

**Predictors of peritoneal metastasis
in gastric cancer
A literature review**

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Dedicatória

Quando comecei a escrever este trabalho não pensei que o iria dedicar a alguém. Talvez porque os tempos fossem outros. Mas tudo muda sempre mais rápido do que esperamos.

Ficaste tão perto de me ver terminar mais uma etapa desta minha caminhada. Sei que ficarás feliz de me ver dar mais este passo. Esta dedicatória é um obrigado. Um obrigado por todos estes anos que te pude ter como um modelo. Todos estes anos em que me pude orgulhar de te ter como avô.

A arte da medicina tem um lado humano que a torna a mais bela das ciências. Não eras médico. Mas ensinaste-me mais de medicina do que alguma vez poderia esperar. A excelência do carácter está no meio termo das coisas. Pautar-me-ei por procurar sempre esse meio, seguindo o teu exemplo.

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Abstract

Background. Gastric cancer is the fifth most common type of cancer worldwide and one of the main factors for the decrease in overall survival and poor prognosis is the occurrence of peritoneal metastasis. This work aims to review the available literature that approaches biomarkers that can be predictors of the presence of peritoneal metastasis in these patients.

Methods. This literature review was performed through searches on PubMed, identifying relevant publications related to peritoneal metastasis in gastric cancer and its predictors. Both free text and MeSH terms were employed. No past date limit was imposed and the upper limit date was February of 2020. Publications in languages other than English were excluded. Rayyan QCRI tool was used to help on the first step of eligible studies selection. A total of 209 studies were found in this first step, and only 37 were included. Additionally, the most relevant publications in the reference list of the included studies were also searched. The articles were categorized in broad categories considering the indicator they report: Tumor markers, Systemic Inflammatory Response markers (SIR markers) and Other molecular markers.

Results. Regarding the tumor markers, CA125 showed the best results in serum (sCA125) and CEA was the most useful measure in the peritoneal lavages (pCEA) with a sensitivity range from 38.78% to 79.1% and 58% to 84.9%