leads to obstruction and distension of the appendix which consequently ruptures

allowing mucinous material with or without neoplastic epithelial cells to access the peritoneal cavity (22). The hallmark of PMP is that its characterized by the redistribution phenomenon, a term initially introduced by Sugarbaker (24) to describe how large volumes of tumour will be present at predetermined anatomical sites and absent from others. Following this, the route through which peritoneal implantation takes place in PMP is via the translymphatic route meaning that mucinous deposits will form at areas of the peritoneal cavity characterized by the presence of lymphatic stomata (i.e greater omentum, appendices epiploicae of the colon, inferior surface of the diaphragms, falciform ligament, Douglas pouch and small bowel mesentery) (22). Lymphatic stomata are areas or gaps between mesothelial cells that are communicated with lymphatic capillaries where peritoneal fluid reabsorption takes place (25). The other main conditioning factor of the redistribution phenomenon is gravity, explaining why tumour aggregates are commonly found in the pouch of Douglas, the paracolic gutters and retrohepatic space (26).

1.3 DIAGNOSIS

1.3.1 Clinical features

PMP progresses in an indolent manner, therefore patients tend to be asymptomatic or complain of diffuse and unspecific abdominal pain. Because of this, it is often found incidentally in patients undergoing investigations or surgery for other reasons. Symptoms occur as the disease progresses and, once present, they reveal an advanced stage of the disease. Localized disease is frequently found in the setting of acute appendicitis, or investigations for right iliac fossa pain that may reveal a pelvic mass secondary to a distended appendix or localized mucinous deposits. In cases of advanced disease, the classical clinical picture of a "jelly belly" can be observed. In these cases, patients show an increase in abdominal girth caused by the accumulation of mucinous ascites. Often, this is accompanied by onset inguinal and umbilical hernias that appear as a consequence of increased intraabdominal pressure. Episodes of bowel obstruction may occur due to the involvement of the small bowel and usually represents final stages of the disease (20).

In 2000, Esquivel and Sugarbaker (27) evaluated the most common presentations in their series of 217 patients. The most common presentation was found to be acute appendicitis (27%), followed by abdominal distension (23%), vague abdominal pain (17%) or diagnosis of new onset hernia (14%).

1.3.2 Imaging modalities and endoscopy.

Many patients end up having endoscopic evaluation of the digestive track as part of the investigations carried out for vague abdominal symptoms. Endoscopies are inevitably normal although in the rare occasion where mucin can be seen seeping through the appendix orifice can be pathognomonic. In the setting of PMP or an incidental finding of a mucinous neoplasm, the European and American guidelines recommend endoscopic evaluation in order to rule out the presence of synchronous colorectal neoplasia (28), (29). A German multicentre study described rates of synchronous colorectal neoplasia in 8.9% of patients with a mucinous neoplasm of the appendix (11).

The most commonly used diagnostic modality for PMP is computed tomography (CT) scan of the chest, abdomen and pelvis with intra-venous and oral contrast (30). The PMP expert panel agreed with a 94% consensus that CT-imaging is the preferred preoperative imaging modality (28). The benefits of CT imaging include its accessibility, cost and easier interpretation by radiologists not experienced in peritoneal malignancies.

The classical radiologic features of PMP include omental caking, mucinous ascites and scalloping of the liver. These features however, are only present in the setting of advanced disease. CT imaging can also reveal a mucocele of the appendix, which refers to the radiological image of a mucin-filled and distended appendix which may be accompanied by peripheral calcification. In the setting of acute appendicitis, a retrospective study including 65 patients reported a sensitivity of 95% in the detection of appendiceal tumours in this context using CT imaging when taking morphologic criteria (i.e cystic dilation or presence of a soft tissue mass) or an appendiceal diameter greater than 15mm (31).

On the other hand, the detection of peritoneal disease represents an ongoing challenge with current imaging techniques. The detection of peritoneal deposits is determined by their size

and location. The sensitivity of CT to detect lesions greater than 5cm ranges from 59 to 94% but drops to 19 to 28% in lesions smaller than 1 cm and to 11 to 28% in lesions smaller than 0.5cm (28), (32). Therefore, CT underestimates the extent of peritoneal disease later on encountered intraoperatively, to the extent that the PCI calculated intraoperatively doubled that determined preoperatively by CT in a retrospective study (32) or increased by a median of 12 points as per the findings of another retrospective study (33). As mentioned previously, sensitivity also depends on the involved area. One retrospective study found that lesions in the ileocaecal area had the lowest sensitivity for detection (11-28%), followed by lesions in the right subdiaphragmatic area (11-22%), and the omentum and transverse colon (25%). The detection rate of lesions in the small bowel and/or its mesentery had a range of 18 to 55% (34).

Recently, the use of diffusion-weighted magnetic resonance imaging (DW-MRI) has shown to improve both sensitivity and specificity in the detection of peritoneal metastasis with a sensitivity of 85-90% in cases of peritoneal deposits smaller than 1cm (35). A study published by Low et al. comparing preoperative MRI with CT reported that MRI predicted tumour volume accurately in 91% of the patients and CT only in 50% (36). Additionally, MRI was able to detect disease in the small bowel in 92% of the cases whereas CT in only 48% (36). These findings were backed up by the results from a larger study that however also concluded that MRI requires experienced radiologist for its accurate interpretation, specially when evaluating the small bowel (37).

The presence of disease in certain locations is associated with worse prognosis due to the reduced likelihood of achieving optimal CRS. These locations are the small bowel and/or its mesentery, the porta hepatis and hepatoduodenal ligament, ureteric encasement, biliary obstruction, presence of gross ascites (38). Such observations led to the study and

development of radiologic scores that would aid predicting the resectability of the disease. One example of these is the simplified preoperative assessment for appendix tumour (SPAAT) score (39). This score was developed to predict the ability of complete CRS in patients with low-grade peritoneal disease originated the appendix based on the following findings on preoperative CT imaging: scalloping of liver, pancreas, spleen or portal vein (1 point each) and the presence or absence of mesenteric foreshortening of the small bowel (from 0 to 3 points). The score was externally validated on a larger cohort, and scores <3 would accurately predict a complete CRS in 97.1% of the cases (39). Another example is the simplified radiographic score (SRS) (40). This score takes into consideration the thickness of the disease (measured in milimeters) in 5 regions of the upper abdomen (inferior vena cava to portal vein, right hepatic lobe to left hepatic lobe, left hepatic lobe to lesser sac, Spiegel lobe to left hepatic lobe, Spiegel lobe to right crus). A sum greater than 28mm predicts incomplete CRS with a positive predictive value of 85% and negative predictive value 59% in their validation cohort (40). The discriminative capacity of these scores has been further investigated by other study groups. The usefulness of the SPAAT score was questioned after observing a positive predictive value of 50% with a sensitivity of 40%, even though the cut-off of three was associated with optimal CRS in the binary regression model (33). A second study group aimed to evaluate the utility of both scores and observed positive predictive values of the SPAAT and SRS scores of 67% and 75%, respectively (41).

These scoring systems, however, have not been widely adopted and the reporting of radiology imaging of patients with peritoneal disease is variable. With the aim of standardizing reporting of patients with peritoneal disease, the Peritoneal Malignancy Institute (PMI) in Basingstoke proposed the PAUSE method (42). This acronym stands for: P: PCI score; A: abdominal wall and ascites; U: unfavourable sites of disease (periportal,

root of the mesentery, ligament of Treitz, pelvic side wall disease and disease involving the sacrum); S: small bowel and mesenteric disease and E: extraperitoneal disease. Following this method, the radiology report will contain information that will aid in the selection of patients that will benefit from CRS+HIPEC at the MDT as well as facilitating research across different centres.

1.3.3 Tumour markers

The prognostic implication of tumour markers in PMP have been broadly studied by different study groups over the course of time. The tumour markers associated to PMP are: carcinoembryonic antigen (CEA), cancer antigen 19-9 (Ca19-9) and cancer antigen 125 (Ca-125). The results obtained have not been homogeneous across the different study groups, therefore the meaning of preoperative tumour marker elevation is still difficult to interpret. However, current guidelines (28), (29) recommend tumour markers to be included as part of the preoperative work-up for these patients.

The elevation of all three tumour markers has been associated with a higher volume of disease (43) and with reduced probability of achieving optimal CRS (44), (45). The study group from the peritoneal malignancy unit in Milan concluded in 2013 that CEA, Ca19-9 and Ca-125 should be used as predictors of optimal CRS (45) and reported that Ca19-9 and Ca-125 were more powerful predictors of prognosis than histology. Similar findings were observed by the study group in Sydney (46). The elevation of tumour markers preoperatively have also been associated with shorter OS and DFS outcomes (47), (44). CEA elevation has been associated with shorter DFS (48), other study groups observed Ca19-9 to be predictor of shorter DFS (49), (46).

1.3.4 Preoperative histological evaluation

Increased awareness of appendiceal PMP and developments in cross-sectional imaging have increased the detection capacity of appendiceal lesions. Therefore, it is currently under debate whether histological confirmation is necessary in the setting of typical radiological findings (28), (29), (50). In case of diagnostic doubt, histological confirmation is recommended, preferably by means of diagnostic laparoscopy as aspiration with fine core needle biopsy usually fails to sample representative tissue frequently resulting in acellular mucin (28), (29), (50).

Exploratory laparoscopy should be carried out in a referral centre and by placing the trocars in a midline position (50). The advantages of laparoscopy include the possibility of taking a proper biopsy under direct vision, assessment of small bowel and mesenteric involvement and estimation of the PCI. European guidelines do highlight that staging with a proper evaluation of the small bowel and its mesentery plays an important role in patients with high-grade disease where initial treatment with systemic chemotherapy (SCT) might be indicated (28).

1.4 MANAGEMENT

In the past, peritoneal carcinomatosis was considered to be an incurable condition identical to that of distant metastases. Lack of response to the systemic treatments available at the time led to the development of aggressive locoregional procedures based on the hypothesis that peritoneal carcinomatosis is a loco-regional disease. In 1980, the first cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (CRS+HIPEC) procedure was

performed by John Spratt on a PMP patient at the University of Louiseville in Missouri (51).

The combination of CRS+HIPEC has been established to be the gold standard of care for PMP patients as per the recently published European (28) and American treatment guidelines (29). Given the low incidence of PMP, randomized controlled trials (RCT) evaluating the benefit of CRS+HIPEC in this setting are missing. Therefore, the evidence supporting the use of CRS+HIPEC is mainly based on results from retrospective series. Concerns regarding the use of CRS+HIPEC arose after the results of the PRODIGE-7 trial were published in which adding HIPEC with oxaliplatin to CRS did not provide any survival advantage but did increase postoperative morbidity in the treatment of peritoneal metastases from colorectal cancer (52). This triggered the need to investigate the efficacy of HIPEC treatment in PMP patients. Consequently, a large multicentre cohort study from the PSOGI registry that included patients with PMP treated with CRS alone (n=376) or CRS+HIPEC (n=1548) concluded that HIPEC was associated with better survival outcomes (hazard ratio (HR) 0.65 (95% CI 0.50-0.83), p=0.001) without increased risk of morbidity throughout the entire series (HR 0.94 (95% CI 0.65-1.37), p=0.76) (53).

Nonetheless, CRS+HIPEC is a complex procedure with associated morbidity and mortality rates that cannot be overlooked. Recent prospective randomized trials have estimated morbidity and mortality rates to stand between 25-27% and 0-2%, respectively (52), (54). In the setting of PMP, a recently published meta-analysis including 13 studies reporting the rate of Clavien-Dindo complications of a grade greater than 3 (55) for 1747 patients treated with CRS+HIPEC of 32.9% (95% CI 30.5 to 35.4%) (56). Therefore, careful patient selection is paramount. Decision to proceed with CRS+HIPEC must take place at a

multidisciplinary meeting with expert radiologist, pathologist, surgical oncologist and medical oncologists in order to aid individualized treatment strategies.

1.4.1 Cytoreductive surgery (CRS)

The main aim of CRS is to eradicate all macroscopic tumoral deposits, therefore, it is the principal component of curative treatment. Surgery begins with a long midline laparotomy from the xiphisternum to the pubis then, an exhaustive evaluation of the peritoneal cavity can be performed (see Figure-1). The size of the peritoneal implants and their distribution is recorded as per the peritoneal carcinomatosis index (PCI) (57). Briefly, the abdominal cavity is divided into 13 regions; each region is assessed for peritoneal deposits and a score ranging from 0-3 is assigned based on the size of the biggest lesion (0- no visible tumour, 1 for nodules <0.5cm, 2 for nodules ranging between 0.5cm to 5cm and 3 for nodules >5cm in diameter or coalescing lesions forming a plaque). The total score is then calculated, and it ranges from 0 to 39. The PCI score has shown to be one of the most important factors when dealing with peritoneal surface malignancies as it is representative of the disease volume and distribution which correlates to the likelihood of achieving complete cytoreduction, therefore its evaluation should be included in intraoperative decision making.

Involved peritoneum is excised by means of performing peritonectomies as described by Sugarbaker (57) (58). In the original paper, five peritonectomies were described:

1. Greater omentectomy with or without splenectomy.
2. Left upper quadrant peritonectomy which included peritonectomy of the left diaphragm.

3. Right upper quadrant peritonectomy which included the stripping of the peritoneum of the right diaphragm, the retro- and subhepatic space (i.e Morrison´s pouch), electrofulguration of Glissons capsule.
4. Lesser omentectomy and cholecystectomy with the stripping of the omental bursa with or without an antrectomy.
5. Pelviperitonectomy including the peritoneum of the iliac fossas associated or not to an anterior resection plus/minus hysterectomy and bilateral salpingoophorectomy.

Additional visceral resections are performed as required in each case: appendectomy, cecal pole excision or right hemicolectomy (determined by the primary tumor's histology).

The completeness of cytoreduction score (CC) is calculated at the end of surgery by measuring the diameter of the largest remaining tumoral deposit (57). Optimal cytoreduction is considered for CC scores of 0 or 1. A score of 0 defines those cases where complete removal of macroscopic disease is achieved and residual disease <0.25cm represents CC score of 1. When residual disease is between 0.25cm to 2.5cm a CC2 is given, and residual disease >2.5cm is scored as CC3. CC2 and CC3 represent suboptimal cytoreduction, and this is associated to worse prognosis (59), (60).

When complete CRS is not possible, a maximum tumour debulking can still achieve survival and significant quality of life improvement. The Peritoneal Malignancy Institute (PMI) at Basingstoke reported overall survival rates of 47%, 30% and 22% at 3-, 5- and 10 years in 205 patients that had a maximum tumour debulking procedure with a median overall survival of 32.8 months (61).

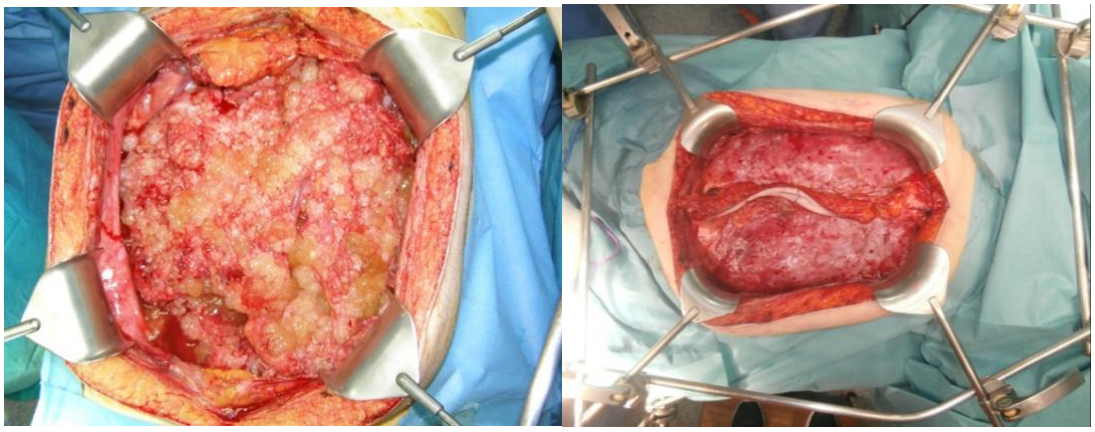

Figure 1. Image on the left showing an omental cake. Image on the right showing peritonectomy of the anterior abdominal wall bilaterally with inclusion of previous scar tissue and umbilicus. Both images also show intraoperative set up with the Thompson retractor (in our centre) in order to achieve adequate exposure to the peritoneal cavity.

1.4.2 Hyperthermic intraperitoneal chemotherapy (HIPEC)

Once cytoreduction is complete, the peritoneal cavity is flooded with the heated chemotherapy solution. The aim of HIPEC is to act upon the microscopic residual neoplastic disease through 2 main pathways: one, the direct administration of the chemotherapy agent (62), (57) and the second, hyperthermia.

1.4.2.1 Intraperitoneal intraoperative chemotherapy

As previously mentioned, the peritoneum plays an essential role as a selective permeability membrane necessary in order to maintain fluid homeostasis. This characteristic of the peritoneum constitutes the known peritoneal-plasma barrier which allows a high intraperitoneal concentration of a chemotherapy agent while maintaining a low plasma concentration (dose-intensification) (63), (64). The peritoneal membrane permeability for the chemotherapy agent depends on its molecular weight and it is lower than the plasma clearance rate, this results in the development of high concentrations of chemotherapy

within the peritoneal cavity whilst sparing systemic concentrations and toxicity (63). Consequently, residual tumour cells are in direct contact with high doses of chemotherapy agent, allowing for a penetration of tumour nodules of up to 3mm in depth (65). This constitutes the two-compartment model known as de Dedrick diffusion model in which the peritoneal clearance rate of the chemotherapy agent is inversely proportional to the square root of the molecular weight (63). The area under the curve (AUC) ratio of a chemotherapy agent is used as a value to estimate the proportion of the drug being exposed to the peritoneal nodules over the plasma compartment. However, major limitations of this model include that specific penetration of the chemotherapy agent into the tumour nodule cannot be predicted (66). The concentration of the chemotherapy agent reached within the tumour nodule is also determined by pharmacodynamic variables such as the density of the tumour nodule, its size and vascularity (67). The interest in pharmacodynamic variables has increased over time. This has brought changes into the definition of suitable chemotherapy agents by establishing the concentration achieved within the tumour nodule as the pharmacological end point rather than AUC ratios (66).

Another reason behind the use of HIPEC is explained by the "tumour cell entrapment" theory. During CRS, and especially in the case of peritoneal metastases, surgical resection sites can be contaminated by the seeding of cancer cells which are posteriorly trapped by fibrinous material and scar tissue forming as part of the inflammatory process (68). Sugarbaker postulated that these cancer cells could be eliminated from the peritoneal surfaces by an intraoperative chemotherapy lavage or the same within the first five postoperative days (69).

1.4.2.2 The rationale behind the use of hyperthermia

The hypotheses supporting the use of hyperthermia are various.

Firstly, hyperthermia on its own has been observed to cause a direct thermal cytotoxic effect on tumour nodules by means of impairing DNA repair, increasing lysosome instability, inhibiting angiogenesis and inducing apoptosis (70) and by reducing blood perfusion leading to an acidic, hypoxic and nutritionally poor environment (71). Secondly, hyperthermia has been reported to have a synergic effect on certain chemotherapy agents (cisplatin, paclitaxel, oxaliplatin, mitomycin-C) (70), (68). Agents like cisplatin display an increase in cytotoxicity proportional to the rise in temperature, whereas agents like mitomycin C have a threshold for maximal amplification of cytotoxicity (68). Thirdly, hyperthermia increases the depth of tissue penetration of the chemotherapy agent (72).

Finally, heat exerts a modulatory effect on the immune system by activation of the adaptative immune response. In vitro studies observed that the activation of the immune system is mediated by the expression of heat-shock proteins to the plasma membrane on cancer cells. This led to the formation of complexes between antigens and heat shock proteins which, once processed by antigen presenting cells led to the activation of T cells in a more efficient manner than antigens alone (73). A small pilot study conducted by Fiorentini et al (74) observed an increase in T helper and T cytotoxic cells at baseline and 30 days after HIPEC administration in patients with peritoneal metastasis from colorectal cancer.

1.4.2.3 HIPEC regimens.

The technique, chemotherapy agent, dose and duration varies amongst referral centres, with at least 60 different HIPEC regimes observed for the treatment of peritoneal metastases from colorectal cancer (75).

Accepted HIPEC regimens included in the European guidelines (28) are oxaliplatin and mitomycin-C based. Three oxaliplatin-based regimens are recognised: the Elias high dose (oxaliplatin 460mg/m^2 for 30 minutes), the Glehen medium dose (oxaliplatin 360mg/m^2 for 30 minutes) and the Wake Forest University regimen (oxaliplatin 200mg/m^2 for 120 minutes). The mitomycin-C regimens included are the following: Sugarbaker regimen (mitomycin-C at 10mg/m^2 in women and 12mg/ m^2 in men), the Dutch high dose or triple dosing regimen (mitomycn-C at 35mg/m^2 for 90 minutes), the American Society of Peritoneal Surface Malignancy low dose mitomycin-C or concentration based regimen (40 mg of mitomycin-C for 90 minutes) and the PMI Basingstoke regimen (mitomycin-C at 10mg/m^2 for 60 minutes). The most frequent regimen was the Glehen medium dose oxaliplatin regimen (28.6%) followed by the American Society of Peritoneal Surface Malignancy low dose mitomycin-C regimen (14.3%) and the PMI Basingstoke low dose mitomycin-C based regimen (10.7%). The American guidelines (29), on the other hand recommend either mitomycin-C 40mg for 90 minutes (fixed dose), mitomycin-C at 30mg/m^2 for 90 to 120 minutes, mitomycin-C at 15mg/m^2 plus doxorubicin at 15mg/m^2 for 90 minutes and oxaliplatin at 300mg/m^2 for 30 minutes. Results from the multicentre cohort using the PSOGI registry observed that in the treatment of PMP patients there was a survival benefit of CRS+HIPEC with the HIPEC regimens of oxaliplatin plus intravenous fluorouracil-leucovorin or cisplatin combined with mitomycin-C , but not mitomycin-C alone (53). Only one RCT has been published comparing survival outcomes and toxicity of two closed-HIPEC regimens (oxaliplatin 200mg/m2 vs mitomycin-C 40mg for 120 minutes) in the treatment of patients with PMP (76). Results showed no difference in survival nor in toxicity rates. Therefore, future controlled trials are warranted in order to clarify the ongoing questions regarding optimal chemotherapy agent, dosage and duration.

In our centre, the open "coliseum" technique is used. The current chemotherapeutic agent is Mitomycin C at a dose of 35 mg/m^2 (body surface area based regime), heated to 42-43ºC and divided into three doses for 90 minutes. A heating pump machine is used to circulate the perfusate throughout the peritoneal cavity. The perfusate solution employed was peritoneal dialysis solution with 1.5% dextrose. The chemoperfusate volume used was 2L/m^2 the body surface area.

1.4.3 Early postoperative intraperitoneal chemotherapy (EPIC)

Early postoperative intraperitoneal chemotherapy (EPIC) was administered as part of the standard Sugarbaker protocol to treat peritoneal surface malignancies after CRS+HIPEC (69), (77). The target of EPIC was to eliminate tumour cells that had escaped HIPEC treatment to prevent them from being incorporated into postoperative fibrinous adhesions leading to early peritoneal recurrence as per the tumour entrapment hypothesis. Scar tissue and adhesions typically form within 7 to 10 days after the surgical procedure, as a result, EPIC was administered immediately after CRS+HIPEC treatment, usually starting on the first postoperative day and was continued during the next four consecutive days. The EPIC protocol involves administering the chemotherapy agent (typically 5-fluorouracil or 5-fluorouracil combined with mitomycin-C, doxorubicin, the combination of cisplatin and doxorubicin, paclitaxel, …) in 1L of perfusate solution through a Tenckhoff catheter placed at the time of CRS+HIPEC procedure alongside four outflow drains placed in each quadrant of the abdomen (78). After infusion, the drains and the Tenckhoff catheter were clamped for 23 hours during which the chemotherapy solution remained in the peritoneal cavity. After this, the drains were unclamped and the solution was allowed to drain for one hour before the next cycle began. Typically, this process was repeated over the course of 5 days. This procedure warranted careful patient monitorization in intensive care or high

dependency units, as well as, trained nursing staff in the safe administration, and disposal of the chemotherapy solution. However, many peritoneal malignancy units have discontinued their use of EPIC as there are still many concerns regarding its association with increased postoperative morbidity and no strong scientific evidence supporting its use.

The benefits of treatment with intraperitoneal 5-fluorouracil were initially established in a RCT including 66 patients that underwent initial surgery for a high-risk colorectal cancer (79). Patients were randomized to receive adjuvant 12 cycles treatment with intraperitoneal or intravenous 5-fluoruoracil. Results from this trial showed that the administration of intraperitoneal 5-fluorouracil significantly reduced peritoneal recurrence ($p=0.003$) without conferring any survival advantage possibly due to the progression of extraperitoneal disease. Additionally, one second trial demonstrated an histological response in patients with mucinous tumours from colorectal and appendiceal origin after treatment with intraperitoneal chemotherapy with mitomycin C and 5-fluorouracil (80). This led to the development of 5-fluorouracil EPIC protocols for patients at risk of peritoneal recurrence.

Several retrospective studies have reported the use of EPIC in addition to CRS+HIPEC for PMP. Two large referral centres have in recent times reported improved survival outcomes with the administration of EPIC following CRS+HIPEC compared to HIPEC alone. The group at St Georges University Hospital in Sydney have observed improved 5-year OS survival rates of 93% vs 64.5% ($p<0.0001$) with the administration of EPIC following CRS+HIPEC in cases of LAMN with peritoneal dissemination (81). This same group published improved OS and DFS outcomes in patients with high-grade peritoneal disease originated from a mucinous adenocarcinoma of the appendix (82). The 5-year OS observed in this case were 62.3% vs 30.2%, respectively. The administration of EPIC was found to be a protection factor in the multivariate analysis for both OS and DFS. This group

advocates for the use of EPIC after CRS+HIPEC treatment given the survival benefit observed in their cohorts without causing additional mortality nor morbidity (81), (82). However, one must consider the high percentage of postoperative complications in the CRS+HIPEC group which could explain the lack of significant differences when compared to the EPIC group: 44.6% vs 48.3% (p=0.593) (81) and 47.9%vs 53.7% (p=0.444) (82), respectively. The other large referral centre that has recently published favorable results of EPIC is the PMI in Basingstoke (78). This constitutes the largest cohort (n=637) published so far evaluating the impact of adding EPIC to the treatment of PMP patients. In this case, an advantage in DFS was observed in the EPIC group; 5-year DFS rates of 63.1% vs 55.4%, p=0.025. No subgroup analysis nor multivariate was performed, however, therefore the impact of EPIC on different histological subgroups was not assessed. Additionally, the EPIC group was associated to longer intensive care stays and overall admission. Interestingly, descriptive analysis of major morbidity and readmission rates revealed that these were actually lower in the EPIC group.

The survival benefits reported by these studies (78), (81), (82) must be interpreted with caution. One of the major limitations of these studies is the fact that they are based on retrospective cohorts from single centres, therefore selection bias is inevitable. The CRS+HIPEC control group was formed by cases where EPIC was withheld. The reasons for not completing treatment with EPIC in the St George Hospital were extensive CRS procedures were proceeding with EPIC carried a reasonable risk of complications, leakage of intraperitoneal chemotherapy, haemodinamic instability, and major organ impairment (81), (82). Similarly, EPIC was withheld by the Basingstoke group in cases requiring extensive CRS procedures involving multiple bowel anastomoses (78). This point was raised by Sugarbaker in his recent review where the title itself conveys the message of abandoning the use of EPIC (83). The experience from the Washington Cancer Institute

with the use of EPIC for the treatment of PMP was also reported throughout this review (awaiting publication). In this cohort, Sugarbaker does not report any survival benefits derived from the use of EPIC in the treatment of patients with low- and high-grade PMP while postoperative complications were more likely (83).

Overall, there exists a lot of controversy regarding the use of EPIC in the treatment of PMP patients; specially regarding its potential benefits and associated increase in postoperative morbidity. This has resulted in a reduced proportion of patients receiving it (78) even in centres that are currently still offering it. Results from the ICARuS (Intraperitoneal Chemotherapy after cytoreductive Surgery) trial (NCT01815359) will be expected to provide further knowledge on the impact of adding EPIC to CRS+HIPEC treatment on patients with peritoneal disease originated from colorectal or appendiceal primaries.

1.4.4 Systemic chemotherapy (SCT).

The role of SCT in the treatment of patients with PMP is unknown. There exists an overall lack of evidence regarding SCT treatment in terms of selecting which patients would benefit from it and which regime. This task is made difficult by the overlapping and confusing terminology that surrounded this pathological entity in the past, and it's low incidence that obstructs the possibility of conducting randomized clinical trials. Additionally, experts recommend the use of same treatment regimens that are approved for colorectal cancer, even though, the natural course and biological and molecular origin of this disease is different (84), (85).

One important contribution were the results of a large retrospective analysis using the national cancer database (NCBD) including 5971 mucinous tumours of the appendix (86). Their relevant observation was that, SCT did benefit patients with stage IV moderately to poorly differentiated disease (median OS 2.99 vs 1.64 years, respectively) whereas, it did not influence those with stage IV well-differentiated disease (86). Reinforcing this, a later publication using the NCDB including only patients metastatic low-grade mucinous appendiceal adenocarcinoma showed no survival advantage when SCT was associated to the treatment of these patients (87). These results have dictated recommendations in treatment guidelines. Both the European (PSOGI/EURACAN) (28) and North American guidelines (Chicago consensus) (29) do not recommend the use of SCT in low-grade disease, but contemplate the use of SCT in high-grade disease, both in the neoadjuvant or adjuvant setting although it is specified that the level of evidence to support these recommendations is low (28). The guidelines coincide in favoring neoadjuvant SCT in cases with initially unresectable disease (28), (29).

Despite this lack of evidence, the use of SCT in this setting is still quite common. Results from a retrospective NCBD evaluating different treatment strategies revealed at that up to 32.4% of patients with PMP were treated with CRS+HIPEC and SCT (88) out of which 18.7% had well-differentiated disease.

There have been few studies investigating whether neoadjuvant SCT provided any survival benefit in the treatment of PMP with some disparity amongst the results. Optimistic results were reported by Bijelic et al (89). The rate of histologic response observed in this retrospective series of high-grade PMP patients was 29%; this associated improved OS ($p=0.003$). Neoadjuvant SCT also associated lower PCIs (19 vs 28, $p=0.003$). Additionally, Spiliotis et al (90) published higher OS and DFS in high-grade PMP patients following a

neoadjuvant SCT and CRS+HIPEC treatment scheme (median OS 19 vs 10 months, p=0.042 and median DFS 10 vs 0 months, p=0.039). However, a similar study by Turner et al (91) reported a radiological response rate of 58%, that did not translate into significant changes in PCI score, CC score, nor survival outcomes. When analyzing the subgroup of patients with poorly-differentiated PMP with SRC, Lieu et al (92) observed improved DFS rates, while Milovanov et al (93) described better OS rates with 3-year OS of 22% vs 14%, p=0.028.

On the other hand, the study by Votanopoulos et al (94) found preoperative SCT treatment to be a factor of worse prognosis for both low- and high-grade PMP patient. Recently, a report including the largest number of patients from a referral centre in China (N=720) also concluded that preoperative SCT was associated to worse survival outcomes in low-grade PMP patients, PCI<20, and in optimal CRS. In addition, it did not provide any survival advantage for patients with high-grade PMP, PCI>20 nor in cases of suboptimal CRS (95).

The results from studies evaluating the effect of adjuvant SCT are similarly inconclusive. Blackham et al (96) investigated the effect of perioperative SCT in the treatment of low- and high-grade PMP patients. The results from previous study groups were reinforced, as the SCT response rate observed in low-grade PMP was 0%. In cases of high-grade PMP, the DFS was higher in patients receiving adjuvant SCT (13.6 vs 7.0 months, p=0.03), but did not translate into an improvement in OS (36.4 vs 19.4 months, p=014). Therefore, they concluded that high-grade PMP patients could benefit from the use of adjuvant SCT, and would recommend using SCT in the neoadjuvant setting only in borderline resectable cases. These same recommendations can be seen in the European (PSOGI/EURACAN) (28) and North American guidelines (Chicago Consensus) (29).

1.5 PATHOLOGY

The development of classification systems for PMP based on histopathological findings and prognosis has been challenged by the existence of confusing and overlapping terminology.

In 1995, Ronnet et al (97) published their pivotal study where three different pathological subgroups with their respective prognosis were defined. Disseminated peritoneal adenomucinosis (DPAM) was used to refer to peritoneal lesions formed by abundant extracellular mucin and scant indolent epithelial cells. Peritoneal mucinous carcinomatosis (PMCA) on the other hand, was used to describe peritoneal lesions composed by abundant mucinous epithelium, with high mitotic activity and cytological features of carcinoma. An intermediate category grouped lesions with inconsistent or discordant lesions (PMCA-I/D).

Consequently, Bradley et al (98) reviewed the histology of 101 PMP cases with the aim of evaluating the prognostic implications of the three-tiered classification proposed by Ronnet (97). No differences were found between the survival outcomes of the DPAM and PMCA-I groups. Therefore, a two-tiered classification system was proposed and the terminology they advocated for was low-grade mucinous carcinoma peritonei (MCP-L) and high-grade mucinous carcinoma peritonei (MCP-H).

The term low-grade appendiceal mucinous neoplasm (LAMN) was introduced by Misdraji et al (99) to refer to mucinous lesions of the appendix that lacked infiltrative invasion characteristic of adenocarcinomas, but could disseminate through the peritoneal cavity.

Other relevant findings in the development of the PMP classification as we know it today, were those observed by Davison et al (100) and Shetty et al (101). The first, identified

pathological features that were correlated to worse survival outcomes such as: destructive invasion, high cytologic grade, high tumour cellularity, angiolymphatic invasion, perineural invasion and the presence of signet ring cells (SRC). They gave pathologic descriptions to the three-tiered classification of the American Joint Cancer Committee (AJCC); grade G1 included cases without adverse features; G2, those with one adverse feature except SRC that would be inherent to G3. The classification proposed by Shetty et al (101) mirrored that proposed by Davison et al (100).

Significant progress in the pathological reporting of appendiceal mucinous neoplasms and PMP took after the Peritoneal Surface Oncology Group International meeting in 2016. In this meeting, experts in PMP from around the world voted on pathological terminology and its corresponding descriptions following a Delphi process (102). In the definition of primary lesions, the group included the terms of LAMN and high-grade appendiceal mucinous neoplasm (HAMN) that should be distinguished from appendiceal mucinous adenocarcinomas by their patterns of invasion (i.e pushing invasion versus infiltrative invasion, respectively). Also, mucinous adenocarcinoma with SRC (< 50% SRC) and mucinous signet ring cell carcinoma (>50% SRC) were included in the description of primary lesions. In the setting of peritoneal dissemination, four different categories were distinguished: acellular mucin (AM), PMP with low-grade histologic features (LG-PMP), PMP with high-grade histological features (HG-PMP) and PMP with SRC (HG-PMP with SRC), in order of biological aggressiveness. AM mainly consists of mucinous deposits without an epithelial component. The epithelial component in LG-PMP is scarce (<20%) and indolent looking with low-mitotic activity. In the HG-PMP subgroup, higher cellularity is found (>20%) showing signs of marked atypia and higher proliferative capacity. HG-PMP with SRC is defined by the presence of SRC which are a marker of worse prognosis.

Advances in terminology from the PSOGI consensus (102) were incorporated in the most recent edition of the AJCC classification (103) were G (grade) and M (metastatic) categories defined stage. Disseminated disease (stage IV) was divided in three groups. Stage IVa included patients with peritoneal disease formed by AM (M1a) or low-grade atypia (M1bG1); stage IVb encompassed patients with high-grade peritoneal deposits (M1bG2) and those with SRC (M1b G3). Stage IVc included patients with metastasis to distant sites other than the peritoneum (M1c).

1.6 PROGNOSTIC FACTORS

As previously highlighted, histology is the factor that has been most commonly correlated to prognosis, and, it is also the most frequently studies factor. However, several others have been identified.

It is not surprising that PCI and CC score have been repeatedly associated with survival outcomes by several study groups. At least four study groups found PCI score to correlate with OS in the multivariable analysis with cut-offs of PCI>20 (104), (105), (106) and PCI >22 (107). Similarly, CC score of 2-3 was associated with worse survival outcomes by several study groups (104), (105), (107), (108), (109), (110). LN status is another factor that has been associated with OS in several studies (104), (105), (110). Overall, these factors should be taken into consideration when selecting patients candidate to CRS+HIPEC. Solomon et al (106) in their study with SRC cases, argued that high PCI does not contraindicate CRS+HIPEC, but MDT discussion is warranted in order to evaluate whether optimal CRS is achievable. The study group of Levinsky et al (110) come to a similar conclusion; CRS+HIPEC can be considered in SRC patients given the absence of LN metastasis and if CC0/1 can be achieved. However, PCI, CC score and LN status are factors that are either determined intra- or postoperatively. Prognostic factors that are determined preoperatively are needed in order to aid patient selection process (104).

Other factors that were found to be associated with prognosis by isolated study groups were severe postoperative complications (106), (111) preoperative SCT (107), elevated Ca19-9 (112) and intraoperative transfusion (112).

JUSTIFICATION, HYPOTHESIS AND OBJECTIVES

2. JUSTIFICATION, HYPOTHESIS AND OBJECTIVES

2.1 JUSTIFICATION

Mucinous neoplasms of the appendix have been differently classified over time, making it difficult to compare data across different the working groups in this field and to establish for each case. Even though there still exists some disparity across the literature when referring to this disease, in 2016, the PSOGI group proposed a standardized way of reporting the histopathological findings from both the primary lesion of the appendix and the peritoneal implants resulting in a four-tiered histologic classification (102).

A historical perspective of the different classification systems of these tumours is essential for the understanding of the evolution of concepts and histopathological definitions that have led up to the present moment (Paper I).

The introduction of new classification systems has posed the challenge of verifying how they adapt to our casuistry and which one defines best the prognosis of our patients (Paper II).

The known prognostic factors of mucinous tumours of the appendix do not accurately determine the evolution of some patients, so the identification of new prognostic factors and the development of risk calculation mechanisms using nomograms that are capable of predicting recurrence and prognosis of the disease could be useful in the development of more precise management and surveillance protocols (Paper III).

2.2 HYPOTHESIS

On this basis, the hypotheses of this thesis is that the PSOGI nomenclature regarding peritoneal lesions (AM, LGMCP, HGMCP and HGMCP-SRC) is the main conditioning factor of prognosis, but, when associated to other biological indicators of aggressiveness into a nomogram predicting disease recurrence after CRS+HIPEC, the patient stratification according to prognosis is better than histopathology alone.

2.3 OBJECTIVES

This project is presented in the form of articles with the following main objectives:

- To obtain a wider understanding of histopathological characteristics of both the primary appendicular lesions and peritoneal deposits that have led to the evolution of the different histopathological classifications (Paper I).
- To evaluate the impact of histopathological characteristics on survival outcomes (including OS and DFS) and how this is reflected on the two most recent classification systems (PSOGI 2016 (102) and AJCC 8th edition (103)) (Paper II).
- To identify factors correlated with DFS in our series in order to construct a clear and simple to use nomogram predicting the risk of recurrence for each patient after optimal treatment with CRS+HIPEC. Subsequently, to externally validate the performance of this nomogram on another cohort from a referral centre treating PMP (Paper III).

MATERIALS AND METHODS

3. MATERIAL AND METHODS.

3.1 STUDY DESIGN

The first article consists of a systematic review following the PRISMA guidelines (113).

The other two articles are based on a retrospective observational study performed over a prospectively maintained database. All consecutive patients treated with CRS+HIPEC for a mucinous appendiceal tumour with peritoneal dissemination at the Hospital Universitario Gregorio Marañón (HGUGM) from 1st of January 2009 to the 31st of December 2018 were included. Second interventions performed during this period of time for disease recurrence were excluded.

Additionally, the third article counted with an external validation cohort which included patients treated for PMP with CRS+HIPEC at the Fondazione IRCCS Istituto dei Tumori (INT) in Italy between January 1995 to December 2019.

3.2 STUDY POPULATION

Patient inclusion criteria

-Adults (age above or equal 18 years) with a confirmed histopathological diagnosis of an appendiceal mucinous neoplasm with peritoneal dissemination undergoing CRS+HIPEC treatment at Hospital General Universitario Gregorio Marañón.

-Revision of histological samples at reference centre (Hospital General Universitario Gregorio Marañón).

-Any pathological subgroup included in the PSOGI classification: AM, LGMCP, HGMCP, HGMCP-SRC.

-Primary or recurrent cases are included, but second interventions performed during this period of time are excluded ensuring that each patient is only included once.

Patient exclusion criteria

-Any other type of appendiceal neoplasm with or without peritoneal dissemination (except appendiceal mucinous neoplasms).

3.3 TREATMENT OF STUDY SUBJECTS

Clinical management

Patients were treated as per the standards of care at our centre.

Each case was reviewed at the multidisciplinary team (MDT) meeting where debate on whether CRS+HIPEC was indicated took place. Case discussion included an extensive clinical history of the patient including ECOG status, serum tumour marker status (CEA, Ca19-9 and Ca-125) and revision of venous and oral contrast enhanced CT scans of the chest/abdomen/pelvis. Expert pathologists would also review tissue samples when available (from appendicectomy specimens, radiologically guided biopsies or previous debulking surgeries).

CRS required a long midline laparotomy and would begin with an intraoperative assessment of the volume, extent and distribution of peritoneal disease which was registered as per PCI (57). Briefly, the abdominal cavity was divided into 13 anatomical regions. A score from 0

to 3 was given to each region according to the volume of peritoneal disease; 0 indicates that there was no visible peritoneal disease; 1- tumour nodules <0.5cm, 2- tumour nodules from 0.5 to 5cm and 3- tumour nodules >5cm. Therefore, the PCI score ranges from 0 to 39. The goal during CRS is to remove all macroscopic tumour deposits. Targeted peritonectomy procedures were performed as described by Sugarbaker (62) and visceral resections took place as required according to the distribution of the disease. The majority of the cases would require an appendicectomy, caecal pole excision or right hemicolectomy (determined by the histology of the primary appendiceal lesion), greater omentectomy, recto-sigmoidectomy and hysterectomy and bilateral salpingo-oophorectomy in women. Ovarian preservation could be contemplated in selected cases of young premenopausal women with a gestational wish and an indolent histological subtype. Once CRS was complete, intestinal anastomoses when required were performed prior to the administration of the chemoperfusate agent. Residual disease was recorded as per the CC score: CC-0 for cases without macroscopic residual disease, CC-1 if persisting tumour nodules are <2.5mm in size, CC-2 from 2.5mm to 2.5cm and CC-3 >2.5cm (57). In PMP cases, CC0/1 scores were considered to be optimal CRS.

The combination of HIPEC to CRS aims to treat residual microscopic disease by the direct administration of a single high dose of heated chemotherapy agent. In our centre, HIPEC was delivered using the open "coliseum technique" using an extracorporeal machine pump. The dosage protocols were based on the body surface area (BSA). Chemotherapeutic regimes used were: combination of intravenous 5-fluouracil 400mg/m^2 and folinic acid 20mg/m^2 administered 20 minutes before intraperitoneal oxaliplatin 460mg/m^2 for 30 minutes or mitomycin-C 35mg/m^2 for 90 minutes (administered in three doses). The perfusate solution used was the peritoneal dialysis solution with 1.5% dextrose. The chemoperfusate volume used was 2L/m^2.

After the HIPEC has been delivered, the abdominal cavity was washed with 10 liters of warm saline. A thorough inspection of the small bowel for any suction related injuries was then performed and hemostasis was checked. Surgical drains were routinely placed and patients were transferred to the high-dependency unit for at least 48-hours postoperative monitoring. Parenteral nutrition and mechanical and pharmacological anti-thrombotic prophylaxis were initiated in all cases. Perioperative mortality and complications were recorded following the Clavien-Dindo classification (55) and the day of discharge was recorded.

After complete pathological evaluation of the specimens, each case was rediscussed at the MDT. Cases with HGMCP or HGMCP with SRC were considered for adjuvant SCT after review by the medical oncologists. Follow-up occurred every three months during the first year; twice a year during the second to fourth years and yearly after the fifth, up until the tenth year. Follow-up was carried out by treating surgeon or medical oncologist at our or referral center. It included physical examination, TM and radiologic CT-scan evaluation. If recurrence occurred; date, site and treatment offered was registered. The date of death (regardless of the cause) was also registered.

Pathological evaluation.

All pathology slides were reviewed and analyzed by two experienced pathologist in peritoneal malignancies (Y.G and M.J.F.A) who classified primary appendiceal tumours and peritoneal implants following the criteria set by the PSOGI classification (102).

Primary appendiceal lesions were divided into benign lesions, LAMN, HAMN and mucinous adenocarcinoma. LAMN and HAMN lesions were distinguished from mucinous adenocarcinoma by the type of invasion: pushing invasion versus infiltrative invasion, respectively. Mucinous adenocarcinomas with SRC (w/SRC) and signet ring cell carcinoma (SRCC) were defined by the presence of <50% and >50% of SRC respectively. The presence of cells with neuroendocrine differentiation (positive chromogranin/synaptophysin immunohistochemical staining) classified a lesion as Goblet cell carcinoma, thus were excluded from the analysis.

Patients were classified into the following categories after analysis of pathological features of peritoneal lesions: AM, LGMCP, HGMCP and HGMCP-SRC. Mucin and a granulation-like response in the peritoneum in the absence of epithelial cells defined AM. Mucinous deposits with <20% of low-grade epithelial cells correspond to LGMCP category. When cellularity was more abundant (>20%) and cells showed high-grade features, patients were classified as HGMCP and into HGMCP-SRC when at least >10% of SRC are present.

Using the histopathological definitions from the PSOGI classification (102), patients were additionally stratified according to the AJCC 8th edition classification (103). AM and LGMCP peritoneal implants were grouped into stage IVa and HGMCP and HGMCP-SRC were grouped into stage IVb.

3.4 STUDY VARIABLES

Demographic and preoperative variables

Demographic variables for each patient have been registered. These included gender, age and ECOG status at the time of CRS+HIPEC treatment.

Additionally, other preoperative data regarding the treatment of the current episode has been registered such as whether the disease is primary or recurrent, preoperative value of CEA, Ca19-9 and Ca-125, and whether preoperative SCT has been administered.

Intraoperative and postoperative variables

The date of CRS+HIPEC has been registered. Intraoperative variables that have been taken into consideration include the PCI and CC scores and the HIPEC protocol applied. Postoperative variables that have been registered include the LOS (in days) and postoperative morbidity recorded according to the Clavien-Dindo classification (55).

Histopathological variables

Primary appendiceal lesions (when available) and peritoneal implants will be classified as per the PSOGI classification (102):

-Primary appendiceal lesions: LAMN, HAMN, mucinous adenocarcinoma, mucinous adenocarcinoma with SRC or SRCC.

-Peritoneal implants: AM, LGMCP, HGMCP or HGMCP-SRC. Using the classification of the AJCC 8th edition (103): stage IVa (included AM and LGMCP) and stage IVb (included HGMCP and HGMCP-SRC).

Other histopathological factors considered were the presence of metastatic lymph node disease.

Follow-up variables

The administration of adjuvant SCT was recorded. The status of the patients at the time of completion of the study has been evaluated and categorized as living free of disease, alive with disease or dead. The date of last review and the date of death was recorded in those cases where appropriate. In cases of recurrence, the location and treatment of the same was recorded.

Survival outcomes (OS and DFS) were calculated from the day of CRS+HIPEC to the day of recurrence and death (regardless of the cause).

3.5 STATISTICAL ANALYSIS

The statistical analysis has been carried out using the SPSS software V.23.0 (IBM) and R software (www.R-project.org).

Briefly, means of continuous variables that followed a normal distribution were compared with the Student-T test or the analysis of variance test. Non-parametric tests (i.e Mann-Whitney U test or Kruskal-Wallis test) were used to compare continuous variables that did not follow a normal distribution. Categorical data, on the other hand, was compared using Pearson´s chi-square test. Survival analysis was performed using the Kaplan-Meier method, and group comparisons were performed using the Log-rank test. Patients with incomplete follow-up were censored at the day of last visit. Multivariate analysis was performed using a Cox-regression model. The aim of multivariate analysis was to assess for the existence of possible confounding variables and to construct a prediction model with significant

variables. Missing data was handled via deletion methods. Statistical significance was defined at $p<0.05$.

The R software was used to design a nomogram based on the prediction model derived from the Cox-regression analysis. The nomogram function included in the “rms” package was used. The nomogram was set to estimate DFS at 1, 3 and 5-years after treatment with CRS+HIPEC.

The predictive performance of both pathological classification systems (PSOGI and AJCC) and the prediction model was evaluated using the Harrell concordance index (C-index). The C-index is a goodness of fit measure used in survival studies that consists of a proportion between the concordant pairs divided by the total number of possible evaluation pairs. Its value ranges from 0.5 (random chance) to 1.0 (total discrimination). A model is said to have acceptable discrimination with values >0.7. The C-index was calculated using the cindex function included in the "dynpred" package in the R software. Additionally, the calibration of the prediction model designed with the nomogram was assessed by plotting the predicted versus observed probabilities of DFS at 1-, 3- and 5-yeards using the calibration plot function included in the “rms” package of the R software.

3.6 DATA HANDLING AND RECORD KEEPING

Patient´s personal data has been handled under the principles of transparency, accountability and security provided by the Data Protection Act, 2018. According to this legislation, the subjects have the right to access, modify, oppose and cancel data. The data has been collected from the computerized clinical history of the patient in a password protected database using the SPSS software. The data has been collected in a pseudo-

anonymous manner so that only the principal and collaborating investigators are able to relate said data with the patient´s medical history.

The principal investigator alongside the collaborating investigators have acted as data controllers and have ensured that the data has been handled in compliance with the data protection law with the strictest confidentiality during the period of this study.

3.7 ETHICS APPROVAL

This study has been approved by the ethics committee at the HGUGM and has been carried out respecting the principles and basic ethical standards that have their origin in the current revision (revised version of 64th Assembly Fortaleza, in October 2013) of the Declaration of Helsinki approved by the World Medical Assembly, the Oviedo Convention.

Due to its observational and retrospective nature, participation in this study did not imply risks for the patients as no interventional changes in their management that could alter the course of the disease have been made. As part of the application and finality of the article 14, paragraph 5, letter b of the General Data Protection Regulation (EU) 2016/679, a specific informed consent for the present study was not required.

RESULTS

4. RESULTS.

4.1 PAPER I: DEFINING STAGE IN MUCINOUS TUMOURS OF THE APPENDIX WITH PERITONEAL DISSEMINATION: THE IMPORTANCE OF GRADING TERMINOLOGY: SYSTEMATIC REVIEW.

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Impact factor: 3.875 (Q1)

Citations: 2

Abstract

Background: Mucinous appendiceal neoplasms with peritoneal dissemination (PD) show a wide spectrum of clinical behaviour.

Histological grade has been correlated with prognosis, but no universally accepted histological grading has been established. The aim of this systematic review was to provide historical insight to understand current grading classifications, basic histopathological features of each category, and to define which classification correlates best with prognosis.

Methods: MEDLINE and the Cochrane Library were searched for studies that reported survival across different pathological grades in patients with mucinous neoplasm of the appendix with PD treated with cytoreductive surgery and hyperthermic intraperitoneal chemotherapy. PRISMA guidelines were followed.

Results: Thirty-eight studies were included. Ronnett's classification was the most common (9 studies). Classifications proposed by the Peritoneal Surface Oncology Group International (PSOGI) (6 studies) and the seventh or eighth edition of the AJCC (7 studies) are gaining in popularity. Nine studies supported a two-tier, 12 a three-tier, and two a four-tier classification system. Three studies demonstrated that acellular mucin had a better prognosis than low-grade pseudomyxoma peritonei in the PSOGI classification or M1bG1 in the eighth edition of the AJCC classification. Four studies demonstrated that the presence of signet ring cells was associated with a worse outcome than high-grade pseudomyxoma peritonei in the PSOGI classification and M1bG2 in the eighth edition of the AJCC.

Conclusion: There is a great need for a common language in describing mucinous neoplasms of the appendix with PD. Evolution in terminology as a result of pathological insight turns the four-tiered PSOGI classification system into a coherent classification option.

Keywords: appendiceal mucinous neoplasms, pseudomyxoma peritonei, acellular mucin, appendiceal mucinous neoplasms with signet ring cells, cytoreductive surgery and hyperthermic intraperitoneal chemotherapy, grading pathology and classification.

4.1.1 Introduction

Primary appendiceal tumours have a low incidence of 2.6 per million people per year (8), (9). Epithelial tumours of the appendix are subdivided into benign lesions (adenomas, serrated polyps), mucinous neoplasms, invasive mucinous adenocarcinoma, nonmucinous adenocarcinoma, goblet cell adenocarcinoma, and appendiceal carcinoids (well differentiated neuroendocrine tumours). Recent reports (10), (114) based on the Surveillance, Epidemiology, and End Results (SEER) database have stated that mucinous tumours are the most frequent histological subtype. This review focuses on this last subtype.

Mucinous tumours of the appendix exhibit a tendency towards transcelomic spread into the peritoneum causing peritoneal mucinous carcinomatosis (PMCA) or a mucinous ascites referred to as pseudomyxoma peritonei (PMP). The definition of PMP is nowadays limited to the clinical indolent entity characterized by the grossly evident diffuse intra-abdominal accumulation of mucus following the redistribution phenomenon (24). It is a malignant condition most frequently originating from the appendix, but it should not be used as a histological diagnostic entity.

Mucinous appendiceal tumours with peritoneal dissemination (PD) show a wide spectrum of clinical behaviour ranging from slow-growing lesions with no recurrence after cytoreductive surgery (CRS) plus hyperthermic intraperitoneal chemotherapy (HIPEC) to highly aggressive adenocarcinomas associated with decreased overall survival (OS). Several studies (97), (98), (99), (115), (116), (117) have identified histological grade as one of the most important prognostic factors. However, no definitive grading terminology has been established despite several past attempts. This has resulted in the existence of several confusing and overlapping terminologies across the literature, which makes it difficult to develop management protocols and compare outcomes across different series.

The aim of this systematic review was to provide sufficient historical insight to understand current grading classifications, basic histopathological descriptions of each category, and to define the classification that correlates best with prognosis.

4.1.2 Methods

The systematic review was done according to PRISMA guidelines (113).

Data search

The PICO data search strategy was employed. The following Medical Subject Heading (MeSH) terms were used for each category: under the P (population) category—'pseudomyxoma peritonei', 'appendiceal mucinous neoplasms', 'appendix cancer', 'appendiceal neoplasms', 'peritoneal dissemination', 'acellular mucin', and 'signet ring cells'; under the I (intervention) category—'cytoreductive surgery', 'intraperitoneal injections', and 'cytoreductive surgery and hyperthermic intraperitoneal chemotherapy'; under the C (comparison) category—'pathology' and 'grading pathology'; and under the O (outcome) category—'classification', 'prognosis', 'recurrence', 'disease free survival', 'survival analysis', and 'survival rate'. The literature was reviewed throughout MEDLINE and Cochrane Library platforms. MeSH terms were combined with 'AND'/'OR'. Only studies published in English were considered and an abstract had to be available. Classification schemes, consensus guidelines, and studies that influenced grading criteria were retrieved manually from reference lists. Some of these did not meet the eligibility criteria, but were included because of their historical relevance (97), (99), (118).

Eligibility criteria

Studies that dealt with patients with PD from mucinous tumours of the appendix treated with CRS+HIPEC and reported OS or disease-free survival (DFS) with reference to pathological grading were included. The additional inclusion of other primary tumours of the appendix or even other gastrointestinal tumours (such as colorectal lesions) with PD was not a criterion for exclusion per se if survival results for tumours of the appendix with PD were reported separately. Results had to be reported independently in the form of median OS or 5-year OS rates, and/or median DFS or 5-year DFS rates, for each histological grade of the peritoneal implants. At least two different histological grades of peritoneal implants had to be compared in univariable or multivariable analysis.

No selection based on how pathological grade was assigned. In some studies, pathology slides were reviewed, whereas in others the classification was based on pathology reports or on information coded into large databases. No selection was made with respect to the classification system used to grade the pathology of peritoneal implants (Ronnett's, WHO, Peritoneal Surface Oncology Group International (PSOGI) or AJCC). The search included reports from January 2000 to February 2020.

Case reports and reviews were excluded. Other exclusion criteria were: fewer than 100 patients, no CRS+HIPEC treatment, and exclusive analysis of primary appendiceal lesions without PD. Studies that centered on ovarian involvement and the differential diagnosis between ovarian cancer and PMP of appendiceal origin were also excluded.

Study selection

Two authors assessed the titles and abstracts for eligibility throughout the search and reference lists, followed by full-text screening. Whether studies met the inclusion criteria was discussed between the two authors before inclusion.

The studies included were retrospective case–control studies. Consensus and staging guidelines (5) and retrospective studies (3) not fully meeting eligibility criteria were extracted manually from reference lists, of which one (97) was published before the time interval set for the search.

Each article was analysed systematically. Initially, a search was made for the histology of the primary appendiceal tumour, then for the histopathological grading of the peritoneal implants. The pathological description provided for each grade was recorded. Next, it was identified whether a two, three- or fourtiered classification system was supported. Finally, median OS and/or DFS rates for each tier were recorded based on results of survival analysis.

4.1.3 Results

A total of 849 records were identified, of which 98 were screened fully by abstract or full-text screening. Reasons for exclusion are shown in Fig. 2. Finally, 38 studies that met the eligibility criteria were included (59), (86), (98), (99), (100), (101), (104), (105), (107), (108), (109), (110), (112), (116), (117), (119), (120), (121), (122), (123), (124), (125), (126), (127), (128), (129), (130), (131), (132), (133) of which are summarized in Table 1. Classification systems (6), (28), (102), (103), (134), (135), and two observational studies (97), (118) were not included in Table 1. Most relevant classification systems are summarized in Table 2.

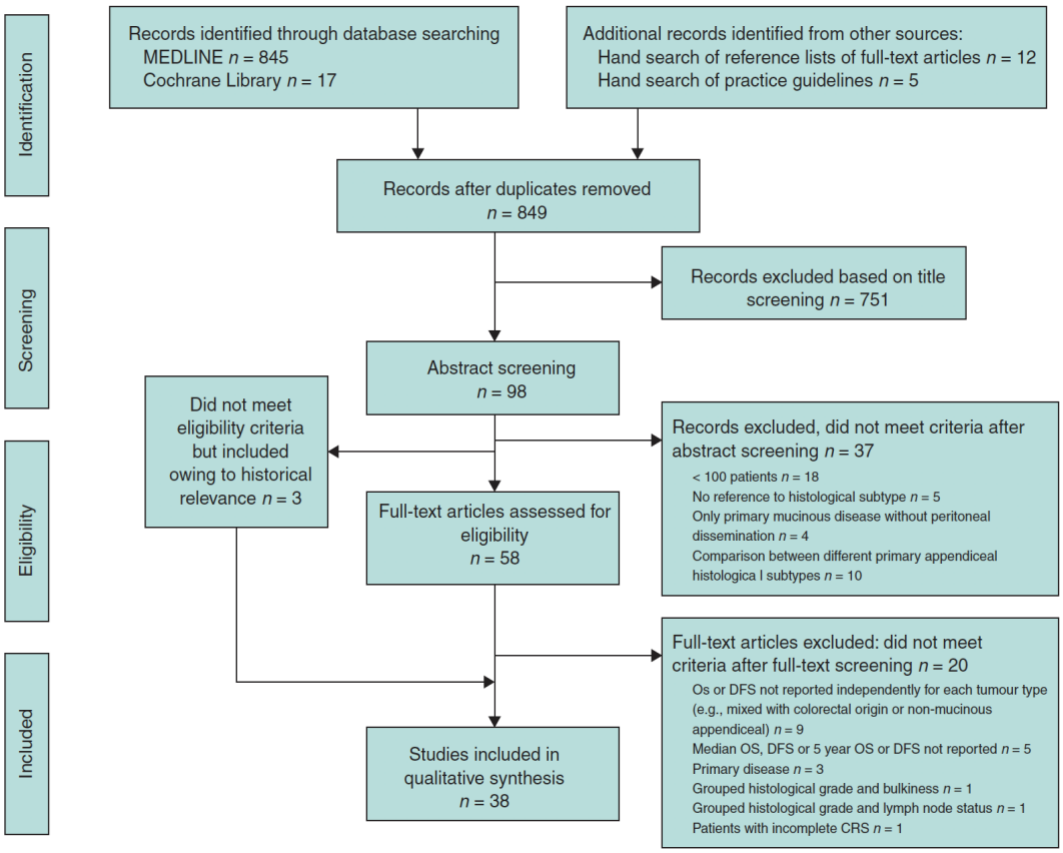

Figure 2. Flow chart showing selection of studies for review
OS-overall survival; DFS- disease-free survival, CRS- cytoreductive surgery.

Initial classification systems

Several study groups have aimed to distinguish and define relevant prognostic groups in patients with PMP.

In 1995, Ronnett and colleagues (97), (116) studied 109 peritoneal lesions defined as PMP and identified three different histological groups based on the pathological characteristics of primary and peritoneal lesions. Primary tumours were classified into: adenoma (villous adenoma or cystadenoma), ruptured adenoma, and adenocarcinoma (invasion of the muscularis accompanied by stromal response) with or without signet ring cells (SRC). Peritoneal lesions were subdivided into: disseminated peritoneal adenomucinosis (DPAM), PMCA, and peritoneal mucinous carcinomatosis with intermediate or discordant features

(PMCA-I/D). Peritoneal lesions in DPAM were defined as scant strips of simple or focally proliferative epithelium with minimal to moderate cytological atypia and no significant mitotic activity with abundant extracellular mucin. A primary appendiceal adenoma was found in 57% of patients with DPAM. Peritoneal lesions in PMCA consisted of a larger component of proliferative mucinous epithelium-forming glands, or organized in nests or individual cells; SRC were included in this group. The cells demonstrated marked cytological atypia and architectural complexity. Most cases of PMCA were found alongside a primary appendiceal or colonic adenocarcinoma. In intermediate PMCA, there were focal areas of mucinous carcinoma immersed within areas resembling DPAM where primary lesions could be well differentiated mucinous adenocarcinomas or adenomas. Cases of discordant PMCA had peritoneal lesions with features of mucinous carcinoma with or without SRC differentiation originating from an atypical adenoma of the appendix with high-grade dysplasia or an intramucosal adenocarcinoma (Table 2).

Ronnett et al (116) identified three prognostic groups. Patients with DPAM had a significantly more favourable prognosis than those with PMCA-I/D or PMCA (5-year OS 75 per cent versus 50 and 14 per cent respectively; $P<0.001$). They also concluded that PMP should not be used as a pathological diagnostic term but rather as a clinical entity. They argued that DPAM was a benign peritoneal lesion and were against using well differentiated mucinous carcinoma to refer to these lesions. However, they included 13 tumours of colonic origin, one of small bowel origin, and 7 of unknown origin (colonic versus appendiceal).

Misdraji and co-workers (99) reviewed 107 appendiceal mucinous tumours, of which 53 had PD. SRCs were excluded from this study. They introduced the term low-grade appendiceal mucinous neoplasm (LAMN) into the literature to refer to primary appendicular lesions lacking infiltrative invasion of the appendicular wall that could,

however, disseminate through the peritoneal cavity. LAMNs demonstrated low-grade cytological atypia (nuclear enlargement, scarce nuclear stratification, and rare mitotic figures) and minimal architectural complexity (uniform, flat epithelial proliferation forming small papillary excrescences/ outgrowths). On the other hand, mucinous adenocarcinomas of the appendix (MACAs) were defined by infiltrative invasion of the appendicular wall with high cytological atypia (full-thickness nuclear stratification, vesicular nuclei with prominent nucleoli, and brisk mitotic figures). When PD was present, the terms LAMNs involving the peritoneum and MACAs involving the peritoneum were used (Table 2). Misdraji et al defined a two-tiered system in which LAMNs involving the peritoneum had a better prognosis than MACAs involving the peritoneum (5-year OS 86 versus 44 per cent; P=0.04).

In 2006, Bradley and colleagues (98) revised the histology of 101 cases of PMP originating from the appendix, and reclassified them according to Ronnett's DPAM, PMCA-I, and PMCA. Appendiceal tumours were evaluated independently and classified into adenomas/LAMNs or adenocarcinomas. The tumours classified as DPAM, which originated from adenomas in Ronnett's classification, were associated with a primary LAMN, whereas PMCAs (high-grade atypia and/or SRCs) were associated with moderate or poorly differentiated appendiceal adenocarcinomas. There was no significant difference in 5-year OS between the DPAM group (61.8(9.2) per cent) and the PMCA-I group (68.2(12.2) per cent). The PMCA group did, however, have significantly worse 5-year OS (38 per cent; P=0.004). Therefore, Bradley and co-workers supported a two-tiered classification system whereby SRCs were included in the PMCA subgroup. They advocated use of the terms low-grade mucinous carcinoma peritonei (MCP-L) instead of Ronnett's DPAM and high-grade mucinous carcinoma peritonei (MCP-H) for Ronnett's PMCA.

Pai et al (122) suggested that both primary tumours and peritoneal implants should be described using the following scheme: presence of neoplastic epithelium, degree of cytologic atypia (low versus high), architectural complexity (simple versus complex), and presence of invasion. The presence of SRCs was considered to indicate high-grade disease. They proposed a grading system based on cytological features and disease extension. The term mucinous adenoma was given to low-grade proliferative lesions confined to the appendix. A three-tiered classification was proposed for tumours with PD. Low-grade mucinous neoplasm with low risk of recurrence was proposed to refer to a low-grade mucinous epithelial proliferation with acellular mucin outside the appendix. The term low-grade mucinous neoplasm with high risk of recurrence was chosen for the same cytologically bland proliferation associated with extra-appendiceal neoplastic epithelium. When invasion was present, the term mucinous adenocarcinoma was chosen for both primary and disseminated disease. The presence of extra-appendiceal neoplastic epithelium (P=0.006) and high-grade cytology (P=0.001) was associated with decreased OS.

WHO and seventh edition of AJCC classification

In an attempt to unify the diagnostic terminology surrounding appendiceal mucinous tumours, both the fourth edition of the WHO Classification of Tumors of the Digestive System (133) and the seventh edition of the AJCC Staging Manual (135) in 2010 made a distinction between low- and high-grade peritoneal disease. The WHO classified primary appendicular tumours into: LAMN, MACA, SRC carcinoma, and undifferentiated appendicular carcinoma. Peritoneal lesions were divided into low- and high-grade disease. Low-grade disease consisted of scanty or missing cells forming small islands or strands, with low cytological and nuclear atypia, and rare mitoses. High-grade disease was defined by the presence of high-grade atypia with cells organized into strands, islands or cribriform

structures, and a higher frequency of mitoses. The presence of SRCs led to classification of a lesion as high grade. However, the WHO still considered PMP to be a pathological diagnosis and a borderline malignant entity.

Carr and co-workers (117) attempted to validate the prognostic implications of the two-tiered classification system proposed by the fourth edition of the WHO classification. They described significant differences in OS between low-grade and high-grade PMP (5-year OS 84 and 48 per cent after treatment with CRS+HIPEC; $P<0.001$). However, they argued against the use of the term carcinoma to describe lesions derived from the peritoneal spread of a LAMN, as these lesions did not show conventional histological features of malignancy.

The seventh edition of the AJCC (135) separated appendiceal carcinomas from the classification of colorectal carcinomas, and distinguished between mucinous and non-mucinous histological subtypes. They advocated a three-tiered classification system for primary lesions: well differentiated (G1), moderately differentiated (G2), and poorly differentiated (G3) tumours. Histological grade was taken into consideration in the staging of stage IV disease. However, only two histological prognostic groups were recognized: low grade, which included well differentiated (G1) mucinous adenocarcinomas, and high-grade, which consisted of both moderately (G2) and poorly differentiated (G3) mucinous adenocarcinomas. The combination of moderately and poorly differentiated disease into the same prognostic group was not supported by a large retrospective database study (124). The outcomes for moderately differentiated and poorly differentiated stage IV mucinous adenocarcinoma were observed to be different; hazard ratios (HRs) compared with the well differentiated counterpart were 1.56 (95 per cent c.i. 1.08 to 2.25) and 5.15 (3.45 to 7.68) respectively.

Consequently, the debate continued about whether a two- or three-tiered classification system should be supported. A large retrospective multi-institutional registry by the PSOGI, in which 2298 patients with PMP of appendiceal origin were analysed, found only two relevant histological groups: low- and high-grade disease. Chua and colleagues (59), along with Bradley et al (98), were unable to find differences between DPAM and hybrid groups. On the other hand, two large retrospective studies based on the SEER database (124) and National Cancer Database (NCDB) (86) identified three histological prognostic groups. Overman and colleagues (124) analysed 1375 appendiceal mucinous adenocarcinomas and found that histological grade was the strongest predictor of survival in patients with PD. The differences in overall cancer specific survival across the three-tiered grade classification system were statistically significant. Asare and co-workers (86), in an analysis of 11 871 appendiceal carcinomas, of which 5971 were mucinous, also supported a three-tiered grading scheme (well, moderately, and poorly differentiated).

Nonetheless, in 2014, Davison et al (100) facilitated staging by defining how to grade tumours in their revised staging of 151 patients with PD. They found destructive invasion, high cytological grade, high tumour cellularity, angiolymphatic invasion, perineural invasion, and SRCs to be associated with worse OS in univariable analysis. SRCs had to be invasive and represent at least 10 per cent of the tumour cellularity. AJCC grade G1 was reserved for cases without adverse histological features; AJCC grade G2 for those with at least one adverse feature excluding SRCs, which were representative of AJCC grade G3. Patients with grade G2 and G3 had a 2.7- and 5.1-fold increased risk of death respectively compared with patients with G1 disease. Therefore, a three-tiered grading system was supported. Similar results were obtained by Shetty and colleagues (101) in an analysis of 211 cases of PMP of appendiceal origin. They developed a three-tiered histological grading

system comprising PMP 1, PMP 2, and PMP 3. PMP 1 included patients with copious mucin and scant columnar epithelium without dysplasia, whereas PMP 3 was defined by any SRC component, and PMP 2 by all other in characteristics between. Survival analysis found each group to be of prognostic relevance with 5-year survival rates of 85.7, 63.1, and 32.2 per cent for PMP 1, PMP 2, and PMP 3 respectively ($P<0.001$).

Current classification systems: PSOGI and AJCC eight edition

The PSOGI reviewed the classification of mucinous appendicular tumours in 2016 (102). The Group supported the terms LAMN and high-grade appendicular neoplasm (HAMN) for primary tumours and discarded the terms adenoma and cystadenoma, only considering use of the term serrated polyp for a mucinous lesion with an intact muscularis mucosae. They highlighted the contrast between pushing-like invasion displayed by LAMNs and HAMNs, in which cells expand into the surrounding tissue without destructive features, and infiltrative or destructive invasion which characterizes adenocarcinoma. LAMN was defined by low-grade cytology and any of the following histological features: loss of lamina propria and muscularis mucosae, fibrosis of the submucosa, pushing-like pattern of growth into the wall, dissection of acellular mucin into the wall, or mucin and/or neoplastic mucinous epithelium outside the wall of the appendix. HAMN was accepted for tumours with LAMN architectural features but with high-grade cytological atypia. However, its prognostic significance remained unknown. Mucinous adenocarcinomas showed infiltrative invasion characterized by tumour budding and/or small, irregular glands within a desmoplastic stroma response. They were classified into well, moderately or poorly differentiated types. SRCs were recognized to be representative of aggressive disease with poor clinical outcomes. Two types of primary lesion with SRCs were identified: mucinous adenocarcinoma with SRCs (less than 50 per cent tumour cells) and mucinous SRC

carcinoma (more than 50 per cent tumour cells). In the stage IV scenario, the grade of the peritoneal disease determined prognosis. The following four prognostic groups were identified: acellular mucin, PMP with low-grade histological features (LG-PMP), PMP with high-grade histological features (HG-PMP), and PMP with SRCs (HG-PMP with SRCs). Acellular mucin lies on the least aggressive extreme of the scale, whereas HG-PMP with SRCs is the most aggressive. The two remaining intermediate categories are reserved for cellular peritoneal deposits with low cellularity (less than 20 per cent) and low proliferative activity (LG-PMP) and cellular peritoneal deposits with marked atypia, higher cellularity, and proliferative activity but without SRC (HG-PMP) (Table 2). The groups with an epithelial cell component are parallel to the G1, G2, and G3 previously by described Davison et al (100), and to the PMP 1, PMP 2, and PMP 3 described by Shetty and colleagues (101).

However, the eighth edition of the AJCC classification (103) introduced significant changes: LAMN was included with its specific T category. Tis(LAMN) referred to low-grade mucinous neoplasia that at least obliterated the muscularis mucosae and could extend to the muscularis propria without penetrating it. LAMNs distorted the architecture of the appendiceal wall44 and spread through it with a pushing front instead of infiltrating it. Therefore, the depth of appendiceal wall involvement was not associated with an increased risk of recurrence, making T1 and T2 categories not applicable. LAMN pT3 referred to involvement of the subserosa, and LAMN pT4 to involvement of the serosa as with other carcinomas. HAMNs pursued a more aggressive clinical course, and were classified using the same staging system as adenocarcinomas.

Stage IV disease was defined by M and G categories. The M category was subdivided into: M1a, intraperitoneal spread of acellular mucin; M1b, peritoneal implants containing tumour

cells; and M1c, metastasis to sites other than the peritoneum. The G category was subdivided into three relevant prognostic groups based on cytological features, tumour cellularity, and presence of SRCs. G1 corresponded to a well differentiated adenocarcinoma with low-grade cytological atypia, low cellularity (less 20 per cent) without invasion or SRCs. G2 was defined by a moderately differentiated mucinous adenocarcinoma with a component of high cytological atypia, and higher cellularity (over 20 per cent) without SRCs. Finally, G3 referred to a poorly differentiated adenocarcinoma defined by any component of SRCs. The final classification into the prognostic IVA, IVB or IVC stages relied on G and M categories. IVA was defined by M1a (acellular mucin) or M1b G1 (low-grade atypia); IVB by M1b G2 (high-grade atypia) or G3 (high-grade atypia with any component of SRCs); and IVC by M1c (distant metastases to sites other than the peritoneum) (Table 2).

Other histopathological landmarks

Acellular mucin

Pai and colleagues (122) observed that only 1 of 14 patients with acellular intraperitoneal disease developed recurrence after 45 months. The presence of acellular/cellular peritoneal disease mucin was associated with OS in multivariable analysis. Furthermore, Davison and co-workers (100) noted that 7 per cent of patients in the subgroup with low-grade mucinous neoplasms had acellular mucinous deposits and none of them developed recurrence. These results suggest that patients with acellular disease have much lower risk of disease recurrence and improved OS compared with those with low-grade cellular disease.

Signet ring cells

The presence of SRCs has been a matter of debate. In 1995, Ronnett and colleagues (97) had allowed SRCs to be present in the PMCA-D group, whereas Bradley et al considered (98) them to be inherent to high-grade lesions. In 2014, Sirintrapum and co-workers (118) studied the significance of SRCs in 55 patients with MACA and PD. None of the 11 patients with low-grade adenocarcinoma had SRCs, whereas 29 of the 44 in the high-grade adenocarcinoma group presented with SRCs. The presence of SRCs could be divided into two prognostically significant groups: SRCs floating in mucin pools or tissue-invading SRCs. The 5-year OS for patients with high-grade mucinous adenocarcinoma without SRCs was similar to that of patients with high-grade mucinous adenocarcinoma with SRCs in mucin pools (32 versus 36 per cent respectively; P=0.58). The presence of SRCs invading tissues decreased OS to a median of 0.5 years, compared with 2.9 and 2.4 years for mucinous adenocarcinoma without SRCs (P=0.003) and mucinous adenocarcinoma with floating SRCs (P=0.004). Mucinous adenocarcinoma with SRCs invading tissues had a higher rate of incomplete cytoreductions. It was suggested that their presence could be a potential contraindication to treatment with CRS+HIPEC.

Qualitative analysis of literature review

The most commonly used classification system was Ronnett's (9 studies). However, increasing use of PSOGI (6 studies) and AJCC (7 studies) classifications over time was noted. Nine studies supported a two-tiered, 12 a three-tiered, and two a four-tiered classification system.

Of studies that used Ronnett's classification system, six identified only two prognostically relevant groups in the multivariable analysis, or had no PMCA-I/D group (104). Three

studies (59), (98), (121) grouped PMCA-I and PMCA, whereas the other two (123), (125) grouped DPAM and PMCA-I.

Three studies (128), (129), (130) demonstrated that acellular mucin was associated with better DFS than LG-PMP in the PSOGI classification; however, a fourth study (133) failed to find significant differences. Additionally, in multivariable analysis, four studies (105), (108), (109), (126), associated the presence of SRCs with worse OS compared with HG-PMP in the PSOGI classification and M1bG2 in the eighth edition of the AJCC classification.

The results of the studies included are summarized in Table 1 (59), (86), (98), (99), (100), (101), (104), (105), (107), (108), (109), (110), (112), (116), (117), (119), (120), (121), (122), (123), (124), (125), (126), (127), (128), (129), (130), (131), (132), (133).

4.1.4 Discussion

The diagnostic terminology for appendicular mucinous tumours has evolved based on the acquisition of pathological insights. However, a common language is necessary to aid therapeutic decision-making and design of clinical trials. Much debate remains despite the enormous efforts of pathologists and institutions (WHO, AJCC) in the development of classification systems with prognostic implications.

The eighth edition of the AJCC classification (103) has captured the peculiarities of mucinous tumours of the appendix. However, only two prognostic groups (EIVA and EIVB) were distinguished. The literature suggests that M1a has a lower risk of recurrence than M1bG1 (6), (100), (122), (130), Reghunathan and colleagues (128) observed that only

one in 33 patients with M1a disease developed recurrence, with 13 having DFS of more than 3 years (HR 9.8; P=0.025). Additionally, Choudry et al (129) found that acellular mucin (19 patients) and scant cellularity (less than 2 per cent of epithelial cells) (30 patients) were associated with better DFS than moderate cellularity (2–19 per cent of epithelial cells) (242 patients) with a HR of 4.4 (P=0.02). Regarding stage EIVB, the authors of single-centre retrospective studies (105), (108) have argued that patients with M1bG3 disease have worse OS than those with M1bG2 disease. Ihemelandu and colleagues (108) observed a decrease in median OS from 45.4months in patients with moderate– high-grade histology to 18.9 months in patients with SRCs, with a HR of 1.4 (P<0.001). Munoz-Zuluaga et al (105) reported median OS of 90 months for patients with high-grade mucinous carcinoma peritonei versus 26.4 months for those with high-grade Mucinous Carcinoma Peritonei with Signet Ring Cells (MCP-S), with a HR of 2.9 (P<0.001). Multicentre studies (109), (110) based on large databases obtained similar results: 16.2 (ref. 101) and 32 (ref. 102) months. However, these results must be interpreted cautiously as specific pathologic criteria such as acellular mucin and SRCs are not registered routinely in large databases. Furthermore, pathological discordance between G2 and G3 grades has been recorded (100) owing to "degenerative cells within pools of mucin that mimic SRC", which in the hands of inexperienced pathologists may erroneously lead to disease being classified as G3. In G3, SRCs should be infiltrating and represent more than 10 per cent of the tumour's cellularity (100). Therefore, concrete histological criteria should be set to define this entity, with both the relative percentage of tumour cells and their arrangement taken into consideration.

The prognostic impact of the four-tiered PSOGI classification (102) has been evaluated by two groups recently. In 2017, Huang et al (112) observed that median OS was not reached in acellular mucin and LG-PMP groups; it was 58.2 months in groups with HG-PMP and 31.1 months in HG-PMP with SRCs (HR 3.13; P<0.001). However, in 2018, Baratti and

colleagues (107) found that the two-tiered WHO classification (134) (HR 1.48; P=0.028) correlated better with OS than the PSOGI classification (102) (HR 1.22; P=0.149). They pointed out that having more categories decreases the number of patients in each, which reduces statistical power.

The main limitation of this review is that it is based on retrospective studies, so evidence supporting the PSOGI classification (102) is limited. Publication bias should also be considered as hand-picked studies (97), (99),(118) that did not fully meet the inclusion criteria were included and the 100-patient limit was met by most historically relevant studies. However, publications by Ronnett and colleagues (97), which provided the first histological classification, and Misdraji et al (99), which introduced LAMN into the literature, could not be excluded and setting a patient limit is essential to facilitate the selection process. Furthermore, comparison of modern studies using recent classification systems with older literature is difficult, despite detailed histological descriptions.

The standard treatment option for mucinous appendiceal tumours with PD (28) is CRS+HIPEC. However, this aggressive treatment strategy is associated with high morbidity and mortality rates (136), so patients must be selected carefully. There is enough evidence in the literature to argue in favour of the four-tiered PSOGI classification system (102). However, another international consensus should take place in order to propose a unified classification system. There is great need for a common language to fully convey and understand the prognostic significance, and develop management protocols for this disease.

Table 1 Comparison of oncological results according to the different histological grades

Reference	No. of patients	Histological classification	Histological nomenclature	OS (%)*	DFS (%)*	Impact of histology on OS and DFS in multivariable analysis
Ronnett *et al.*[8]	109	Ronnett's classification	DPAM (65) PMCA-I (11) PMCA (30)	75† 50† 14† (P = 0.001)	n.a.	n.a.
Misdraji *et al.*[9]	107		LAMN with PD (49) MACA with PD (4)	86† 44† (P = 0.004)	n.a.	n.a.
Bradley *et al.*[10]	101		MCP-L (78) MCP-H (23)	62.5† 37.7† (P = 0.004)	n.a.	n.a.
Stewart *et al.*[14]	110	Ronnett's classification	DPAM (55) PMCA-I (18) PMCA (29) HG non-mucinous (8)	77.4‡ 81.5‡ 35‡ 15‡ (P = 0.003)	n.a.	OS: n.s.
Smeenk *et al.*[15]	103	Ronnett's classification	DPAM PMCA-I PMCA	77.4† 40† 0†	n.a.	OS: increased risk of death in PMCA-I (HR 3.4; P < 0.001) and PMCA (HR 10.4; P < 0.001) *versus* DPAM DFS: increased risk of recurrence in PMCA-I (HR 1.9; P < 0.05) and PMCA (HR 4.1; P < 0.01) *versus* DPAM
Elias *et al.*[16]	105	Ronnett's classification	DPAM PMCA-I PMCA	n.a.	35.3† 16.4† (PMCA-I + PMCA) (P = 0.03)	DFS: increased risk of recurrence in PMCA-I + PMCA *versus* DPAM (HR 2.6; P = 0.02)
Pai *et al.*[17]	116		LG-LR LG-HR Mucinous ADC	100† 79† 28† (P < 0.001)	100† 88† 20† (P < 0.001)	Cytological features associated with decreased OS: extra-appendiceal neoplastic epithelium *versus* LG-LR (AM) (P = 0.006) and HG *versus* LG cytology (P = 0.001) Cytological features associated with decreased DFS: extra-appendiceal neoplastic epithelium *versus* LG-LR (AM) (P < 0.001) and HG *versus* LG cytology (P = 0.05)
Elias *et al.*[18]	301	Ronnett's classification	DPAM (136) PMCA-I (71) PMCA (59)	85† 84† 47† (P < 0.001)	n.s.	OS: decreased risk of death in DPAM + PMCA-I *versus* PMCA (HR 0.33; P = 0.02) DFS: n.s.
Chua *et al.*[19]	2298	Ronnett's classification	DPAM (1419) PMCA-I (140) PMCA (700)	82† 79† 59† (P < 0.001)	n.a.	OS: increased risk of death in PMCA *versus* DPAM + PMCA-I (HR 1.69; P < 0.001) DFS: increased risk of recurrence in PMCA *versus* DPAM + PMCA-I (HR 1.9; P < 0.001)
Carr *et al.*[11]	274	4th edition WHO	LG-PMP (207) HG-PMP (50)	84† 48† (P < 0.001)	69† 36† (P = 0.001)	n.a.
Overman *et al.*[20]	2469	7th edition AJCC	MAC (1375, stage IV): G1, G2, G3 SRCC (234, stage IV)	71†, 51†, 0†	n.a.	OS: increased risk of death in G2 (HR 1.56) and G3 (HR 5.15) *versus* G1

(continued)

Table 1. (continued)

Reference	No. of patients	Histological classification	Histological nomenclature	OS (%)*	DFS (%)*	Impact of histology on OS and DFS in multivariable analysis
						DFS: increased risk of recurrence in G2 (HR 1.73) and G3 (HR 1.93) *versus* G1
Shetty *et al.*[21]	211		PMP 1 (80) PMP 2 (75) PMP 3 (50)	85.7† 63.1† 32.2† ($P < 0.001$)	n.a.	OS: increased risk of death in G2 (HR 2.7) and G3 (HR 5.1) *versus* G1 ($P = 0.008$)
Davison *et al.*[22]	151	7th edition AJCC	PMP1 PMP2 PMP3	91† 61† 23† G1 *versus* G2 ($P < 0.001$) G2 *versus* G3 ($P = 0.07$)	n.a.	
Jimenez *et al.*[23]	202	Ronnett's classification	DPAM (77) PMCA (125)	83† 41† ($P < 0.001$)	58† 34† ($P = 0.003$)	OS: increased risk of death in PMCA *versus* DPAM (HR 3, 95% c.i. 1.4 to 6.1) DFS: increased risk of recurrence in PMCA *versus* DPAM (HR 2.1, 1.2 to 3.7)
Shaib *et al.*[24]	165	Ronnett's classification	DPAM (60) PMCA-I/D (15) PMCA (88)	98 months§ 39 months§ 28 months§ ($P < 0.001$)	n.a.	OS: increased risk of death in PMCA + PMCA-I/D *versus* DPAM (HR 3.53; $P = 0.007$)
Ihemelandu *et al.*[25]	494		PMCA (361) PMCA-S (80) PMCA-A (53)	38† 22† 15† ($P < 0.001$)	n.a.	OS: increased risk of death in PMCA-S *versus* PMCA (HR 1.4; $P = 0.033$)
Milovanov *et al.*[26]	208	Ronnett's classification and 7th edition AJCC	DPAM (84) IVA PMCA (47) IVB PMCA (77)	88† 67† 27† DPAM *versus* PMCA IVA ($P = 0.002$)	71† 43† 15† DPAM *versus* PMCA IVA ($P = 0.04$)	OS: increased risk of death in PMCA IVB *versus* PMCA IVA (HR 3.7; $P < 0.001$) and in HG *versus* LG histology (HR 3.1; $P = 0.001$) DFS: increased risk of recurrence in HG *versus* LG histology (HR 2.4; $P = 0.011$)
Asare *et al.*[27]	3105 stage IV	7th edition AJCC	G1 G2 G3	56.7† 31.5† 11.3†		OS: increased risk of death in G2 (HR 1.92) and G3 (HR 3.71) *versus* G1 ($P < 0.001$)
Grotz *et al.*[28]	265	7th edition AJCC	G1 (201) AM (34) G2 (45) G3 (19)	94† 100† 71† 21† ($P < 0.001$)	66† 93† 21† 20† ($P < 0.001$)	OS: increased risk of death with increasing grade (HR 1.8; $P = 0.008$) DFS: increased risk of recurrence with increasing grade (HR 2.8; $P = 0.01$)
Huang *et al.*[29]	444	PSOGI classification	AM (44) DPAM (232) PMCA (119) PMCA-S (49)	95.2† 83† 47† 12† ($P < 0.001$)	n.a.	OS: increased risk of death with increasing grade (HR 3.13; $P < 0.001$)
Reghunathan *et al.*[30]	197	PSOGI classification	AM (33) LG-MCP (114) HG-MCP (44)	n.a.	n.r.§ 34.4 months§ 16.8 months§ ($P < 0.001$)	DFS: increased risk of recurrence in LG-MCP (HR 9.8; $P = 0.025$) and in HG-MCP (HR 24.6; $P = 0.002$) *versus* AM
Baratti *et al.*[31]	265	PSOGI classification	AM (26) LG-PMP (197) HG-PMP (38) SRC-PMP (4)	89.3† 77.5† 51† 0†	n.a.	OS: increasing grade not associated with increased risk of

(continued)

Table 1. (continued)

Reference	No. of patients	Histological classification	Histological nomenclature	OS (%)*	DFS (%)*	Impact of histology on OS and DFS in multivariable analysis
						death (HR 1.22; P = 0.149)
Choudry *et al.*[32]	310	8th edition AJCC	All G1 PMP: AM (19) Scant cellularity (30) Moderate cellularity (242)	n.a.	100† 83† 27†	OS: n.s. DFS: increased risk of recurrence in moderate cellularity *versus* scant cellularity (HR 4.4; P = 0.02)
Munoz-Zuluaga *et al.*[33]	406	PSOGI classification	LG-MCP (Ex) HG-MCP (86) HG-MCP-S (65)	64† 25† (P < 0.001)	48† 14† (P < 0.001)	OS: increased risk of death in HG-MCP-S *versus* HG-MCP (HR 2.9; P < 0.001)
van Eden *et al.*[34]	225	PSOGI classification	AM (36) LG-PMP (149) HG-PMP (40)	93† 69.8† 55† (P < 0.001)	n.r. 41.9 months§ 28.1 months§	OS: n.s. difference in risk of death in LG-PMP (HR 3; P = 0.139) and HG-PMP (HR4.61; P = 0.052) *versus* AM DFS: n.s. difference in risk of recurrence in LG-PMP (HR 2.21; P = 0.06) and HG-PMP (HR 2.06; P = 0.139) *versus* AM
Masckauchan *et al.*[35]	109	Ronnett's classification	DPAM (35) PMCA-I (55) PMCA (19)	100† 78.1† 40.1† (P < 0.001)	n.a.	OS: increased risk of death in PMCA *versus* DPAM (HR 5.4; P = 0.009); n.s. in PMCA-I (HR 2.18; P = 0.149)
Narasimhan *et al.*[36]	175	PSOGI classification	AM (38) LG-PMP (119) HG-PMP (18)	100 months§ 36 months§ (P < 0.001)	34 months§ 22 months§ (P < 0.001)	OS: increased risk of death in HG-PMP *versus* LG-PMP (HR 10; P = 0.004)
Solomon *et al.*[37]	156	8th edition AJCC	All LAMNs: AM (25) G1 (127) G2 (2) G3 (2)	n.a.	82† 78† (P = 0.549)	DFS: n.s.
Legué *et al.*[38]	986		AC (56) MAC (83) SRCC (45)	13.3 months§ 31.2 months§ 16.2 months§	n.a.	OS: MAC has lower risk of death *versus* AC (HR 0.42, 95% c.i. 0.28 to 0.62), but differences between AC and SRCC n.s.
Levinsky *et al.*[39]	514		AC non-SRC (389) AC SRC (125)	91.4 months 32 months	32.4 months 17.1 months	OS: n.s. Subgroup analysis of OS within AC SRC: increased risk of death in G3 *versus* G1 (HR 5.6; P = 0.02)

*Values are †5- or ‡3-year survival rates unless indicated otherwise; §median survival. OS, overall survival; DFS, disease-free survival; DPAM, disseminated peritoneal adenomucinosis; PMCA-I, peritoneal mucinous carcinomatosis—intermediate; PMCA, peritoneal mucinous carcinomatosis; n.a., not applicable; LAMN, low-grade appendiceal mucinous neoplasm; PD, peritoneal dissemination; MACA, mucinous adenocarcinoma of appendix; MCP-L, mucinous carcinoma peritonei—low-grade; MCP-H, mucinous carcinoma peritonei—high grade; HG, high grade; n.s., not significant; HR, hazard ratio; LG-LR, low grade low risk; LG-HR, low grade high risk; ADC, adenocarcinoma; AM, acellular mucin; LG, low grade; PMP, pseudomyxoma peritonei; MAC, mucinous adenocarcinoma; SRCC, signet ring cell adenocarcinoma; PMCA-I/D, peritoneal mucinous carcinomatosis with intermediate/discordant features; PMCA-S, peritoneal mucinous carcinomatosis with signet ring cells; PMCA-A, peritoneal mucinous carcinomatosis with goblet positive periodic acid Schiff staining cells; PSOGI, Peritoneal Surface Oncology Group International; n.r., not reached; MCP, mucinous carcinoma peritonei; SRC, signet ring cell; Ex, excluded; MCP-S, Mucinous Carcinoma Peritonei with Signet Ring Cells; AC, non-mucinous adenocarcinoma.

Table-1. Comparison of oncological results according to different histological grades.

Table 2 Main histological classification systems

Reference/ classification	Stage of disease	Type	Histological nomenclature	Key histological features
Ronnett *et al.*[7]	Primary tumours	Benign lesions	Villous adenoma	Adenomatous epithelium with villous architecture confined to mucosa
			Cystadenoma	Adenomatous epithelium without villous architecture confined to mucosa of a dilated appendix
			Dilated/ruptured adenoma	Glands or strips of adenomatous epithelium within wall or on serosa of a dilated or ruptured appendix without stromal response Dissecting mucin or epithelium extending through wall of appendix
		Invasive lesions	Adenocarcinoma	Adenomatous epithelium invading muscularis of appendix accompanied by stromal response
			Mucinous adenocarcinoma with SRCs	Neoplasms with glandular and SRC differentiation, with or without neuroendocrine features that showed marked cytological atypia and muscularis invasion
	Peritoneal implants		DPAM	Scant strips of simple proliferative epithelium with minimal to moderate cytological atypia and no significant mitotic activity within abundant mucin
			PMCA I/D	Features of DPAM with focal areas of carcinoma +/– SRCs I: arising from a well differentiated mucinous adenocarcinoma D: arising from a villous adenoma with moderate to marked cytological atypia and areas of poorly differentiated carcinoma in wall and serosa of appendix
			PMCA	Abundant proliferative epithelium, glands, nests or individual cells including SRCs, demonstrating marked cytologial atypia and mitotic activity
Misdraji *et al.*[9]	Primary mucinous tumours		LAMN	Low-grade cytological atypia (nuclear enlargement, scarce nuclear stratification, and rare mitotic figures) and minimal architectural complexity (uniform, flat epithelial proliferation forming small papillary excrescences). No infiltrative invasion of appendiceal wall
			MACA	High cytological atypia (full-thickness nuclear stratification, vesicular nuclei with prominent nucleoli and brisk mitotic figures) and infiltrative invasion of appendicular wall
	Peritoneal implants		LAMN with peritoneal dissemination	Low-grade cytological atypia with flat epithelial proliferation forming papillary excrescences, low cellularity
			MACA with peritoneal dissemination	High-grade cytological atypia, destructive invasion of wall of appendix, high cellularity, abundant mitotic figures
PSOGI classification[42]	Primary mucinous tumours	Benign lesions	Serrated polyp with or without dysplasia	Tubular architecture with basal parts of crypts showing serration and dilatation. Muscularis mucosae intact
		Mucinous neoplasms	LAMN	Pushing invasion with loss of muscularis mucosae and fibrosis of submucosa. Filiform villi, undulating and flat. Basally orientated nuclei with minimal atypia and rare mitotic figures
			HAMN	Pushing invasion with loss of muscularis mucosae. Filiform villi, undulating, flat with pseudopapillae. Loss of

(continued)

Table 2. (continued)

Reference/ classification	Stage of disease	Type	Histological nomenclature	Key histological features
				nuclear polarity and frequent mitotic figures that may be atypical
			Mucinous adenocarcinoma	Infiltrating invasion (discohesive single cells or clusters of cells, small irreigular glands within desmoplastic stroma). Variably sized glands and islands, variable nuclear features and frequent mitotic figures that may be atypical. Can be well, moderately and poorly differentiated
			Mucinous adenocarcinoma with SRCs	Infiltrating invasion. Poorly differentiated, with <50% SRCs
			SRC carcinoma	Infiltrating invasion. Poorly differentiated, with >50% SRCs
	Peritoneal implants	No epithelial component	Mucin without epithelial cells	Acellular mucin. Abundant mucin without evidence of neoplastic epithelium. Extensive sampling required to discard presence of neoplastic epithelium
		Epithelial component	LG-PMP	Abundant mucin with low cellularity (< 20% tumour volume composed of neoplastic epithelium). Low-grade cytological features with low proliferative activity
			HG-PMP	Abundant cellularity (>20% tumour volume composed of neoplastic epithelium). High-grade cytological features with high proliferative activity (can be mixed with areas of low-grade cytological features). Infiltrative invasion into subjacenttissues. Must lack SRCs
			HG-PMP with SRC	Abundant cellularity (>20% tumour volume composed of neoplastic epithelium). High-grade cytological features with high proliferative activity. Infiltrative invasion into subjacent tissues. SRC component present
8th edition AJCC[43]	Primary lesions	Benign lesions	Adenoma	LAMN confined to mucosa with intact muscularis mucosae
		Premalignant lesions	High-grade dysplasia	Neoplastic cells confined to crypts that do not invade lamina propria
			Intramucosal adenocarcinoma	Neoplastic cells invade lamina propria with or without extension into, but not through, muscularis mucosae. pTis.
		Mucinous appendiceal neoplasms	LAMN	Neoplastic cells extend through wall of appendix with a pushing front, without features of invasion Tis (LAMN): LAMN confined by muscularis propria, acellular mucin or mucinous epithelium may extend into muscularis propria pT3: involvement of subserosa pT4a: involvement of visceral peritoneum (with acellular mucin or mucinous epithelium) pT4b: direct involvement of adjacent organs or structures
			HAMN	Tumours with architectural features of LAMN with areas of high-grade dysplasia. pT categorization follows that of mucinous adenocarcinoma
			Mucinous adenocarcinoma	Neoplastic epithelium displays infiltrative and destructive growth into wall of appendix, beyond muscularis mucosae. Associated desmoplastic reaction pT1: involvement of submucosa through muscularis mucosa

(continued)

Table 2. (continued)

Reference/ classification	Stage of disease	Type	Histological nomenclature	Key histological features
				pT2: involvement of muscularis propria pT3: involvement of subserosa or mesoappendix pT4a: involvement of visceral peritoneum (with acellular mucin or mucinous epithelium) pT4b: direct involvement of adjacent organs or structures
	Peritoneal implants	EIVA	M1a	Intraperitoneal acellular mucin without neoplastic epithelium in disseminated peritoneal mucinous deposits
			M1bG1	Intraperitoneal dissemination containing tumour cells with low-grade cytological atypia without SRCs. Low cellularity (<20%). No infiltrative invasion of peritoneum, may be involved with pushing front without desmoplastic reaction. Perineural or lymphovascular invasion rarely observed
		EIVB	M1bG2	Intraperitoneal dissemination containing tumour cells with mixture of low- and high-grade cytological atypia without SRCs. High cellularity (> 20%). Infiltrative invasion of peritoneum and into adjacent organs. Perineural or lymphovascular invasion may be present
			M1bG3	Intraperitoneal dissemination with tumour cells displaying adverse histological features. High cellularity (> 20%). Infiltrative invasion of peritoneum, adjacent organs. Perineural or lymphovascular invasion may be present

SRC, signet ring cell; DPAM, disseminated peritoneal adenomucinosis; PMCA, peritoneal mucinous carcinomatosis; PMCA-I/D, peritoneal mucinous carcinomatosis with intermediate/disconcordant features; PMCA, peritoneal mucinous carcinomatosis; LAMN, low-grade appendiceal mucinous neoplasm; MACA, mucinous adenocarcinoma of appendix; HAMN, high-grade appendiceal mucinous neoplasm; LG-PMP, low-grade pseudomyxoma peritonei/mucinous carcinomatosis peritonei; HG-PMP, high-grade pseudomyxoma peritonei/mucinous carcinomatosis peritonei.

Table-2. Main histological classification systems.

4.2 PAPER II: WHICH CLASSIFICATION SYSTEM DEFINES BEST PROGNOSIS OF MUCINOUS NEOPLASMS OF THE APPENDIX WITH PERITONEAL DISSEMINATION: TNM VS PSOGI?

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Abstract

Aims: Several classification systems are used for pseudomyxoma peritonei. The four-tiered classification system proposed by Peritoneal Surface Oncology Group International (PSOGI) and the two-tiered proposed by the eighth edition of the American Joint Committee on Cancer (AJCC) result from evolution in terminology and pathological insight. The aim is to evaluate the impact of PSOGI and eighth edition of the AJCC classifications on survival.

Methods: Pathological slides were reviewed from a prospectively maintained database including patients treated with cytoreductive surgery and hyperthermic intraperitoneal chemotherapy for an appendiceal mucinous neoplasm with peritoneal dissemination

between January 2009 and December 2019. Patients were reclassified according to PSOGI and AJCC eighth edition criteria. Survival analysis evaluated the impact of each classification system on overall survival (OS) and disease-free survival (DFS) while the concordance-index evaluated their predictive power.

Results: 95 patients were identified; 21.1% were reclassified as acellular mucin, 55.8% as low-grade mucinous carcinoma peritonei, 8.4% as high-grade MCP (HGMCP) and 14 as HGMCP with signet ring cells. Median OS was not reached, 5-year OS and DFS were 86.1% and 51.5%, respectively. Multivariate analysis revealed significant associations with OS (PSOGI: HR 10.2, p=0.039; AJCC: HR 7.7, p=0.002) and DFS (PSOGI: HR 12.7, p=0.001; AJCC: HR 3.7, p<0.001). The predictive capacity of both classification systems was unacceptable for OS and DFS (concordance-index values <0.7).

Conclusions: Both classification systems behaved similarly when stratifying our series into prognostic groups. The PSOGI classification provides better histopathological description, but histology alone is insufficient for adequate patient prognostication.

Keywords: appendix; cancer; chemotherapy; neoplasms; peritoneum; regional perfusion.

4.2.1 Introduction

Confusing terminology has surrounded mucinous tumours of the appendix with peritoneal dissemination (PD), condition previously known as pseudomyxoma peritonei (PMP). Several authors (97), (98), (99), (115), (116), (117) identified a wide range of biological behaviour according to histopathological features. However, lack of standardised histological classification systems delayed the development of treatment protocols.

Evolution in terminology and pathological insight resulted in recent classification systems. The Peritoneal Surface Oncology Group International (PSOGI) in 2016 (102) incorporated the terms low-grade appendiceal mucinous neoplasm (LAMN) (99) and high-grade AMN (HAMN) into the classification of primary lesions and subdivided peritoneal disease into four categories: acellular mucin (AM), low-grade mucinous carcinoma peritonei (LGMCP), high-grade MCP (HGMCP) and HG-MCP with signet ring cells (HGMCP-SRC), in order of biological aggressiveness. In cases with PD, the grade of the peritoneal disease conditioned prognosis. The eighth edition of the American Joint Committee on Cancer (103) and Union for International Cancer Control (137) (AJCC/UICC) defined stage IV disease with M (metastatic) and G (grade) categories. Stage IVa included AM (M1a) and cellular implants with low-grade features (M1bG1). Stage IVb grouped together cellular peritoneal implants with high-grade atypia (M1bG2) with any SRC component (M1bG3). Huang et al (112) found the four-tiered PSOGI classification to be associated with overall survival (OS) (HR 3.13, 2.34–4.39), whereas Baratti et al (107) were unable to reproduce this association (HR 1.22, 0.93–1.59).

Recent European clinical guidelines (28) have adopted the PSOGI's four-tiered classification (102) and reestablished cytoreductive surgery with hyperthermic

intraperitoneal chemotherapy (CRS+HIPEC) to be the gold standard treatment option for mucinous appendiceal tumours with PD as proposed by Sugarbaker (136) even in high-grade cases. The Chicago Consensus Guidelines (29) implemented the fifth edition of the WHO Digestive System Tumours, 2019 (138), (139) classification system and recommended neoadjuvant/adjuvant systemic chemotherapy (SC) in high-grade histology peritoneal implants. A classification system that stratifies patients according to prognosis is needed to develop standardised treatment and follow-up protocols and universalise the language surrounding this entity among the medical community.

The aim of our study was to determine which of the classification systems (PSOGI and AJCC/UICC eighth edition) correlates best with prognosis. Secondary aims were to identify other prognostic factors influencing survival.

4.2.2 Materials and methods

A retrospective study over a prospectively maintained database including consecutive patients treated by the Peritoneal Carcinomatosis Unit at our centre was designed. All patients diagnosed of appendiceal mucinous neoplasms with PD undergoing CRS+HIPEC between January 2009 and December 2019 were selected.

Pathology slides obtained during the CRS+HIPEC procedure were reviewed for the purpose of this study.

Clinical management

Preoperative work-up included clinical history, evaluation of the Eastern Cooperative Oncology Group (ECOG) (140) performance status; venous and oral contrast-enhanced CT scan of chest/ abdomen/pelvis and serum tumour markers (TM): carcinoembryonic antigen (CEA), cancer antigen (Ca) 19–9 and Ca–125. The indication of CRS+HIPEC was discussed at the multidisciplinary tumour board.

Intraoperatively, PD was evaluated and recorded with the Peritoneal Cancer Index (PCI) (57) CRS aimed to eradicate all macroscopic tumour. Targeted peritonectomy procedures were performed as described by Sugarbaker (62) and visceral resections consisted of: appendectomy, cecal pole excision or right hemicolectomy (determined by the primary tumour's histology), excision of the greater omentum, bilateral oophorectomy and hysterectomy (ovarian and uterine preservation could be considered in selected cases of young premenopausal women with gestational wish) and cholecystectomy. Other visceral resections or lymphadenectomies were completed when involved. Intestinal anastomoses were performed prior to chemoperfusate administration. Residual disease was registered according to completeness of cytoreduction score (CC) (57) CC0/1 scores were considered optimal CRS. HIPEC was delivered using the open 'coliseum technique'. Chemotherapeutic agent dosage was body surface area (BSA)-based; 35 mg/m2 for mitomycin-C (MMC) and 460 mg/m2 for oxaliplatin plus intravenous 5-fluorouracil (400 mg/m2) and leucovorin (30 mg/m2). Perfusate solution employed was peritoneal dialysis solution with 1.5% dextrose. The chemoperfusate volume used was twice the BSA. HIPEC duration varied according to HIPEC regimen: 90 min for MMC or 30 min for oxaliplatin.

Perioperative mortality and surgical complications were registered with the Clavien-Dindo classification (55) All cases were discussed at the multidisciplinary tumour board once pathology results were available. Postoperative SC was considered according to treatment

guidelines available at the time. Follow-up occurred every 3 months during the first year; two times a year during the second to fourth years and yearly after the fifth, up until the tenth year. Follow-up was carried out by treating surgeon or medical oncologist at our or referral centre. It included physical examination, serological (TM) and radiological CT-scan evaluation. If recurrence occurred; date, site and treatment offered was registered. The date of death (regardless of the cause) was registered.

Pathological evaluation

All slides from the intervention; primary tumour (if available) and peritoneal implants were reviewed by two pathologists at our centre: YG and MJF-A. Pathology slides were reassessed according to pathological criteria set by the PSOGI 2016 consensus (102).

Microscopic evaluation of the appendix distinguished between benign lesions, LAMN, HAMN and mucinous adenocarcinoma (MAC). Benign lesions (eg, serrated polyps) display no invasion. LAMN and HAMN are characterised by pushing invasion, whereas MAC demonstrate infiltrative growth (100). Cases with <50% of SRC were classified as MAC with SRC (w/ SRC) and cases with >50% of SRC as SRC carcinoma (SRCC). Goblet cell carcinoma was differentiated from SRCC because cells with neuroendocrine differentiation (chromogranin/synaptophysin positive immunohistochemical staining) are present among goblet cells (141) pT staging of the primary tumour (103), (137) was not evaluated.

Microscopic evaluation of peritoneal implants classified patients into AM, LGMCP, HGMCP and HGMCP-SRC (102) The absence of epithelial cells accompanied by a granulation-like response of the peritoneum characterised AM. Deposits with cytologically bland epithelial cells representing <20% of the tumour volume were classified as LGMCP,

and as HGMCP when neoplastic epithelium was more abundant (>20%) with high-grade cytological features. HGMCP-SRC had >10% SRC component. SRC were distinguished from degenerative SRC-like tumour cells. The location of SRC was also recorded: floating within mucin pools or invading tissues.

Patients were also classified following criteria of the eighth edition of the AJCC/UICC into stages IVa or IVb (103), (137). AM and LGMCP peritoneal implants were considered stage IVa and stage IVb included HGMCP and HGMCP-SRC groups.

Statistical analysis

Statistical analysis and data management was done using SPSS V.23.0 (IBM) and RStudio. Means of continuous variables with normal distributions were compared using the analysis of variance test. Non-parametric tests (Mann-Whitney U test or Kruskal-Wallis test) were used with continuous variables without normal distributions or few cases. Categorical data were analysed using Pearson's $\chi 2$. OS was calculated from the day of CRS+HIPEC until the patient's death regardless of the cause; disease-free survival (DFS), until diagnosis of recurrence. Survival analysis was performed using the Kaplan-Meier method and the log-rank test. Censored observations were used for patients lost during follow-up. Age and PCI cut-off values were chosen by receiver operating characteristic analysis. Multivariate analysis allowed identification and management of possible confounding variables (PCI, neoadjuvant/adjuvant SCT, CC score…), it was performed using a Cox-regression model. Deletion methods were used to handle missing data; therefore, models including variables with missing data were based on fewer observations. Statistical significance was defined at $p<0.05$. The predictive performance of both classification systems was evaluated using the Harrell Concordance Index (c-index). The c-index measures discrimination in survival

models; its value ranges from 0.5 (random chance) to 1.0 (total discrimination). Acceptable discrimination is considered with values >0.7.

4.2.3 Results

A total of 100 patients underwent CRS+HIPEC for a mucinous neoplasm of the appendix with PD between January 2009 and December 2019. After pathological evaluation, five patients were excluded: two revealed a MAC of colorectal origin; one, a serrated polyp; one, a goblet cell carcinoma and another lacked neoplastic epithelium and mucin. The final cohort consisted of 95 patients.

The appendix was available for pathological review in 66 cases. Reclassification according to PSOGI's criteria revealed 21 LAMN (31.8%); 36 MAC (54.5%); 5 MAC w/SRC (7.6%) and 4 SRCC (6.1%). No cases of HAMN were found. Review of peritoneal implants revealed 20 AM cases (21.1%); 53 LGMCP (55.8%), 8 HGMCP (8.4%) and 14 HGMCP-SRC (14.7%). There was concordance between appendiceal and peritoneal lesions ($p<0.001$). Seven LAMN were associated to AM (33.3%) and 14 to LGMCP (66.7%); 7 MAC (6 well-differentiated and 1 moderately-differentiated) were associated to AM (19.4%), 23 (17 well-differentiated and 6 moderately-differentiated) to LGMCP (63.9%), 5 moderately-differentiated to HGMCP (13.9%) and 1 poorly-differentiated to HGMCP-SRC (2.8%). All MAC w/SRC or SRCC associated HGMCP-SRC.

The mean age was 57.1 years. 63.2% were female patients. The rate of CC0/1 CRS was 92.7%. Oxaliplatin-based HIPEC regimens were most frequent (69.8%), followed by MMC-based regimens (30.4%). The clinical characteristics of the 95 patients are summarised in table 3. Median intraoperative PCI score was 23 (IQR 12–33); AM

associated lower PCI's (median 12; IQR 9–24, p=0.044). Nine patients had at least one pathological regional lymph node (LN). LN positivity related to high-grade primary tumours (p=0.003); 50% in MAC, 12.5% in MAC w/SRC and 37.5% in SRCC. Fourteen patients received preoperative SC; 11 received FOLFOX (+bevacizumab in 2) and 3 FOLFIRI with bevacizumab. Median number of cycles was 6. Twenty-eight patients received postoperative SC (31.1%); 2 received 5-fluorouracil, 16 FOLFOX (+bevacizumab in 2) and 5 FOLFIRI (+bevacizumab in 2). Preoperative and postoperative SC were significantly more frequent in HGMCP and HGMCP-SRC (see table 3). No differences regarding TM positivity (CEA, Ca 19–9 or Ca–125) were found across the different histological subtypes.

Perioperative 30-day mortality was 2.1%. One patient died on the 8th postoperative day due to a massive pulmonary thromboembolism; the second, on the 13th postoperative day from a septic shock secondary to mitral endocarditis. The incidence of perioperative grade III/IV Clavien-Dindo complications was 41.4%. Both postoperative mortality and morbidity occurred regardless of histological subtype.

Median follow-up was 49.2 months. Six patients were lost and 11 out of 89 patients died. Median OS of the whole cohort was not reached; 5-year OS was 86.1%. Disease recurred in 39 of 89 patients. Median DFS was 64.7 months (46.1–83.4) with 5-year and 10-year DFS rates of 51.5% and 43.8%, respectively.

Preoperative and postoperative SC, PCI, CC score, LN status and PSOGI and AJCC/UICC classifications influenced survival in the univariate analysis (see table 4 and figure 3). Median OS was not reached in AM nor LGMCP, and was 41.4 months in HGMCP and 56.3 months in HGMCP-SRC (p=0.002). Pairwise comparisons revealed significant differences

between AM and HGMCP-SRC (p=0.006) and between LGMCP and HGMCP (p=0.001). Similarly, median OS was not reached in stage IVa patients and was 56.3 months in stage IVb (p<0.001). In order to avoid collinearity effects, PSOGI and AJCC/UICC classifications were not included into the same model nor were primary tumours. Both classifications significantly influenced prognosis with similar HR; 10.2 (p=0.039) and 7.7 (p=0.002), respectively, and had similar discriminative capacities (c-index values of 0.685 and 0.669, respectively). Only PCI >21 retained its significance in the multivariate analysis with a HR of 11.4, p=0.022 (see table 4).

Preoperative SC, PCI, CC score, preoperative elevated TM, LN status and PSOGI and AJCC/UICC classifications were associated with DFS in the univariate analysis (see table 5 and figure 4). Median DFS was not reached in patients with AM, but was 60.9 months in LGMCP, 13.6 months in HGMCP and 8.8 months in HGMCP-SRC (p<0.001). In pairwise comparisons, differences were significant between AM and LGMCP (p=0.029), HGMCP (p<0.001) and HGMCP-SRC (p=0.002); and between LGMCP and HGMCP (p=0.008) and HGMCP-SRC (p=0.013). Median DFS of stage IVa patients was not reached and was 9.1 months in IVb patients (p<0.001). In multivariate analysis, both classifications were significantly associated with DFS with respective HR of 12.7 (p=0.001) and 3.7 (p<0.001). AM had significantly lower recurrence rates than every other histological subgroup (AM vs LGMCP (HR 4.95, p=0.03); AM vs HGMCP (HR 17, p=0.001) and AM vs HGMCP-SRC (HR 12.7, p=0.001)), whereas no differences were found between HGMCP and HGMCP SRC (p=0.6). However, similar c-index values indicated similar discriminative power of both classifications (0.669 and 0.623, respectively). Other risk factors of lower DFS were postoperative SC, PCI >21 and elevated TM (see table 5).

The location of recurrence was identified in the 39 patients. The most frequent site of recurrence was the peritoneum (in 29 patients, 74.4%), followed by multiple recurrence (>2 sites) in 6 patients (15.4%), followed by hepatic metastasis in 3 patients (7.7%) and 1 pleural involvement (2.6%). Systemic metastases occurred in 9 patients; 1 AM and 2 LGMCP patients developed liver metastases. Multifocal relapse occurred in 3 LGMCP and 3 HGMCP-SRC patients. Overall, peritoneal and systemic metastases occurred regardless of histological subtype (p=0.341).

4.2.4 Discussion

Mucinous neoplasms of the appendix with PD show a wide range of clinical outcomes. Histology is a major factor determining the disease's clinical course (97), (98), (99), (115), (116), (117). Recent classification systems (102), (103), (137) define concrete histological subgroups in an attempt to universalise the terminology used and standardise treatment protocols.

Much debate still stands on whether a two-tiered, three-tiered or four-tiered classification correlates best with prognosis. The PSOGI classification system (102) advocates for a four-tiered classification, whereas the AJCC/UICC eighth edition (103), (137) acknowledges two-tiers.

The categories with an epithelial component proposed by PSOGI (102) resemble those described by Davison et al (100) and Shetty et al (101). Davison et al (100) even mentioned a subgroup of patients with AM deposits that displayed more favourable prognosis. Several study groups have made efforts to evaluate the prognostic significance of the four-tiered classification. Huang et al (112) supported the four-tiers as histological subtype was

associated to OS in the multivariate analysis. The 5-year OS observed were 95.2% in AM, 83% in LGMCP, 47% in HGMCP and 12.6% in HGMCP-SRC. Baratti et al (107) found significant differences only in univariate analysis with 5-year OS across the different subgroups of 89.3%, 77.5%, 51% and 0%, respectively. They argued that more categories decreased the number of patients in each which reduced statistical power of tests. Additionally, Rufián-Andujar et al (142) concluded that the PSOGI classification correlated best with OS than the Ronnett classification with a HR of 2.47 (1.096–5.581).

The results from our series appear to concur with the two prognostic groups defined by the eighth edition of the AJCC/UICC (103), (137). AM and LGMCP showed excellent long-term Prognosis with respective 5-year OS rates of 95% and 94% (p=0.760) comparable to stage IVa (94.3%). HGMCP and HGMCP-SRC displayed significantly worse prognosis with respective 5-year OS rates of 50% and 30% (p=0.434) comparable to stage IVb (39.7%). However, the following factors must be emphasised.

First, our series could not reinforce the negative impact of SRC on survival. The AJCC seventh edition (135) had already been criticized for grouping patients with and without SRC into the same prognostic group (124). Ihemelandu and Sugarbaker (108) and Munoz-Zuluaga et al (105) described median OS in patients with SRC of 18.9 (HR 1.4, p<0.001) and 26.4 (HR 2.9, p<0.001) months, respectively. Failure to reproduce these results was likely due to the reduced number of patients with SRC (n=14) and unexpectedly higher median survival (56.3 months). Additionally, no conclusions could be drawn on the location of SRC because only 12 patients were available for the analysis and the 1 patient with SRC floating within mucin pools died prematurely at 13.4 months. Therefore, we could not provide evidence in favour or against the findings of Sirintrapun et al (118). Second, when recurrence is taken into consideration, our study highlights the higher recurrence rates of

high-grade tumours, as previously reported by many groups, (128), (129), (130) alongside the exceptionally low tendency of AM to recur when compared with cellular counterparts. Patients with AM displayed higher 5-year DFS rates (82.2%) when compared with LGMCP (51.2%) with a HR of 4.95 (p=0.03). Although the AJCC/UICC (eighth edition) introduced an independent category to exemplify the unique behaviour of this subgroup of patients (M1a), the singularity is later lost when grouped together with LGMCP into the same prognostic group (IVa).

Histology, as the main determinant factor of prognosis, conditions treatment and follow-up strategies. For example, SC has been found to benefit only high-grade histologies (86). Therefore, recent clinical guidelines (28), (29) consider neoadjuvant SC in high-grade tumours (initially not amenable to complete CRS+HIPEC) and not in low-grade histologies. A recent publication by Peritoneal Malignancy Institute (PMI) Basingstoke also recommends different follow-up protocols based on tumour histology (143). The Manchester study group (144) observed a 3% recurrence rate (at 12 and 56 months) in patients with AM arising from LAMN primary tumours. They advocate for a reduced surveillance time in this setting limiting annual CT scans to 5 years. In general, we support this view, as strict follow-up protocols may not be efficient in AM. However, we would like to highlight that the discriminative power of histology alone is not sufficient as none of the classification systems had a c-index value higher than 0.7 predicting OS and DFS. Therefore, we consider that other prognostic factors (serological TM, biological markers…) should be taken into consideration for a better patient stratification. Additionally, some histological landmarks are yet to be properly defined; the number of slides that should be evaluated to confidently label a patient as AM is controversial (145).

Our study is limited by its retrospective nature and small size (three out of four categories have <20 patients). This reduces statistical power to detect significant differences. Also selection bias may explain the good OS results of our HGMCP-SRC subgroup. Additionally, many patients treated at our centre are referred from other centres spread across the country. Follow-up is in many cases done at referral centre contributing to data loss.

In conclusion, both the AJCC/UICC eighth edition and the PSOGI classification systems behave similarly in terms of stratifying patients into prognostic groups in our series. Though, the PSOGI classification provides a better histopathological description and slightly better prognostic stratification when taking into consideration both OS and DFS. However, the discriminative power of histology alone is insufficient. Further studies with larger number of patients are required to establish the real impact of histology on survival and to identify other prognostic factors to aid a better patient stratification needed to develop more efficient treatment and follow-up schemes.

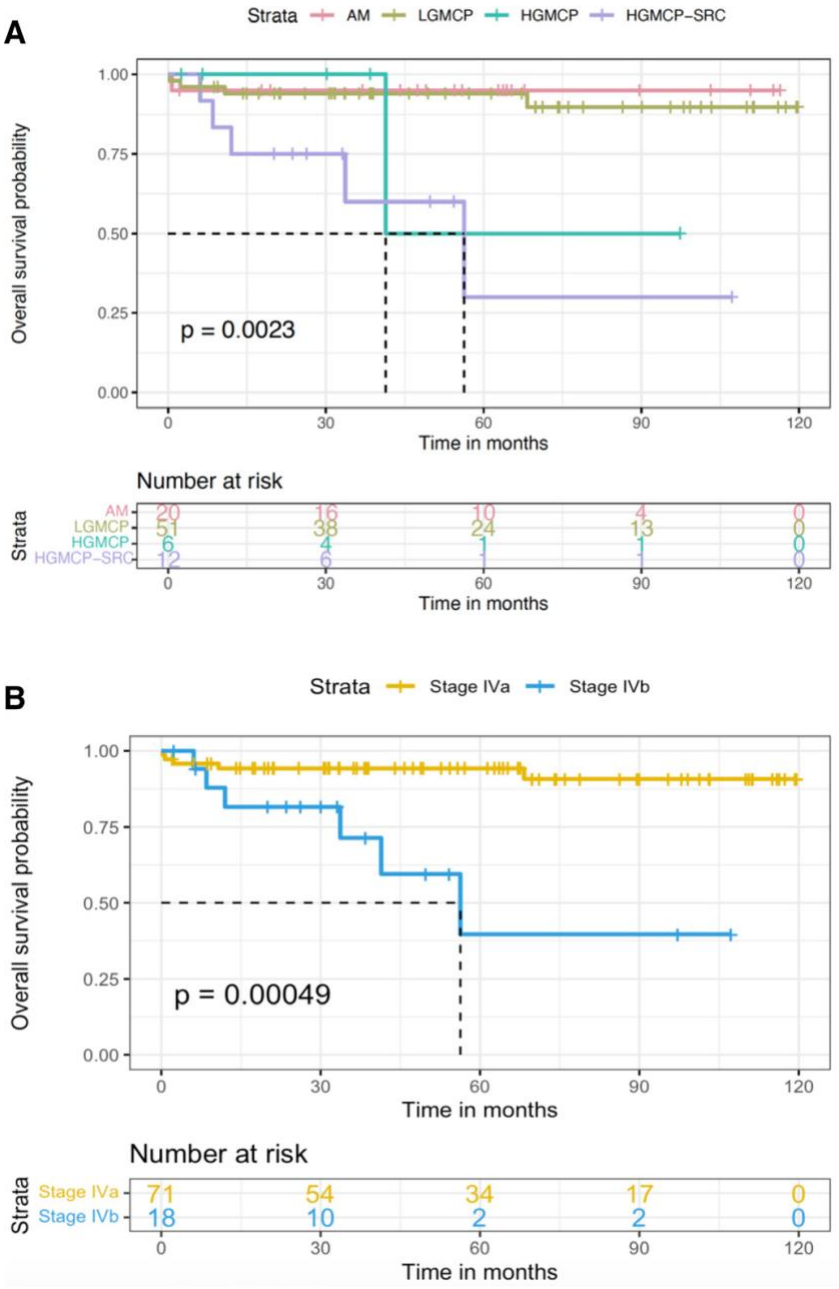

Figure-3. Overall survival according to Peritoneal Surface Oncology Group International classification of peritoneal implants (A) and eighth edition of the American Joint Committee on Cancer (B).
AM, acellular mucin; HGMCP, high-grade mucinous carcinoma peritonei; HGMCP-SRC, HGMCP with signet ring cells; LGMCP, low-grade MCP.

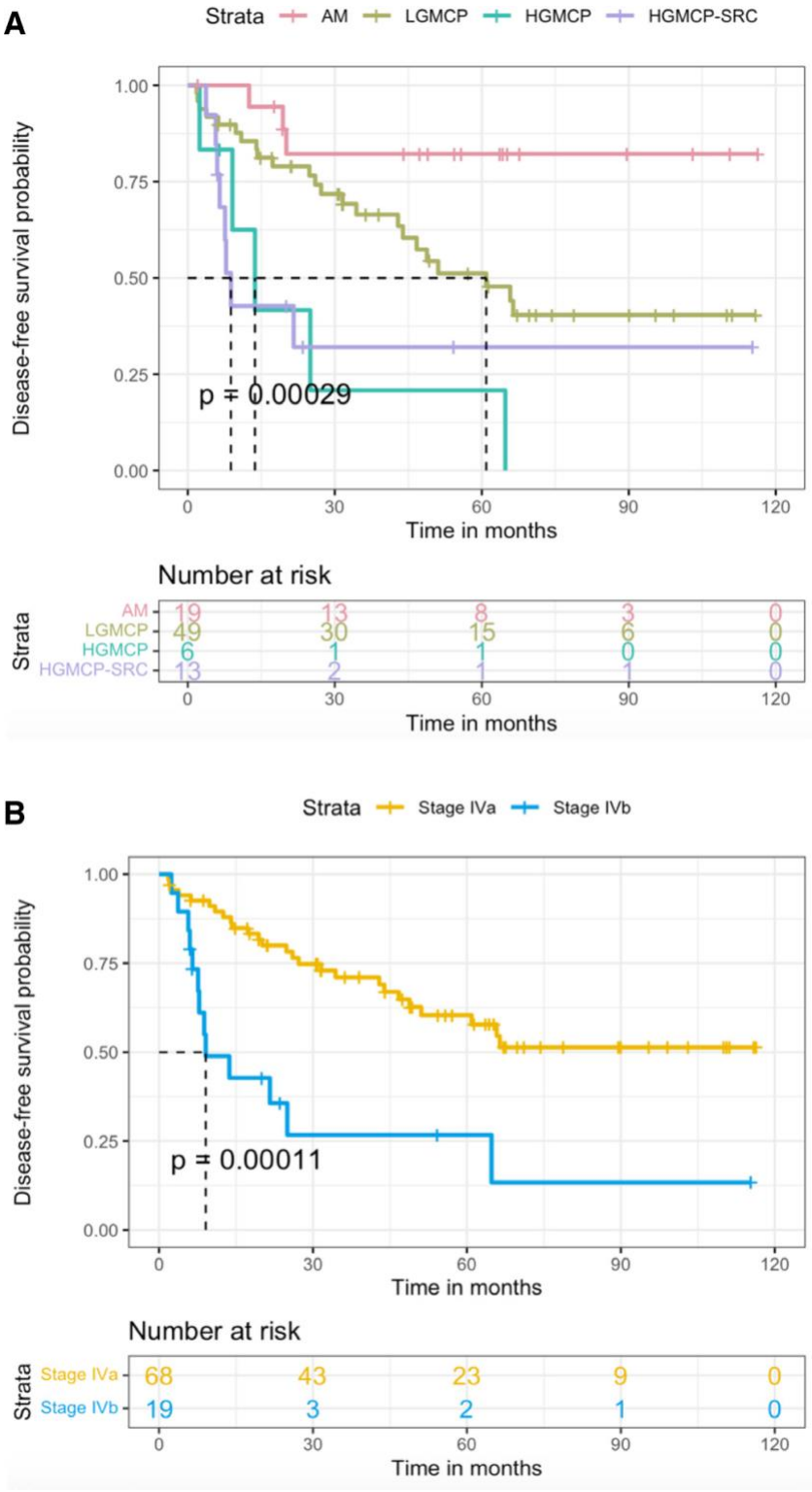

Figure-4. Disease-free survival according to Peritoneal Surface Oncology Group International classification of peritoneal implants (A) and eighth edition of the American Joint Committee on Cancer (B).

AM, acellular mucin; HGMCP, high-grade mucinous carcinoma peritonei; HGMCP-SRC, HGMCP with signet ring cells; LGMCP, low-grade MCP.

	Whole series (N=95)	AM (n=20)	LGMCP (n=53)	HGMCP (n=8)	HGMCP-SRC (n=14)	P value
Gender						P=0.920*
Male	35 (36.8)	8 (40)	18 (34)	3 (37.5)	6 (42.9)	
Female	60 (63.2)	12 (60)	35 (66)	5 (62.5)	8 (57.1)	
Age						P=0.112†
Mean (SD)	57.1 (12.1)	59 (3)	59 (2)	53 (5)	51 (3)	
ECOG score						P=0.996*
0	48 (65.8)	12 (70.6)	25 (61)	4 (66.7)	7 (77.8)	
1	21 (28.8)	4 (23.5)	14 (34.1)	1 (16.7)	2 (22.2)	
2	3 (4.1)	1 (5.9)	2 (4.9)	0	0	
3	1 (1.4)	0	0	1 (16.7)	0	
Preoperative SC	14 (14.7)	2 (10)	5 (9.4)	2 (25)	5 (35.7)	P=0.017*
Postoperative SC	28 (31.1)	3 (15.8)	8 (16)	5 (71.4)	12 (85.7)	P<0.001*
Primary	51 (53.7)	11 (55)	30 (56.6)	3 (37.5)	7 (50)	P=0.567*
Recurrent	44 (46.3)	9 (45)	23 (43.4)	5 (62.5)	7 (50)	
PCI						P=0.044‡
Median (IQR)	23 (12–33)	12 (9–24)	26 (19–34)	26 (7–37)	23 (11–32)	
CC score						P=0.131*
0	50 (52.6)	15 (75)	24 (45.3)	5 (62.5)	6 (42.9)	
1	37 (38.9)	5 (25)	24 (45.3)	2 (25)	6 (42.9)	
2–3	8 (8.4)	0	5 (9.5)	1 (12.5)	2 (14.3)	
CEA (>5 ng/mL)	35 (53.8)	4 (44.4)	22 (56.4)	2 (40)	7 (58.3)	P=0.743*
CA 19–9 (>37 UI/mL)	20 (34.5)	3 (33.3)	9 (27.3)	3 (75)	5 (41.7)	P=0.331*
Ca–125 (>35 UI/mL)	24 (36.9)	3 (33.3)	15 (37.5)	3 (60)	3 (27.3)	P=0.855*
Perioperative death	2 (2.1)	1 (5)	1 (1.9)	0	0	P=0.304*
Severe postoperative complications	39 (41.4)	8 (40)	20 (37.7)	3 (37.5)	8 (57.1)	P=0.320*
PSOGI classification 1° appendiceal lesion						P<0.001*
NA	29					
LAMN	21 (31.8)	7 (50)	14 (37.8)	0	0	
HAMN	0	0	0	0	0	
ADC	36 (54.5)	7 (50)	23 (62.2)	5 (100)	1 (10)	
ADC w/SRC	5 (7.6)	0	0	0	5 (50)	
SRCC	4 (6.1)	0	0	0	4 (40)	
LN						P=0.015*
N0	84 (90.3)	20 (100)	50 (96.2)	6 (85.7)	8 (57.1)	
N1a	6 (6.6)	0	2 (3.8)	1 (14.3)	2 (21.4)	
N1b	1 (1.1)	0	0	0	1 (7.1)	
N2	2 (2.2)	0	0	0	2 (14.3)	
LVI	2 (2.2)	0	0	0	2 (14.3)	P=0.005*
Perineural invasion	3 (3.2)	0	0	0	3 (21.4)	P<0.001*

*χ^2 test.
†Analysis of variance.
‡Kruskall-Wallis.
ADC, mucinous adenocarcinoma of the appendix; ADC w/SRC, ADC with signet ring cells; AM, acellular mucin; Ca, cancer antigen; CC, completeness of cytoreduction; CEA, carcinoembryonic antigen; ECOG, Eastern Cooperative Oncology Group; HAMN, high-grade appendiceal mucinous neoplasms; HGMCP, high grade mucinous carcinoma peritonei; HGMCP-SRC, HGMCP with signet ring cells; LAMN, low-grade appendiceal mucinous neoplasms; LGMCP, low-grade mucinous carcinoma peritonei; LN, lymph node status; LVI, lymphovascular invasion; NA, not available; PCI, Peritoneal Carcinomatosis Index; PSOGI, Peritoneal Surface Oncology Group International; SC, systemic chemotherapy; SRCC, signet ring cell carcinoma.

Table-3. Patient´s features.

	Median OS (months)	2-year OS	5 year OS	P value (log rank)	Multivariate analysis (Cox regression)
Sex				P=0.535	
Male	NR	93.6	89.2		
Female	NR	90.7	84.5		
Age				P=0.635	
≤52	NR	96.6	82.9		
>52	NR	89.6	86.6		
ECOG				P=0.258	
0	NR	97.6	91.2		
1	NR	81	75.2		
>2	NR	66.7	66.7		
Preoperative SC				P=0.005	HR 1.59 (0.373–6.78), p=0.531.
No	NR	91.8	91.8		
Yes	68.3	92.3	58.7		
Postoperative SC				P=0.002	HR 1.06 (0.103–10.62), p=0.970.
No	NR	96.6	96.6		
Yes	NR	91.5	70.1		
CEA (>5 ng/mL)				P=0.740	
No	NR	93	81.3		
Yes	NR	91.1	85.7		
CA 19–9 (>37 UI/mL)				P=0.142	
No	NR	91.7	85.1		
Yes	NR	90	71.6		
Ca–125 (>35 UI/mL)				P=0.869	
No	NR	89.9	85.7		
Yes	NR	100	82.5		
PCI				P=0.008	HR 11.4 (1.43–91.4), p=0.022
≤21	NR	97.4	97.4		
>21	NR	91.4	81.3		
CC score				P=0.011	HR 3.56 (0.65–19.4), p=0.143
CC0 (reference)	NR	97.8	94.2		
CC1	NR	82.1	76.6		
CC2–3	41.4	100	50		
HIPEC				P=0.267	
MMC	NR	96.2	96.2		
Oxaliplatin	NR	89.8	83		
PSOGI classification primary appendiceal tumour				P=0.020	
LAMN	NR	94.7	94.7		
ADC	NR	91.3	86.5		
ADC w/ SRC	33.7	80	0		
SRCC	56.3	75	0		
PSOGI classification peritoneal disease				P=0.002	HR 10.2 (1.13–91.1), p=0.039
AM (reference)	NR	95	95		
LGMCP	NR	94	94		
HGMCP	41.4	100	50		
HGMCP-SRC	56.3	75	30		
AJCC eighth edition				P<0.001	HR 7.71 (2.2–27.6), p=0.002
IVA	NR	94.3	94.3		
IVB	56.3	81.6	39.7		
LN				P<0.001	HR 2.89 (0.51–14.16), p=0.190.
N0	NR	93.3	89.5		
N+	56.3	75	37.5		

ADC, mucinous adenocarcinoma of the appendix; ADC w/SRC, ADC with signet ring cells; AJCC, American Joint Committee on Cancer; AM, acellular mucin; Ca, cancer antigen; CC, completeness of cytoreduction; CEA, carcinoembryonic antigen; ECOG, Eastern Cooperative Oncology Group; HGMCP, high grade mucinous carcinoma peritonei; HGMCP-SRC, HGMCP with signet ring cells; HIPEC, hyperthermic intraperitoneal chemotherapy; LAMN, low-grade appendiceal mucinous neoplasms; LGMCP, low-grade mucinous carcinoma peritonei; LN, lymph node status; MMC, mitomycin-C; NR, not reached; OS, overall survival; PCI, Peritoneal Carcinomatosis Index; PSOGI, Peritoneal Surface Oncology Group International; SC, systemic chemotherapy; SRCC, signet ring cell carcinoma.

Table-4. Univariate and multivariate analysis of risk factors of overall survival.

	Median DFS (months)	2-year DFS	5-year DFS	P value (log rank)	Multivariate analysis (Cox regression)
Sex				P=0.375	
Male	64.7	57.7	52.4		
Female	NR	76.6	51.3		
Age				P=0.088	
≤52	48.8	58.8	45		
>52	NR	75.4	55.5		
ECOG				P=0.732	
0	NR	69	59		
1	51.1	77.8	45.7		
>2	6.2	50	50		
Preoperative SC				P=0.009	HR 2.1 (0.93–4.87), p=0.076.
No	66.4	73.8	58.7		
Yes	13.7	46.2	27.7		
Postoperative SC				P=0.001	HR 4.1 (2.1–7.9), p<0.001.
No	150.3	98.3	85.3		
Yes	15.4	88	58.4		
CEA (>5 ng/mL)				P=0.029	HR 2.5 (1.03–6.1), p=0.043.*
No	NR	81.4	56.9		
Yes	26	55.5	38.4		
CA 19–9 (>37 UI/mL)				P<0.001	HR 5.5 (2.2–13.4), p<0.001.†
No	NR	75.9	63.4		
Yes	12.5	36.6	12.3		
Ca–125 (>35 UI/mL)				P=0.021	HR 2.5 (1.18–5.4), p=0.016.‡
No	NR	78.3	57.3		
Yes	25	56.6	27.2		
PCI				P=0.028	HR 1.52 (0.59–3.89), p=0.382.
≤21	NR	78.3	74.9		
>21	42.9	64.1	39		
CC score				P=0.004	HR 1.4 (0.32–6.14), p=0.652.
CC0 (reference)	65.8	74.9	59.4		
CC1	NR	71.8	52		
CC2–3	5.9	33	16.7		
HIPEC				P=0.672	
MMC	NR	64.3	57.8		
Oxaliplatin	65.8	73.9	55.2		
PSOGI classification primary appendiceal tumour				P=0.003	
LAMN	NR	86.1	64.6		
ADC	34.3	63.6	44.2		
ADC w/SRC	8.8	0	0		
SRCC	5.9	25	25		
PSOGI classification peritoneal disease				P<0.001	HR 12.7 (2.7–60.4), p=0.001.
AM (reference)	NR	82.2	82.2		
LGMCP	60.9	78.9	51.2		
HGMCP	13.6	41.7	20.8		
HGMCP-SRC	8.8	32.1	32.1		
AJCC eighth edition				P<0.001	HR 3.7 (1.8–7.3), p<0.001.
IVA	NR	79.9	60.4		
IVB	9.1	35.6	26.7		
LN				P=0.017	HR 1.8 (0.61–5.42), p=0.287.
N0	66.4	75.2	57.9		
N+	21.7	38.9	19.4		

*Model with 61 patients.
†With 40 patients.
‡With 35 patients.
ADC, mucinous adenocarcinoma of the appendix; ADC w/SRC, ADC with signet ring cells; AJCC, American Joint Committee on Cancer; AM, acellular mucin; Ca, cancer antigen; CC, completeness of cytoreduction; CEA, carcinoembryonic antigen; DFS, disease-free survival; ECOG, Eastern Cooperative Oncology Group; HGMCP, high-grade mucinous carcinoma peritonei; HGMCP-SRC, HGMCP with signet ring cells; HIPEC, hyperthermic intraperitoneal chemotherapy; LAMN, low-grade appendiceal mucinous neoplasms; LGMCP, low-grade mucinous carcinoma peritonei; LN, lymph node status; MMC, mitomycin-C; NR, not reached; PCI, Peritoneal Carcinomatosis Index; PSOGI, Peritoneal Surface Oncology Group International; SC, systemic chemotherapy; SRCC, signet ring cell carcinoma.

Table-5. Univariate and multivariate analysis of risk factors of disease-free survival.

4.3 PAPER III: VALIDATION OF A NOMOGRAM TO PREDICT RECURRENCE IN PATIENTS WITH MUCINOUS NEOPLASMS OF THE APPENDIX WITH PERITONEAL DISSEMINATION AFTER CYTOREDUCTIVE SURGERY AND HIPEC.

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Abstract

Background: Survival of patients affected by mucinous appendiceal neoplasms with peritoneal dissemination (PD) is mainly related to histopathological features. However, prognostic stratification is still a concern, as the clinical course of the disease is often unpredictable. The aim of this study is to construct and externally validate a nomogram predicting disease-free survival (DFS) in mucinous appendiceal neoplasms with PD treated by cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (CRS/HIPEC).

Patients and methods: Patients treated in two referral centers were included: Hospital General Universitario Gregorio Marañón, Madrid, Spain (derivation cohort) and

Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy (validation cohort). Cox regression analysis identified factors associated with shorter DFS in the derivation cohort. The nomogram performance was externally evaluated in the validation cohort using concordance index and calibration plots. Histology was classified according to the Peritoneal Surface Oncology Group International (PSOGI).

Results: The derivation cohort included 95 patients, and the validation cohort 348. Five-year DFS rates were 51.5 and 62%, respectively. Cox regression analysis (derivation cohort) identified PSOGI histology of the peritoneal components, number of preoperative elevated tumor marker, and peritoneal disease extent, as assessed by peritoneal carcinomatosis index, to be predictors of DFS. The model's predictive capacity was higher than that of PSOGI classification alone, with respective concordance indexes of 0.702 ± 0.023 and 0.610 ± 0.018 (validation cohort). The nomogram approximated the perfect model in the calibration plots at 3- and 5-year DFS.

Conclusions: An easy-to-use model that provides better prognostic stratification than histopathological features has been constructed. This nomogram may help clinicians in individualized survival predictions and informed clinical decision-making.

4.3.1 Introduction

Mucinous neoplasms of the appendix constitute a heterogeneous rare subgroup of malignancies with a tendency toward transcoelomic spread, causing the accumulation of mucin within the peritoneal cavity (14), (146). Recent consensus guidelines (28), (29) have established cytoreductive surgery plus hyperthermic intraperitoneal chemotherapy (CRS+HIPEC) as initially described by Sugarbaker et al (136) to be the gold-standard treatment option for these patients. However, despite achieving optimal cytoreduction, survival outcomes vary greatly among these patients (147), (148), (149).

Since the seminal study of Ronnett et al (97) it has been acknowledged that histopathological features of the peritoneal implants are the main factor determining prognosis, conditioning overall management and surveillance regimes (28), (29). Nevertheless, controversies regarding the terminology and pathologic classification of appendiceal primaries and their peritoneal dissemination (PD) have long persisted (99), (98), (122). Only in the year 2016 did an international consensus process by the Peritoneal Surface Oncology Group International (PSOGI) address these inconsistencies and develop a uniform pathological classification (102).

Several study groups (107), (112), (142), (150) have demonstrated a positive correlation between this recently established classification and overall survival (OS) and disease-free survival (DFS). However, histopathologic features alone have not been able to explain why some cases follow an aggressive clinical course (147), and others recur soon after optimal CRS+HIPEC treatment (148), (149). Other factors such as tumoral (47), (49), (151) and biological markers (85), (152), (153) must also influence biological aggressiveness and

should, therefore, be considered for adequate patient stratification, counseling, and treatment/surveillance strategy planning.

Mathematical prediction models have been used in patient stratification. Nomograms are easy-to-use and reliable prediction tools derived from these mathematical prediction models. The use of nomograms has been widely expanding, especially as a prognostic prediction tool in oncologic patients (154), (155), (156).

Taking advantage of our institutional cohort of appendiceal mucinous tumors with peritoneal dissemination (PD), the aim of the present study is to construct a nomogram predicting the risk for recurrence after CRS+HIPEC, and to externally validate it in a series from Istituto Nazionale dei Tumori at Milan, Italy.

4.3.2 Patients and methods

Derivation and Validation Cohorts

Patients treated with CRS+HIPEC for appendiceal mucinous neoplasms with PD at two tertiary HIPEC referral centers were selected. The derivation cohort included patients treated between January 2009 and December 2019 at the Peritoneal Carcinomatosis Unit of the Hospital General Universitario Gregorio Marañón (HGUGM), Spain. The external validation cohort included patients treated between 1995 and December 2019 at the Fondazione IRCCS Istituto Nazionale dei Tumori (INT), Italy.

In both centers, patient demographics alongside histological and treatment-related characteristics were collected in a prospectively maintained database. Patients undergoing

palliative debulking procedures were excluded from the analysis, as were patients with macroscopically incomplete cytoreductions (defined by completeness of cytoreduction (CC) score 2/3) (57) experiencing in-hospital postoperative death, or without available follow-up.

Local ethical approval was obtained for each of the participating centers.

Clinical Management

All patients underwent extensive preoperative work-up with clinical history, physical examination, venous and oral contrast-enhanced computed tomography (CT) scan of the chest, abdomen, and pelvis, and serum tumor markers (TM): carcinoembryonic antigen (CEA), carbohydrate antigen (CA) 19-9, and cancer antigen CA-125. TM determination in the HGUGM cohort occurred during preoperative assessment clinic (<1 month prior to CRS+HIPEC), and in the INT cohort, at time of surgical admission. TM were measured by immunoradiometric assay. Reference values were ≤ 5 ng/mL for CEA, ≤ 37 U/mL for CA 19-9, and ≤ 35 U/L for CA-125 for both centers alike. The indication of CRS+HIPEC was discussed at the respective multidisciplinary tumor meeting for all patients.

The operative technique performed at both centers has been previously described in detail (107), (150). Briefly, peritoneal dissemination was assessed and documented with the peritoneal cancer index (PCI) (57). CC score was classified as macroscopically complete (CC0); nearly complete: residual disease ≤ 2.5 mm in any region (CC1); or suboptimal: residual disease > 2.5 mm and ≤ 25 mm (CC2), or > 25 mm (CC3). The objective of CRS was to achieve optimal CRS (CC0/1) (57) by means of peritonectomy procedures (62) and (multi)visceral resections as required per case. In the derivation cohort (HGUGM), HIPEC

was administered using the open ‘‘coliseum technique.’’ Chemotherapeutic regimes used were: combination of intravenous 5-fluouracil 400 mg/m2 and folinic acid 20 mg/m2 administered 20 min before intraperitoneal oxaliplatin 460 mg/m2 for 30 min or mitomycin-C 35mg/m2 for 90 min. Chemoperfusate volume was calculated as twice the body surface area. In the validation cohort (INT), HIPEC was administered following the closed-abdomen technique with the extracorporeal circulation device Performer HRT (RAND, Medolla, Italy). Chemotherapeutic agents used were a combination of cisplatin (25 mL/m2 /L of perfusate) plus mitomycin-C 3.3 mg/m2 /L of perfusate until 2017, and, after, mitomycin-C 35 mg/m2 for 60 min. The perfusate volume used was 4–6 L.

Pathological Assessment

The PSOGI classification (102) subdivided peritoneal disease into four categories: acellular mucin (AM), low-grade mucinous carcinoma peritonei (LGMCP), high-grade MCP (HGMCP), and HG-MCP with signet ring cells (HGMCPSRC), by biological aggressiveness order.

Pathologic slides were reviewed by two pathologists (Y.G. and M.J.F.A.), and cases were reclassified in the derivation cohort according to the pathologic criteria set by the PSOGI 2016 consensus (102). In the validation cohort, pathology reports were reviewed and cases were retrospectively recategorized following the PSOGI classification. A detailed description of the pathological evaluation carried out at each center is provided elsewhere (107), (150). Cases with heterogeneous peritoneal histology (i.e., mostly LGMCP but with focal areas of high-grade dysplasia) were not found in the HGUGM cohort and were excluded from the INT cohort.

Statistical Analysis

DFS time was calculated from date of CRS+HIPEC up to date of recurrence. Patients were censored at date of last available follow-up.

Statistical analyses were performed using the R software (www.R-project.org) with the addition of the ‘‘survival’’ and ‘‘rms’’ packages. Statistical significance was set at $p<0.05$.

Nomogram Derivation (Derivation Cohort)

A multivariate, proportional hazard Cox model was performed to identify the variables in the derivation cohort independently associated with DFS alongside their hazard ratios (HR). The analyzed variables included: Eastern Cooperative Oncology Group (ECOG) performance status, age, gender, preoperative CEA, CA 19-9 and CA-125 status (normal/elevated), preoperative elevated TM (only when all three TMs were available), PCI, CC score (CC0/ CC1), pathology of peritoneal implants (PSOGI classification) (102), lymph node (LN) status, administration of pre and postoperative SCT, and Clavien–Dindo C 3 complications. Possible correlations between independent variables were studied to identify multicollinearity and confounding variables (categorical variables with the chi-square test and continuous variables with non-parametric Mann–Whitney U test). Introduction of multiple combinations of independent variables into the model was performed to evaluate possible confounding variables. Deletion methods were used to handle missing data. Variables with $p>0.1$ were removed from the model except for administration of systemic chemotherapy (SC), which was not included as it was considered to be time and location dependent.

Consequently, a nomogram was designed with the prognostically relevant variables using the nomogram function included in the ‘‘rms’’ package. The nomogram was set to facilitate estimations of DFS at 1, 3, and 5 years after CRS+HIPEC according to the individual sum score for each patient on the basis of the included variables.

Defining Low- and High-Risk Groups

A decision to divide the cohort into two opposite risk groups was taken with the aim of simplifying patient stratification at the cost of losing specificity, especially in patients with intermediate scores being classified into opposite extreme risk groups. A simple risk stratification is more useful from a clinical perspective; patients at high risk of recurrence may benefit from additional treatment, while patients at low risk could follow shorter and more relaxed surveillance protocols. However, the individual risk of recurrence at 1, 3, and 5 years could be calculated according to the nomogram.

The 50th centile sum score value of the derivation cohort was used as the cut-off value to define low- and high-risk groups. This same sum score value was used to categorize the validation cohort into the same prognostic groups. The Kaplan–Meier method and the log-rank test were used to evaluate and compare the survival probabilities of the model-estimated prognostic groups.

Nomogram Performance Evaluation (Internal and External Validation)

The nomograms’ discriminative ability and calibration were assessed (157). Discrimination refers to its ability of properly categorizing patients into prognostic groups, and is investigated by Harrell’s concordance index (C-index). The C-index is a proportion

between all pairs of subjects whose survival time can be ordered so that the subject with the longest predicted survival survives longer (158). Its value ranges from 0.5 (random chance) to 1.0 (total discrimination). Acceptable discrimination is considered with values >0.7. The Nagelkerke R2 statistic evaluates the amount of explained variation; values >0.4 define an acceptable model. The nomogram's calibration was assessed by plotting predicted versus observed probabilities.

Discrimination and calibration were evaluated in the external validation cohort to minimize overfitting of internal validation.

4.3.3 Results

Derivation and Validation Cohort

The derivation (HGUGM) cohort comprised 95 patients: 20 AM cases (21.1%), 53 LGMCP (55.8%), 8 HGMCP (8.4%), and 14 HGMCP-SRC (14.7%). Mean age was 57.1 years, and most were female (63.2%). Median PCI score was 23 (IQR 12–33), and rate of optimal CRS was 92.7%. Median follow-up time was 49.2 months (IQR 23–80.8 months). The 5-year OS rate was of 86.1%, and median OS was not reached. Disease recurrence was observed in 39 of the 89 cases (6 patients died during follow-up). Median DFS was 64.7 months (95% CI, 46.2–83.4 months) with 5-year DFS of 51.5%. After exclusion of missing data, CC2/3 patients, and postoperative deaths, data of 56 patients with 23 recurrences were available to construct the model.

The validation cohort (INT) consisted of 348 patients with 39 AM (11.2%), 254 LGMCP (73%), 49 HGMCP (14.1%), and 6 (1.7%) HGMCP-SRC cases. Mean age was 54.5 years,

and the majority were also female (54.9%). The rate of CC0/1 CRS was 97.4% with a median PCI score of 21 (IQR 11–28). Median follow-up time was also 49.2 months (IQR 16.3–103.6 months). Median OS was 162 months (120–203 months) with a 5-year OS rate of 86.4%. A total of 182 disease progressions were observed during follow-up. The median DFS was 44.3 months (95% CI, 30.6–58 months) with a 5-year DFS rate of 62%. The model was tested on 141 recurrences out of 278 patients available without any missing data once CC2/3 patients and perioperative deaths were excluded. All patient characteristics are summarized in Table 6.

Model Development (Derivation Cohort)

The multivariate Cox regression revealed the following factors to be significantly associated with DFS: PSOGI classification of peritoneal implants, PCI score, preoperative elevated TM, and postoperative SC (Table 7).

There was a correlation between PSOGI classification and LN status (AM 0% versus LGMCP 4.3% versus HGMCP 20% versus HGMCP-SRC 50%, $p<0.001$) and postoperative SC (AM 15.8% versus LGMCP 15.6% versus HGMCP 66.7% versus HGMCP-SRC 83.3%, $p<0.001$). Elevated CEA, CA 19-9, and CA-125 also associated with higher median PCIs (CEA: 19 versus 32, $p = 0.001$; CA 19.9: 22 versus 33, $p = 0.007$, and CA-125: 20 versus 31, $p = 0.016$), as did increasing number of preoperative elevated TM (0–1 TM: 20, 2 TM: 30, 3 TM: 33, $p=0.007$, 15–27). Testing of these variables in the multivariate Cox regression model did not change respective regression coefficients. Therefore, none acted as confounding variables, and could be excluded from the final model. However, inclusion of postoperative SC administration into the model [results not shown in Table 7; HR 4.1 (2.1–7.9), $p <0.001$] underestimated regression coefficients of the HGMCP-SRC group as a result

of selection bias. This, and the fact that postoperative SC administration is time and location dependent (rarely indicated at the INT and only indicated within a formal study protocol, EudraCT:2018-000655-40), favored the decision to exclude this variable from the model. On the other hand, inclusion of both preoperative elevated TM and PCI into the same model could induce multicollinearity.

These variables (PCI score, PSOGI classification, and preoperative TM elevation) were entered into a proportional hazard Cox regression model. PCI score had a linear association and was inserted as a continuous variable, whereas PSOGI classification and preoperative TM elevation were inserted as categorical variables. The variables with the strongest association with DFS were the HGMCPSRC subgroup [HR 21.5 (3.39–136), p=0.001] and the presence of all three TM elevated preoperatively [HR 8.02 (2.58–24.9), p<0.001]. A nomogram was constructed according to this model (Fig. 5). Only 1 point (out of 220 points total) separated patients with 0 and 1 elevated TM; therefore, they were grouped for simplification purposes. Regression coefficients, HR with 95% CI, and the corresponding points scored in the nomogram are presented in Table 7. The HR results of the included variables were also calculated for the validation cohort and are also presented in Table 7.

Model Performance (Derivation and Validation Cohort)

The 50th centile sum score value of the derivation cohort (75.8 points) was used as the cut-off value to define low- and high-risk groups. Figure 6 shows the Kaplan– Meier curves for DFS according to (a) the PSOGI classification and (b) low- and high-risk groups as defined by the new model.

The score of this new model could be calculated in 277 patients of the validation cohort (INT). A total of 111 patients were classified as low risk with 3- and 5-year DFS rates of 82.0% and 73.7%, respectively. The high-risk group included 167 patients, and their 3- and 5-year DFS were of 40.6% and 30.3%. Differences in DFS times were significant between both groups ($p<0.001$) (Fig. 7b). The discriminative ability of the new model (as a continuous variable) was internally and externally evaluated.

The discriminatory ability of the PSOGI and AJCC classifications was also assessed in both cohorts to provide reference values. Results are presented in Table 8. In the validation cohort (INT), the explained variation measured with the R2 statistic of the new model was higher than that of the PSOGI classification (0.184 versus 0.106, respectively). Also, the concordance (Harrell's C-index) of the new model was approaching the 0.7 acceptance value (0.702 ± 0.023) and was also higher than the concordance achieved by the PSOGI classification (0.61 ± 0.018).

Figure 8 shows the calibration plots demonstrating the concordance between predicted and observed 1-, 3- and 5-year DFS rates. The diagonal line traced in grey represents a perfect prediction model where the predicted and the observed DFS rates are equal.

4.3.4 Discussion

It is increasingly clear that histology alone, though historically thought to be the main conditioning factor of prognosis, is not able to fully explain the differences in survival outcomes after optimal CRS+HIPEC. Some cases with indolent histopathologic features show an aggressive clinical behavior with a rapidly progressing disease (147), and early recurrences negatively impact OS regardless of histological grade (102), (122).

Nevertheless, updated treatment guidelines mainly take into consideration pathologic grading when dictating surveillance or overall management schemes.

In this study, we constructed and externally validated a prognostic score to identify patients at higher risk of recurrence after initial CRS+HIPEC treatment. The combination of PCI, preoperative TM elevation alongside the PSOGI classification allowed for better patient stratification than PSOGI classification alone. This was demonstrated by the C-index results in both cohorts when compared with that of PSOGI classification. In both cases, the C-index obtained for the new model was higher than the acceptable 0.7 value (0.764 ± 0.06 in the derivation cohort, 0.702 ± 0.023 in the validation cohort). This nomogram can provide a reliable prognostic stratification, which is essential for patient selection and counseling, planning individualized treatment strategies, and risk adapted follow-up protocols.

Despite optimal CRS+HIPEC, the recurrence rate is as high as 30% (159), thus limiting OS. This validated nomogram will allow to identify patients at high risk of recurrence who may benefit from additional treatments such as systemic chemotherapy (86) or from stricter follow-up schemes. Prompt identification of locoregional recurrence is essential to offer iterative CRS+HIPEC procedures that result in OS advantage in selected cases (148), (160).

At the opposite end of the spectrum, radiation exposure secondary to unnecessary imaging may be reduced in low risk patients. This concept has been explored before by the PMI Basingstoke group (143), who advocate for a 5-year limited surveillance protocol in patients with AM. Corroborating this are the findings of Solomon et al (133) where no recurrences were observed in a cohort of patients with both acellular and cellular peritoneal deposits arising from primary low-grade mucinous neoplasm of the appendix (LAMN). However, the Manchester study group did report one early recurrence at 12 months and one late

recurrence at 56 months, near the end of the proposed surveillance period in AM patients (144). Also in the INT series, two patients with AM recurred and died of disease progression (107). This reinforces that histology alone lacks the predictive capacity to modulate the intensity of follow-up regimes in terms of duration and frequency. On the other hand, the nomogram proposed in this study tends to overestimate the risk of recurrence during the first year but then approximates to the ideal model when predicting 3- and 5-year DFS rates as seen in the calibration plots. Hence, its better prognostic accuracy will result in the development of more adequately risk-stratified surveillance regimes with an increased sensitivity for identification of patients at risk of early recurrences owing to its tendency to overestimate during the first year.

The variables included in the nomogram have been widely associated with DFS across existing literature. Histology was found to be one of the main predictors of DFS; the presence of HGMCP-SRC peritoneal deposits was the variable that scored the highest points in the nomogram (100 points). The PSOGI classification has been validated by many study groups with acceptable correlation with OS and DFS outcomes (107), (112), (142), (150). This coincides with the findings of this study as AM scored 0 points, LGMCP 22 points, HGMCP 38 points, and HGMCP-SRC 100 points.

Preoperative TM elevation has also been correlated with prognosis by individual study groups with a heterogeneous set of observations. CA 19-9 elevation has been associated with both decreased OS (151) and DFS (46), (49), (130) even when adjusted to PCI, proving that TM elevation is also representative of aggressive tumor biology, and not only higher disease burden (46). Similar findings were obtained by another study group with CEA elevation (128), while others found both CEA and CA-125 elevation to be predictors of shorter DFS (133). None of the TM showed a stronger association with DFS than the others,

but the combined elevation of all three scored the highest points in the nomogram, as expected. Additionally, the elevation of only one did not confer prognostic implications, therefore CEA, CA 19-9, and CA-125 should be preoperatively assessed in all patients.

The association of PCI with higher risk of recurrence has been widely established (130), (159), (161). PCI determination is the only variable included in the model that is obtained intraoperatively. Therefore, in cases with other high-risk factors (aggressive tumor histology and preoperative elevated TM), a more accurate investigation may be warranted with diffuse-weighted magnetic resonance imaging (DW-MRI) and staging laparoscopy. DW-MRI has shown to be more accurate in the detection of small peritoneal deposits and liver metastases in colorectal cancer patients (162), and staging laparoscopy has shown to decrease the number of open-and-close procedures and allow 74% of complete peritoneal evaluation (163).

The greatest strength of this study is that the performance of the proposed model has been evaluated on an external cohort. Nevertheless, it is subjected to limitations. First, patients treated in both centers represent retrospective and highly selected series that may not be fully representative of the general population of appendiceal lesions with PD. In particular, selection bias might have underestimated the negative impact of HGMCP-SRC. The limitation of missing data must also be considered, as they might have precluded further analyses. Secondly, molecular information shown to be associated with prognosis could not be included in the prognostic model leading to omitted variable bias, and the inclusion of correlated variables (elevated TM and PCI) could result in less reliable inferences due to multicollinearity. Thirdly, there exist differences between the centers in the proportions of patients diagnosed with each of the PSOGI pathological categories and receiving adjuvant SCT (not part of the standard of care at the INT). Also, HIPEC administration techniques

varied between centers in terms of open- versus closed-abdomen technique, dosage, and chemotherapeutic agents. Data comparing HIPEC techniques are inconclusive and drawn mainly from retrospective studies. In colorectal cancer, the open versus closed approach did not impact survival outcomes (164), while in appendiceal mucinous neoplasms with PD, a large retrospective multicenter study reported overall survival advantage in patients receiving a cisplatin plus mitomycin-C regime (53). Differences in HIPEC regime across the cohorts might have decreased the discriminative ability of the model, but this can also be considered as one of the strengths of this study as it reflects existing operative variations among centers (75).

4.3.5 Conclusions

This study constructed and externally validated a prognostic nomogram to predict the risk of recurrence in patients with appendiceal mucinous neoplasms with PD after initial CRS+HIPEC treatment. The nomogram demonstrated better predictive performance than the PSOGI classification. Future studies are warranted to confirm the model validity in large-scale, multicenter, prospective settings and to further increase its prediction accuracy by including molecular markers still under evaluation (i.e., Ki67 proliferation rate).

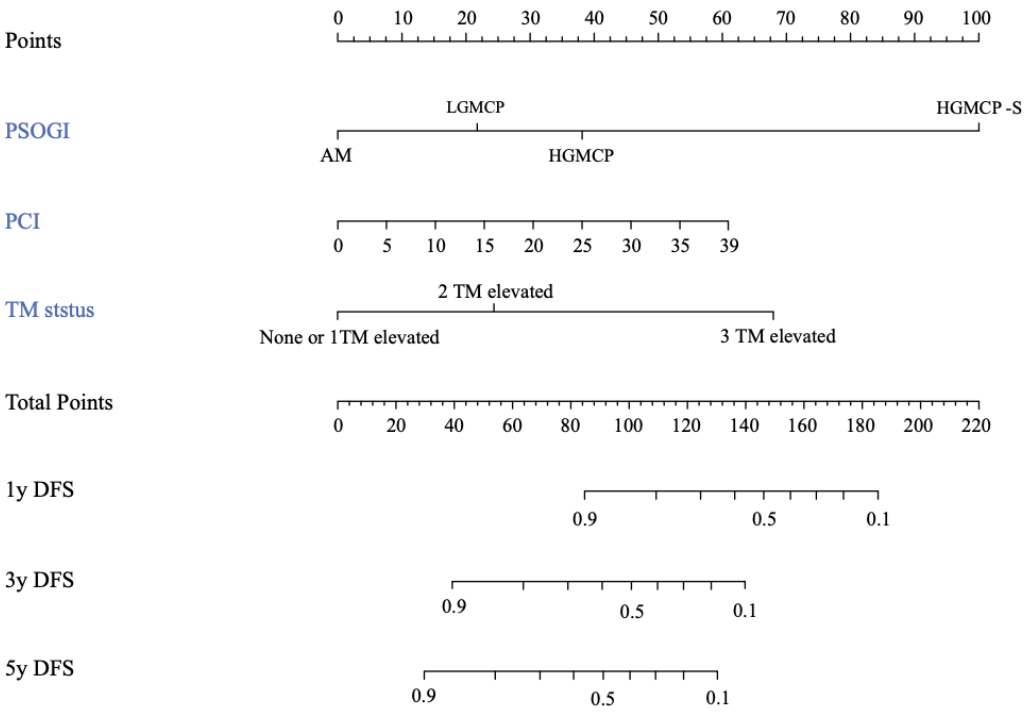

Figure-5. A nomogram to predict 1-, 3- and 5-year disease free survival (DFS) of patients with mucinous neoplasms of the appendix with peritoneal dissemination treated with CRS+HIPEC.
PSOGI-Classification of the Peritoneal Surface Oncology Group International; AM-Acellular mucin; LGMCP Low-grade Mucinous Carcinoma Peritonei; HGMCP High-grade MCP; HGMCP-S HGMCP with signet ring cells, PCI- Peritoneal Carcinomatosis Index, TM- number of elevated Tumour Markers.

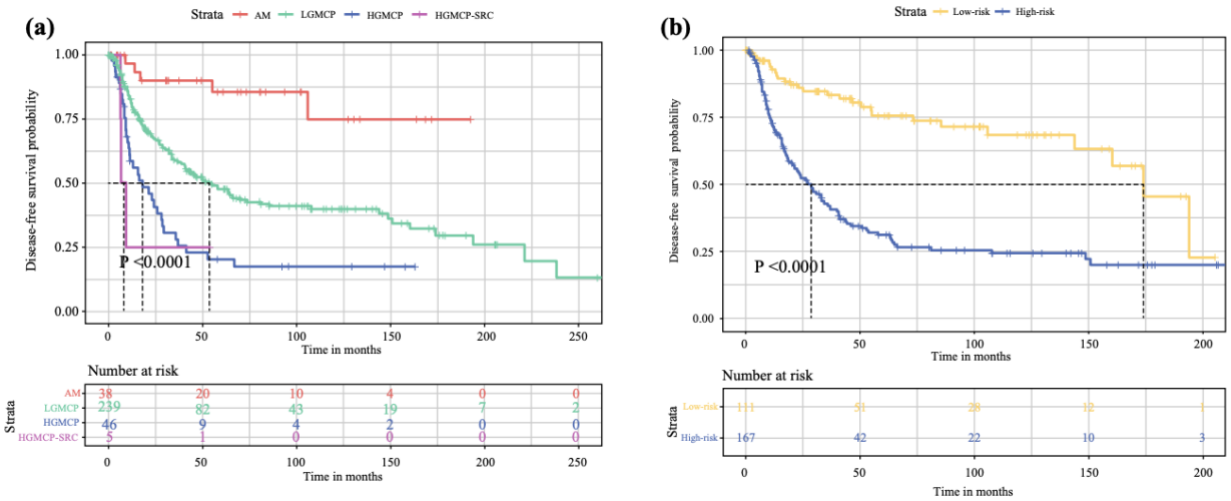

Figure-6. Disease-free survival curves of derivation cohort (HGUGM) according to (a) PSOGI classification and (b) low- and high-risk groups as defined by the new model.
a. Median disease-free survival was not reached in AM patients, but was 65.8 months in LGMPC, 13.6 months in HGMCP and 8.8 months in HGMCP-SRC (p<0.001). b. Median disease-free survival was not reached in low-risk group, and was 24.8 months in the high-risk group (p<0.001).

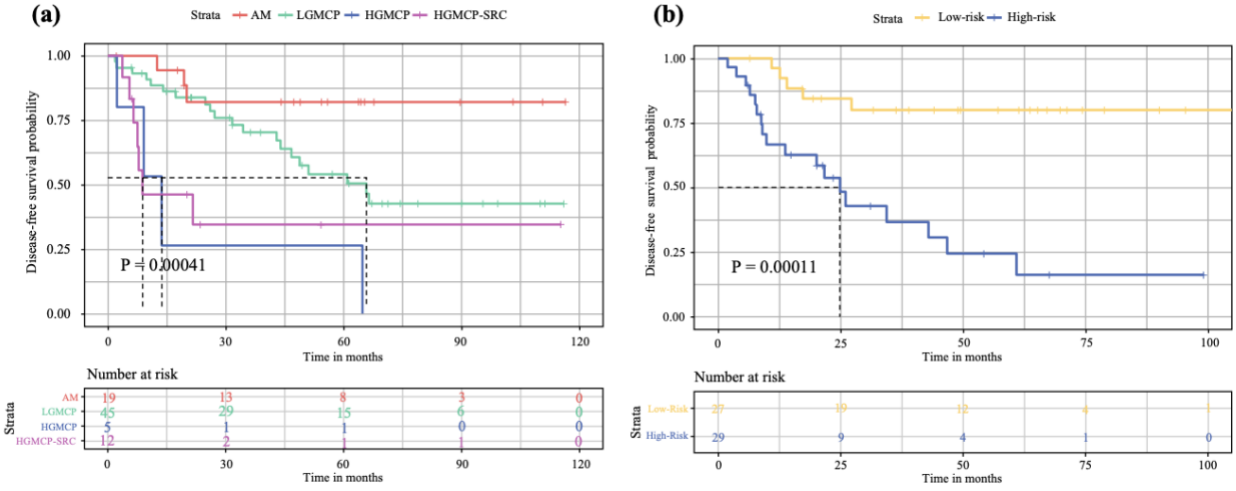

Figure-7. Disease-free survival curves of validation cohort (INT) according to (a) PSOGI classification and (b) low- and high risk groups as defined by the new model.
a Median disease-free survival was not reached in AM patients, but was 53.6 months in LGMPC, 18.1 months in HGMCP and 6.5 months in HGMCP-SRC (p<0.001). b Median disease-free survival was 173.9 months in low-risk group, and 28.6 months in the high-risk group (p<0.001).

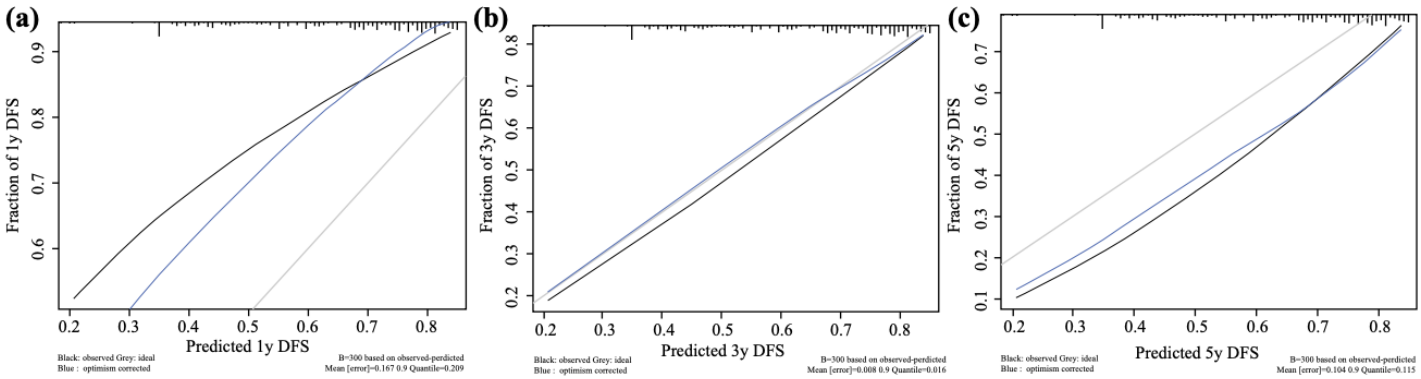

Figure-8. Calibration plots showing the observed against the predicted survival (black line) for (a) 1-year disease-free survival, (b) 3-year disease-free survival and (c) 5-year disease-free survival.
Black and blue (optimism corrected) lines represent the model, the grey line represents the ideal model where observed event are equal to the predicted events.

	Derivation cohort	Validation cohort
Global	95	348
Gender		
Male	35 (36.8)	157 (45.1)
Female	65 (63.2)	191 (54.9)
Age		
Mean (SD)	57.1 (12.2)	54.5 (12.5)
ECOG		
0–1	69 (94.5)	345 (99.1)
2–3	4 (5.5)	3 (0.9)
Primary	51 (53.7)	Missing
Recurrent	44 (46.3)	
CEA		
Available in	66	339
CEA > 5	36 (54.5)	194 (57.2)
CA 19-9		
Available in	62	339
CA 19-9 > 37	20 (32.3)	145 (42.8)
Ca-125		
Available in	66	307
CA-125 > 35	25 (37.9)	136 (44.3)
All three TM available	61	283
Preoperative SC	14 (14.7)	81 (23.2)
Postoperative SC	23 (31.1)	18 (5.2)
PCI		
Median (IQR)	23 (12-33)	21 (11-28)
CC score		
0–1	87 (91.6)	339 (97.4)
2–3	8 (8.4)	9 (2.6)
HIPEC		
OXL	28 (30.4)	
MMC	64 (69.6)	348 (100)[b]
PSOGI classification 1° appendiceal lesion	66 available	239 available
LAMN	21 (31.8)	195 (56)
HAMN	0	34 (9.8)
ADC	36 (54.5)	46 (13.1)
ADC with SRC	5 (7.6)	6 (1.7)[a]
SRCC	4 (6.1)	
PSOGI classification peritoneal implants		
AM	20 (21.1)	39 (11.2)
LGMCP	53 (55.8)	254 (73)
HGMCP	8 (8.4)	49 (14.1)
HGMCP-SRC	14 (14.7)	6 (1.7)
LN		
N0	82 (90.1)	333 (95.7)
N+	9 (9.9)	15 (4.3)
LVI	2 (2.1)	Missing
Perineural invasion	3 (3.2)	Missing
Perioperative death	2 (2.1)	11 (3.2)
Severe postoperative complications	39 (41.1)[c]	124 (35.6)[d]
Median follow-up in months (IQR)	49.2	49.2
OS outcomes		
Median (months) (120.7–203.1)	NR	161.9
2-year OS (%)	91.9	86.4
5-year OS (%)	86.1	73.6
DFS outcomes		
Recurrences	39	182
Median (months)	64.7	(46.1–83.4)
44.3 (30.6–58)		
2-year DFS (%)	70.8	62
5-year DFS (%)	53.6	44.8

[a]Includes both patients with ADC with SRC and SRCC

[b]The first 265 patients were treated with mitomycin C (3.3 mg/m^2/L of perfusate) plus cisplatin (25 mL/m^2/L of perfusate); the following patients were treated only with mitomycin-C 35 mg/m^2 for 60 min

[c]Clavien–Dindo classification > 3

[d]CTCAE classification > 3

ECOG Eastern Cooperative Oncology Group, *CEA* Carcinoembryonic antigen, *CA 19-9* carbohydrate antigen 19-9, *CA-125* Cancer antigen 125, *TM* Tumor markers, *SC* Systemic chemotherapy, *PCI* Peritoneal carcinomatosis index, *CC* Completeness of cytoreduction, *HIPEC* Hyperthermic intraperitoneal chemotherapy, *MMC* Mitomycin C, *OXL* Oxaliplatinum, *NA* Not available, *LAMN* Low-grade appendiceal mucinous neoplasm, *HAMN* High-grade appendiceal mucinous neoplasm, *ADC* Mucinous adenocarcinoma of the appendix, *ADC with SRC* Mucinous adenocarcinoma of the appendix with signet ring cells, *SRCC* Signet ring cell carcinoma, *PSOGI* Peritoneal Surface Oncology Group International, *AM* Acellular mucin, *LGMCP* Low grade mucinous carcinoma peritonei, *HGMCP* High-grade mucinous carcinoma peritonei, *HGMCP-SRC* High-grade mucinous carcinoma peritonei with signet ring cells, *LN* Lymph node status, *LVI* Lymphovascular invasion, *OS* Overall survival, *DFS* Disease free survival, *NR* Not reached, *NA* Not applicable

Table-6. Patient characteristics of derivation and validation cohort.

Variable	Derivation cohort[a]				validation cohort[b]	
	Regression coefficient	Hazard ratio (95% CI)	*P*-value	Points scored in nomogram	Hazard ratio (95% CI)	*P*-value
PCI (PER POINT)	0.047	1.05 (1.00–1.10)	0.056	1.55	1.04 (1.02–1.06)	< 0.001
PSOGI classification peritoneal implants						
AM (reference)				0		
LGMCP	0.667	1.95 (0.42–8.98)	0.340	22	2.22 (0.88–5.61)	0.09
HGMCP	1.168	3.22 (0.38–27.4)	0.300	38	4.17 (1.56–11.1)	0.004
HGMCP-SRC	3.066	21.5 (3.39–136)	0.001	100	11.4 (2.67–48.5)	< 0.001
Preoperative TM status						
0–1 TM elevated (ref.)				0		
2 TM elevated	0.748	2.11 (0.64–7.02)	0.200	24	1.43 (0.90–2.27)	0.13
3 TM elevated	2.082	8.02 (2.58–24.9)	< 0.001	68	1.76 (1.13–2.75)	0.012

[a]Model based on 56 patients with the preoperative values of the three tumor markers available

[b]Model results based on 278 patients with the preoperative values of the three tumor markers available

CI Confidence interval, *PCI* Peritoneal carcinomatosis index, *PSOGI* Peritoneal Surface Oncology Group International, *AM* Acellular mucin, *LGMCP* Low-grade mucinous carcinoma peritonei, *HGMCP* High-grade mucinous carcinoma peritonei, *HGMCP-SRC* High-grade mucinous carcinoma peritonei with signet ring cells, *TM* Tumor markers

Table-7. Cox regression model and points scored in the nomogram for each variable obtained from derivation cohort. Calculation of hazard ratios of the model variables in the validation cohort.

	Derivation cohort (HGUGM)	Validation cohort (INT)
Nagelkerke R^2 PSOGI	0.177	0.106
Nagelkerke R^2 new model	0.425	0.184
Harrell's C-index PSOGI (SE)	0.676 (0.045)	0.601 (0.018)
Harrell's C-index new model (SE)	0.764 (0.060)	0.702 (0.023)

C-index was calculated for patients categorized into low- and high-risk groups

SE Standard error, *PSOGI* Peritoneal Surface Oncology Group International, *HGUGM* Hospital General Universitario Gregorio Marañón, *INT* Istituto Nazionale dei Tumori

Table-8. Comparison of the Nagelkerke R2 and C-indexes between the proposed nomogram and the PSOGI classification in derivation and validation cohorts.

GENERAL DISCUSSION

5. GENERAL DISCUSSION.

This investigation is compounded of three research items with the global aim of analysing why there is such disparity in terms of survival outcomes after optimal CRS+HIPEC treatment for PMP patients.

The investigation adheres to the following structure:

- Paper I is focused on identifying histopathological characteristics of both primary appendicular lesions and peritoneal deposits that have been associated with prognosis by previous study groups and, consequently, have resulted in the development of various histopathological classifications.
- Paper II fixates on analysing the correlation between survival outcomes and two of the most recent classification systems that have been incorporated into recent treatment guidelines (PSOGI 2016 (102) and the AJCC 8th edition (103)).
- Paper III concentrates on combining factors that were associated with DFS in Paper II to develop a nomogram to predict the risk of recurrence after CRS+HIPEC treatment at 1-, 3-, and 5-years. The performance of this model was validated on an external cohort from a referral centre in Italy.

5.1 IN DEPTH ANALYSIS OF HISTOPATHOLOGICAL FEATURES

5.1.1 Primary appendiceal mucinous tumours

Relevant advances regarding the terminology and pathological descriptions of the primary appendiceal lesions originating PMP were achieved by Misdraji and co-workers (99). They established the term low-grade appendiceal mucinous neoplasm (LAMN) to describe

primary appendicular lesions that lacked infiltrative invasion of the appendicular wall but could spread through to the peritoneal cavity. LAMNs exhibited low-grade cytological atypia (nuclear enlargement, sparse nuclear stratification, and rare mitotic figures) and minimal architectural complexity (uniform, flat epithelial proliferation resulting in small papillary excrescences/outgrowths). In opposition, mucinous adenocarcinomas of the appendix (MACAs) were defined by infiltrative invasion of the appendicular wall with high cytological atypia.

The term LAMN was incorporated into the PSOGI classification in 2016 (102). It was distinguished from benign lesions (i.e serrated polyps) by a pushing-type of invasion of the appendiceal wall with loss of the *muscularis mucosae* and fibrosis of the submucosa. The term for the high-grade counterpart (HAMN) was also contemplated in the PSOGI classification. LAMN and HAMN were distinguished from a mucinous adenocarcinoma of the appendix by their characteristic pushing-type invasion and lack of infiltrative destruction of the appendiceal wall characteristic of adenocarcinomas. Mucinous adenocarcinomas could be well, moderately and poorly differentiated. In the presence of SRC, primary lesions could be referred to as mucinous adenocarcinomas with SRC (when adenocarcinomas were poorly differentiated has SRC represented <50% of tumour cellularity) or SRC carcinoma (when SRC represented >50% of total cellularity).

Important advances regarding the classification of primary lesions of the appendix were introduced into the 8th edition of the AJCC classification system (103). LAMN lesions followed pT categorization slightly different to that of HAMN and adenocarcinomas. pT1 and pT2 categories were not included to define LAMN lesions as the *muscularis mucosae* is not present in LAMN lesions, and involvement of the *muscularis propria* is not of prognostic significance in these lesions. pT3 describes lesions extending onto the subserosa

and, pT4a, lesions extending onto the visceral peritoneum (with acellular or cellular mucin). The prognostic significance of this subclassification, and of the presence of acellular versus cellular mucin on the serosal surface is currently under evaluation.

5.1.2 Peritoneal mucinous deposits

In the setting of peritoneal dissemination, the prognosis is mainly determined by the characteristics of the peritoneal implants rather than by that of the primary tumour. In paper I, all the most relevant classification systems were reviewed in chronological order as advances in histopathological descriptions and terminology were made.

Differences in survival were initially correlated with differences at histopathological level by the seminal study published by Ronnett et al (97) in 1995. This study group observed that patients with peritoneal deposits that were made up of abundant extracellular mucin and scarce indolent epithelial cells (DPAM) had better prognosis than those where the peritoneal lesions were composed of abundant mucinous epithelium with high mitotic activity and cytological features of carcinoma (PMCA). They also described a third intermediate category that grouped lesions that were inconsistent or discordant (PMCA I/D). Ronnett's classification was the first and also most widely spread classification system up until recently. However, its prognostic significance was questioned by early study groups. In 2006, Bradley et al (98) evaluated the prognostic implications of the classification proposed by Ronnett in their cohort and found no significant differences between the DPAM and PMCA-I/D subgroup (5-year OS rates of 61.8% and 68.2%, respectively), while prognosis for the PMCA was significantly worse (5-year OS rate 38%, p=0.004).

Davison et al (100) made other significant advances in the development of the PMP classification as we know it today. They described pathological features linked to poorer survival outcomes, such as the presence of destructive invasion, high cytologic grade, high tumour cellularity, angiolymphatic invasion, perineural invasion, and the presence of SRC. SRC needed to be invasive and account for at least 10% of the tumour cellularity. Following this, they provided pathologic descriptions for the AJCC three-tiered classification; grade G1 included cases with no adverse features; G2, included those with at least one adverse feature except SRC, and G3 included those cases with SRC which anticipated worse prognosis. Additionally, they described that 7% of patients in the G1 group had mucinous deposits without any epithelial cells, and these were at a much lower risk of disease recurrence compared to the epithelial counterpart.

Consequently, a Delphi method survey was carried out amongst experts on peritoneal surface malignancies at the PSOGI meeting in 2016. Results from this Delphi method advocated for the existence of four different prognostic subgroups: AM, LGMCP, HGMCP and HGMCP-SRC (102). AM was defined by the absence of epithelial cells, which was accompanied by a granulation-like response of the peritoneum. Deposits consisting of cytologically bland epithelial cells representing up to 20% of the tumour volume were classified as LGMCP, and deposits with neoplastic epithelium with high-grade cytological features, marked atypia and high-proliferative activity were classified as HGMCP. The presence of SRC (at least 10%) within the mucinous deposits corresponded to HGMCP-SRC. AM was considered to be the category with the best prognosis, whereas HGCMP-SRC the most aggressive one.

Lastly, the 8th edition of the AJCC classification (103) adopted terminology derived from the PSOGI consensus (102). M (metastatic) and G (grade) categories were used to define

stage IV disease. M1a represents intraperitoneal spread of acellular mucin; M1b represents peritoneal implants containing tumour cells; and M1c represents metastasis to sites other than the peritoneum. Based on cytological features, tumour cellularity, and the presence of SRCs, the G category was subdivided into three relevant prognostic groups. G1 was a well-differentiated adenocarcinoma with low-grade cytological atypia, low cellularity (less than 20%), and no invasion or SRCs. G2 was defined as a moderately differentiated mucinous adenocarcinoma with high cytological atypia and higher cellularity (more than 20%) but no SRCs. Finally, G3 denoted a poorly differentiated adenocarcinoma defined by any SRC component. Stage IVa grouped together patients with M1a (acellular mucin) and M1bG1 (low-grade atypia), whereas stage IVb grouped together patients with M1bG2 (high-grade atypia) and M1bG3 (high-grade atypia and SRC). Lastly, stage IVc was reserved for patient with distant metastasis to other sites than the peritoneum.

Results from the extensive literature review carried out in paper I seem to support the four-tiered classification system proposed by the PSOGI (102).

Two studies highlighting the lower recurrence risk of AM patients were found. The study carried out by Reghunathan and colleagues (128) found only one recurrence out of 33 patients with AM disease and 13 patients had a DFS of more than 3 years (HR 9.8; P=0.025). Similarly, Choudry et al (129) evaluated the impact of cellularity on disease recurrence. They discovered that acellular mucin (19 patients) and scant cellularity (30 patients) were significantly associated with better DFS than moderate cellularity (2-19% of epithelial cells) (242 patients) with an HR of 4.4 (P=0.02). On the other hand, two single-centre retrospective studies (105), (108) contended that the presence of SRC in high-grade mucinous implants confers worse survival rates compared to HGCMP. Ihemelandu at al (108) observed a decrease in median OS from 45.4 months in patients with moderate-high-

grade histology to 18.9 months in patients with SRCs (HR 1.4, p=0.001). Correspondingly, Munoz-Zuluaga et al (105) recorded a median survival drop from 90 months in patients with HGMCP to 26.4 months in patients with HGMCP-SRC (HR 2.9, p=0.001). The results from these study groups oppose the classification proposed by the 8th edition of the AJCC where AM and LGMCP are grouped into stage IVa and HGMCP and HGMCP-SRC are grouped into stage IVb.

However, a couple of considerations must be taken into account. Firstly, the observations made by these study groups must be interpreted with caution as they are based on single-centre retrospective cohorts which are therefore subject to bias. Secondly, when the prognostic impact of the PSOGI's four-tiered classification system was evaluated by individual study groups, the results revealed a lack of congruency. Only two study groups (107), (112) had evaluated the prognostic significance of the PSOGI classification system by the time paper I was published. The results from the Australian study group in 2017 (112) vouched for the four-tiers as differences across the different subgroups were significant (HR 3.13, p<0.001). In contrast, Baratti et al (107) were unable to reproduce these same results and found that the two-tiered WHO classification (134) (HR 1.48; p<0.028) correlated better with OS than the PSOGI classification (102) (HR 1.22; p<0.149).

Therefore, the review carried out in paper I provides a thorough understanding of how and why the PSOGI classification developed through the primary research supporting its use. However, because this review is based on retrospective studies, the evidence supporting the PSOGI classification (102) is limited. Additionally, publication bias cannot be ignored as well as historical bias where the comparison of more modern studies using recent terminology with older literature was difficult.

5.2 CORRELATION OF THE PSOGI AND AJCC 8TH EDITION CLASSIFICATIONS WITH PROGNOSIS IN OUR COHORT.

Following the review carried out in paper I, paper II focused on analysing the prognostic impact of both of the recent classification systems (PSOGI (102) and AJCC 8th edition (103)) on our cohort.

As previously mentioned, already two study groups had already undertaken the task of validating the PSOGI classification. The results obtained by Huang et al (112) were in agreement with the four-tiered classification proposed by the PSOGI with observed 5-year OS rates of 95.2% in AM, 83% in LGMCP, 47% in HGMCP and 12.6% in HGMCP-SRC. Similar OS rates were reported by Baratti et al (107): 89.3%, 77.5%, 51% and 0%, respectively. However, in distinction to the results obtained by Huang et al (112), the differences in survival in the Italian cohort were not significant in the multivariate analysis, hence, the PSOGI classification could not be validated as the better classification system to adequately stratify patients according to prognosis. One of the main arguing points for these results given was that the presence of more subcategories reduced the number of patients in each which in turn reduced the statistical power of the tests. Interestingly, both of these study groups focused on evaluating the impact of the PSOGI classification on OS and not on DFS.

Prior to the publication of Paper II, one Spanish study group assessed the impact of the PSOGI classification on OS and DFS and compared it to Ronnett's classification (142). They concluded that the PSOGI classification provided better predictive capacity for OS and DFS than the classical classification by Ronnett. Nonetheless, a careful analysis of these results is warranted. Firstly, when survival results were adjusted to CC score, the PSOGI

classification maintained its significant association with OS (HR 2.47 (1.096-5.581)) but not with DFS. Secondly, the values of the AUC obtained by both classifications for OS (Ronnett:0.699; PSOGI:0.697) and DFS (Ronnett:0.620; PSOGI:0.614) were very similar and just below the adequate prediction threshold of 0.7. Thirdly, and most importantly, this study group validated the PSOGI classification on a cohort that consisted mainly of LGMCP and HGMCP patients, AM and HGMCP-SRC patients were excluded from statistical analysis because of their small size (n=3 in each subgroup). As a result, their findings may not apply to other cohorts.

In paper II, the correlation between survival outcomes and the most recent classification systems (PSOGI 2016 (102) and the AJCC 8th edition (103)) was analysed. The OS rates observed across the different subgroups proposed by the PSOGI revealed that AM and LGMCP constituted subgroups with excellent long term prognosis (5-year OS of 95% and 94%, respectively, p=0.760) while outcomes in HGMCP and HGMCP-SRC were significantly worse (5-year OS of 50% and 30%, respectively, p=0.434). Initial interpretation of these results vouches for the classification proposed by the 8th edition of the AJCC (103) defending two prognostic groups (5-year OS of stage IVa was 94.3% and of stage IVb, 39.7%) since no differences were found between AM vs LGMCP, and HGMCP vs HGMCP-SRC subgroups. However, two main points must be taken into account.

First, our series was unable to demonstrate the negative impact of SRC on survival. The literature supporting the poor prognosis of SRC patients has already been reviewed throughout this manuscript (105), (108). Failure to replicate these findings could be attributed to the small size of the SRC sub-cohort (n=14) and the unexpected high median survival observed (56.3 months) as a possible result of a careful patient selection process.

Second, when recurrence is considered, our study highlights the exceptionally low recurrence tendency of AM when compared to cellular counterparts as previously discussed by Reghunathan et al (128) and Choudry et al (129). Patients with AM had a higher 5-year DFS rate (82.2%) than LGMCP (51.2%), with an HR of 4.95 (p=0.03). Although the 8th edition of the AJCC introduced an independent category to exemplify the distinct behavior of this subgroup of patients (M1a), the singularity is lost when combined with LGMCP into the same prognostic group (IVa). Therefore, in the view of these last arguments, we could conclude that, considering both OS and DFS, the PSOGI classification provides a slightly better prognostic stratification. However, histology´s discriminative power is insufficient on its own as none of the classification systems had a c-index value higher than 0.7 predicting OS and DFS.

Since the publication of paper II, further retrospective single-centre studies have aimed to validate the PSOGI classification. Lee et al (111) observed significant differences in the survival outcomes across the different subgroups, although no patients with AM were found in their cohort. The 5-year OS rates were 56.2% in LGMCP, 37.5% in HGMCP and 25% in HGMCP-SRC (p=0.001) while the 5-year DFS rates were 33.1%, 15.7% and 0%, respectively (p=0.024). However, once adjusted to possible confounding factors, the differences in survival were no longer significant. When the PSOGI classification was applied to their cohort, Santullo et al (165) did not observe significant differences in OS nor in DFS. Similarly, Lopes et al (166) observed that the PSOGI classification was associated with significant differences in DFS (p=0.02) but not OS (p=0.15). This cohort, however, lacked patients belonging to the HGMCP-SRC subgroup.

Overall, there still exists a lot of incongruency regarding the validation of the PSOGI classification. One of the main reasons for this is the low-quality of the studies that have

attempted to validate it in terms of cohort size, especially focusing on the size of each of the subgroups, and retrospective nature where factors such as data loss and selection bias cannot be overruled. Larger, prospective, multicentre cohorts might aid in the validation of this classification. Additionally, as mentioned throughout the discussion of paper II, histopathological factors might not be enough to predict the biological behaviour of the tumour. Other factors have been repeatedly associated with survival outcomes throughout different cohort studies, therefore, the combination of these could aid better patient stratification of patients according to prognosis. This idea set the foundation for the next paper in this manuscript.

5.3 CONSTRUCTION AND VALIDATION OF A NOMOGRAM PREDICTING DISEASE RECURRENCE AFTER CRS+HIPEC.

It is becoming increasingly clear that histology, long thought to be the most important determinant of prognosis, cannot fully explain the differences in survival outcomes following optimal CRS+HIPEC. Some cases with indolent histopathologic features exhibit aggressive clinical behaviour with rapidly progressing disease (147), and early recurrences which negatively impact OS (102), (122). Nonetheless, updated treatment guidelines primarily consider pathologic grading when recommending surveillance or overall management plans.

The investigation carried out in paper III created and externally validated a prognostic score to identify patients at higher risk of recurrence after initial CRS+HIPEC treatment. The combination of PCI and preoperative TM elevation, in addition to the PSOGI classification, resulted in better patient stratification than the PSOGI classification alone. The C-index obtained for the new model was greater than the acceptable 0.7 value in both cases (0.764

± 0.06 in the derivation cohort, 0.702 ± 0.023 in the validation cohort). This nomogram could provide reliable prognostic stratification, which is important for patient selection and counselling, and for planning individualized treatment strategies and risk-adapted follow-up protocols.

The DFS was chosen as the primary end point in this investigation because disease recurrence is known to be one of the main limiting factors of OS even in patients with low-grade disease. Disease recurrence after optimal CRS+HIPEC treatment has been observed in up to 30-40% of patients (143), (159). This validated nomogram could allow the identification of patients who are at high risk of recurrence and may benefit from additional treatments such as systemic chemotherapy (78) or stricter follow-up schemes. Early detection of locoregional recurrence is required in order to perform successful iterative CRS+HIPEC procedures that result in OS advantage in selected cases (148), (160).

Simultaneously, the identification of patients at low-risk of recurrence could also be beneficial by the development of less stringent surveillance guidelines, limiting thus the radiation exposure from unnecessary imaging. This concept has been explored by several study groups before, but once again, the determination of congruent and robust results is hindered by the low incidence of this disease and retrospective nature of most study cohorts. In their cohort of 156 PMP patients with both acellular and cellular peritoneal deposits resulting from a primary LAMN, Solomon et al (133) reported no recurrences after the 5-year period questioning the benefit of continuing with surveillance after the 5-year time mark. In fact, only 2 out of 25 patients with AM recurred (8%). On the other hand, the PMI at Basingstoke analysed their large cohort of PMP patients (N=775) with 20 years follow-up available (143). They proposed different surveillance protocols adapted to pathological findings, but still supporting the concept of long-term surveillance. Based on their

observations, DFS in both low- and high-grade disease levelled after 6 years surveillance (5- and 10-year DFS rates of 68.7% and 57.3%, respectively, in low-grade and 26.2% and 18.8%, respectively, in high-grade, p=0.001). However, occasional recurrences after this 6 year period could be observed, therefore, prolonged surveillance protocols were proposed, especially given the indolent nature of the disease. Similarly, the Manchester study group (144) also reported two recurrences in patients with AM deposits: one early, taking place at 12 months, and one late, taking place at 56 months, near the end of the proposed surveillance period by Solomon et al (133). In the INT series, two patients with AM relapsed and died as a result of disease progression (107). Results from these study cohort reinforce the fact that histology alone does not have the predictive power to modulate the intensity of follow-up regimes in terms of duration and frequency. The nomogram proposed in paper III, on the other hand, tends to overestimate the risk of recurrence during the first year but then approaches the ideal model when predicting 3- and 5-year DFS rates, as shown in the calibration plots (Figure-8).

The variables included in the nomogram have been widely linked to DFS in the existing literature. The association between the PSOGI classification and DFS has been thoroughly discussed throughout this manuscript. The results obtained in paper III are in concordance with the PSOGI classification as AM scored no points in the nomogram; LGMCP, 22 points; HGMCP, 38 points, and HGMCP-SRC received 100 points.

PCI and tumour marker elevation have also been correlated with DFS by many previous study groups. Ca19-9 elevation has been linked to decreased DFS (46), , (49,130) even when adjusted to PCI, demonstrating that tumour marker elevation is also indicative of more aggressive disease and not just increase tumour burden (46). Similarly, other study groups have found Ca125 and CEA to be predictors of shorter DFS (128), (133). PCI score has also

been associated with shorter DFS (130), (159), (161). However, the main drawback of relying on PCI score as a prognostic factor is that its calculation is done intraoperatively and no reliable methods for its accurate prediction is currently available.

The main strength of the study carried out in paper III is that the performance of the proposed model has been evaluated on an external cohort. However, it is also subject to many limitations that have been thoroughly discussed throughout the papers. For starters, patients treated in both centers are from retrospective and highly selected series that may not be fully representative of the general population of appendiceal lesions with peritoneal dissemination. Selection bias, in particular, may have underestimated the negative impact of HGMCP-SRC. Missing data must also be considered, as they may have prevented further analysis. Secondly, molecular information shown to be associated with prognosis by other study groups could not be evaluated as it was not available in both cohorts. Third, the proportions of patients diagnosed with each of the PSOGI pathological categories and receiving adjuvant SCT differ between centers (not part of the standard of care at the INT). In addition, the open-versus-closed-abdomen technique, dosage, and chemotherapeutic agents used in HIPEC administration differed between centers. These differences, on the other hand, reflect on the existing operative differences amongst centres treating PMP, which in turn put into question the validity of this nomogram which has been externally validated with a large cohort from one centre. Therefore, ideally, validation using a larger multicentre cohort would assess its performance in a more accurate manner.

5.4 CONTRIBUTION TO THE SCIENTIFIC LITERATURE

The extensive literature review provided in paper I has highlighted a major dilemma surrounding PMP which is the vast amount of different terminology existing to define

similar pathologic entities instead of a common language. It has also provided a historical background to the development of the current classifications of the PSOGI (102) and the 8th edition of the AJCC (103). In this case, there is evidence (although of low quality) throughout the literature to support the four-tiered classification system proposed by the PSOGI consensus in 2016 (102) where AM has a lower risk of recurrence than LGMCP (100), (122), (130) and survival outcomes of patients with HGMCP-SRC are lower in comparison to HGMCP (105), (108).

However, the initial classification system proposed by Ronnett (97) was found to be the favourite one amongst referral centres treating PMP. Although, the uptrend in the use of the PSOGI (102) and AJCC 8th edition (103) classification systems alongside their incorporation into PSOGI/EURACAN guidelines (28) and the Chicago Consensus (29) could represent the transition towards an universal language with prognostic implications.

Limited studies had assessed the prognostic significance of the four-tiered classification proposed by the PSOGI consensus (102). Two study groups found the PSOGI classification to correlate with OS even after adjusting for other possible confounding factors (112), (142), however, another large study group found differences only in the univariate analysis (107). Paper II externally validated the PSOGI classification (102) in terms of OS and DFS, but also provided a direct comparison the PSOGI (102) and the AJCC 8th edition (103) classification systems. Additionally, Paper II has displayed medium and long-term outcomes of PMP patients after CRS+HIPEC treatment at a referral centre in Spain.

The contribution of Paper III has been to develop and design a nomogram in order to predict disease recurrence at 1, 3 and 5-years after CRS+HIPEC based on the results of a prediction model which included the following variables: PSOGI histological group, PCI and

preoperative tumour marker elevation. The prediction model was derived from our study cohort, and collaboration with a referral centre in Italy allowed to validate this model externally on a larger cohort. The results from this paper have demonstrated that the easy-to-use nomogram provides better prognostic stratification than histopathological features alone.

Incorporation of this nomogram into clinical practice may aid clinicians to take personalized and informed clinical decisions. On one end, patients at very low-risk for recurrence, may benefit from shorter surveillance schemes. Two study groups have already proposed limiting surveillance up to 5 years for patients with AM (133), (143). But disease recurrence after the proposed surveillance period has been reported for AM patients in the literature (107), (144). Therefore, we suggest that a combination of histology, PCI and tumour marker evaluation will adequately select those patients eligible to limited surveillance.

On the other hand, we extrapolate that this nomogram may benefit also patients at high risk of recurrence. Disease recurrence even after optimal CRS+HIPEC treatment is still one of the main factors limiting OS (159). Identification of patients at risk may result in early identification of disease recurrence when iterative CRS+HIPEC procedures may convey survival advantage (160). Additionally, one important concept from paper III that we would like to highlight is that the combination of studied prognostic factors provides better patient stratification than the use of one single factor. These concepts have been incorporated into the stratification of other tumour types such as neuroendocrine tumours where the Ki67 proliferation rate is incorporated into tumour staging (167), pancreatic adenocarcinoma where preoperative Ca19-9 elevation (168) and molecular profiling in colorectal cancer (169) influences overall patient management, to name but a few. Therefore, we advocate for the incorporation of tumour markers and molecular markers that are currently under

evaluation into future treatment guidelines with the hope of obtaining risk adapted protocols.

5.5 MAIN CONCLUSIONS OF THE INVESTIGATION

Main results from Paper II revealed that the prognostic implications of both classification systems when dealing with OS were actually very similar. AM and LGMCP showed excellent long-term survival results (5-year OS rates of 95% and 94%, respectively, p=0.760) comparable to those of stage IVa (5-year OS rates of 94.3%). Similarly, OS outcomes for HGMCP and HGMCP-SRC were not so optimistic (5-year OS rates of 50% and 30%, respectively, p=0.434), which once again, were comparable to the outcomes of stage IVb patients (5-year OS rate of 39.7%). Both classification systems were found to be significantly correlated with OS in the multivariate analysis and as expected, they were found to have similar discriminative capacities (c-index values of 0.685 and 0.669, respectively). On the other hand, the outcomes of patient with AM were significantly better than those of LGMCP patients when considering DFS (5-year DFS rate 82.2% vs 51.2%, respectively, p=0.03). But no differences in the outcomes of DFS between HGMCP and HGMCP-SRC subgroups were found (5-year DFS rate of 20.8% vs 32.1%, respectively, p=0.6), and once again, the DFS rate was found to be very similar to that of stave IVb (5-year DFS rate of 26.7%). Both classification systems were significantly associated with DFS in the multivariate analysis, and, once more, the discriminative capacities of both were found to be similar (c-index of 0.669 and 0.623, respectively).

Overall, histology was established to be one of the major conditioning factors of prognosis. Both classification systems were found to have similar discriminative capacities regarding OS and DFS. The lower recurrence rate found in AM was the finding that would favour the

PSOGI classification as this singularity is lost in the 8th edition of the AJCC by grouping M1a and M1bG1 into stage IVa. However, it is necessary to highlight that the discriminative capacity of histology alone is not adequate as the c-index values obtained did not reach the 0.7 threshold for adequate prediction.

Interestingly, other factors predicting shorter DFS were identified. These results laid the foundation for the prediction model developed in paper III. This prediction model was based on the histopathological classification proposed by the PSOGI, the PCI score and preoperative tumour marker elevation (0 or 1 elevated tumour marker, 2 elevated tumour markers or all 3 elevated tumour markers) (see Figure-5). The performance of this model was tested in a larger cohort from a referral centre in Italy and were compared to that of the PSOGI classification on its own to have as reference. The new model was found to have a higher c-index value than the PSOGI classification alone (0.702 versus 0.61). Also, the calibration plots showing the concordance between predicted and observed values were proximate to the ideal model when predicting 3 and 5-year DFS rates (see Figure-8). These findings suggest that other factors (such as PCI scores and preoperative value of tumour markers) should be taken into consideration in clinical guidelines to optimize treatment and surveillance strategies adapted to personalized risk assessment.

5.6 FUTURE RESEARCH

The major limitations of the papers included in this project are the observational and retrospective nature of these. These aspects have been commented on separately, but, briefly, the existence of selection bias cannot be overlooked (specially in HGMCP and HGMCP-SRC cases where only those cases with high probabilities of CC0/1 resections are considered for CRS+HIPEC), and the small cohort size (where 3 out of 4 pathological

subgroups consisted of <20 patients). Additionally, as seen in Paper I, the evidence revolving around PMP is mainly of low quality, as most studies are, once more based on single-centre retrospective cohorts.

Future research projects should focus on first, establishing the PSOGI classification system as a common language amongst PMP referral centres and second, on promoting collaboration between multiple institutions. The low incidence of PMP has always hindered the possibility of producing evidence of high quality. For this reason, multicentre collaboration is necessary in order to work on large cohorts and obtain more robust results.

The results of several multicentre collaboration studies at European level are to be expected soon. One of these focuses on the validation of a modified PSOGI classification taking into consideration the Ki67 proliferation index. The role of the Ki67 proliferation index has been investigated by a study group in Spain (152). They suggested a change in the PSOGI classification based on their findings as the Ki67 proliferation rate divided the HGMCP group into two-well defined subcategories with different OS and DFS outcomes (i.e 5-year OS rate for LGMCP, HGMCP Ki67≤15% and HGMCP Ki67>15% was 100%, 70% and 24% ($p<0.001$) respectively, while the 5-year DFS was 90%, 44% and 0%, respectively ($p<0.001$)). The performance of this new classification system will be evaluated in a large and multicenter European cohort and its results will determine the utility of analyzing Ki67 on all HGMCP patients alongside any potential changes in management. Another large European multicentre cohort that is expected to publish its results soon is that of the LAMNet study. This study is led by the Italian group at the INT and focuses on identifying the risk factors associated with the progression to PMP of a LAMN confined to the appendix or a LAMN associated with localized peritoneal disease (PCI<3). This study was triggered by the low recurrence rate observed in their small prospective cohort of patients

with LAMN that was resected without the administration of HIPEC, even in the presence of limited peritoneal disease (5-year DFS of 95.1%) (170). With the results from the large multicohort study, we expect to find evidence-based answers to when CRS+HIPEC should be performed in these patients as opposed to ongoing surveillance and for how long surveillance should be carried out.

Additionally, the leading European centres treating PMP are collaborating under the Accelerator Award on Appendiceal Mucinous Neoplasms and Pseudomyxoma Peritonei project that aims to build a research infrastructure that simultaneously covers clinic, molecular and cellular biology areas of PMP. The goals within this project include to investigate the biology of PMP through the use of tissue banks and in vitro models, to discover new interactions between neoplastic cells and their microenvironment during peritoneal dissemination to investigate future targets of chemotherapeutic treatments and to assess the impact on long-term survival outcomes of molecular and immune markers.

The use of tissue biobanks and in vitro models will allow to assess the efficacy of the different HIPEC regimes available. As briefly mentioned in the introduction, there are multiple HIPEC regimes that are currently being used to treat PMP in referring centres. These regimes differ from each other not only in the chemotherapy agent being used (mitomycin-C, oxaliplatin, or the combination of mitomycin-C and cisplatin or mitomycin-C and doxorubicin), but also in the dosage per m^2 of the same chemotherapy agent (i.e PMI Basingstoke low-dose mitomycin-C regime (10mg/m^2 for 60 minutes) and the Dutch triple dosing mitomycin-C regime (35mg/m^2 for 90 minutes) as well as in the treatment duration. As mentioned, the pharmacologic end point has now shifted from AUC ratios which would be representative of the concentration of chemotherapy agent in contact with the residual tumour nodules to the actual chemotherapy agent concentration achieved within the tumour

nodule. Basic science research using in vitro tissue models could be the first step of a series of investigations to determine which chemotherapy agent is most effective in terms of tumor nodule penetration and cytotoxic effects. The only clinical study available up to date comparing different regimes available for PMP is that of Kusamura et al (53) published in 2021. This study was based on the retrospective cohort of the PSOGI multicentre registry and within its secondary end points, it aimed to evaluate the efficacy of the different HIPEC drug schedules. Results from the multivariate analysis showed that the only two HIPEC regimes that associated overall survival benefits compared to CRS-alone were the combinations of oxaliplatin and 5-fluorouracil (HR 0.42 95% CI (0.19-0.93), p=0.03) and cisplatin plus mitomycin-C (HR 0.57 95% CI (0.42-0.78), p=0.001). The administration of HIPEC with mitomycin-C or oxaliplatin as single agents was not associated with improved OS in this cohort. Overall, they conclude that HIPEC was found to be associated with better survival outcomes when compared to CRS-alone without a significant increase in postoperative morbidity nor mortality in PMP patients. However, no major conclusions could be drawn regarding the efficacy of the different HIPEC protocols given the limitations of the study. Therefore, the search for the most effective HIPEC protocol is still ongoing. Ideally, results from basic science research would aid to set the foundations of clinical trials to obtain more robust results.

Furthermore, advances in the knowledge of the molecular biology behind PMP would also be expected as a result of the Accelerator Award on Appendiceal Mucinous Neoplasms and Pseudomyxoma Peritonei project. Advances in this field would be expected could aid the development of targeted therapies (both biological and immunotherapies). The wide range of biological behaviors displayed by PMP patients even after optimal CRS+HIPEC treatment has been made evident throughout this study. Enhancing our knowledge on a genomic level may allow the application of precision medicine in this field. Cancer

precision treatment refers to the use of a specific agent that is expected to generate a benefit in only a subgroup of patients with particular characteristics at a genomic level (171). Precision medicine, using molecular profiling technologies, is increasingly being combined with standard clinicopathology evaluations to improve diagnosis, prognosis, and prediction of clinical outcomes. Its application in colorectal cancer, for example, has brought changes into the clinical management of these patients where the obtention of molecular profile is now essential to guide directed therapies (169).

CONCLUSIONS

6. CONCLUSIONS.

1. A universal language for describing mucinous neoplasms of the appendix with peritoneal dissemination is desperately needed. The four-tiered PSOGI classification has developed as a consequence of increased pathological insight into a coherent classification option.
2. The application of both of the classification systems (PSOGI 2016 (102) and AJCC 8th edition (103)) to divide our cohort into different prognostic groups, revealed that they divided patients with similar prognostic accuracy. Although the PSOGI classification provides a more detailed histopathological description, histology alone is insufficient for accurate patient prognosis.
3. A easy to use nomogram has been constructed and externally validated using PCI, preoperative tumour marker status and the PSOGI classification to predict recurrence after optimal treatment with CRS+HIPEC. This nomogram proved to be more accurate in predicting prognosis than the PSOGI classification alone, and could, therefore, help clinicians in individualized survival predictions and informed clinical decision making.

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7. REFERENCES.

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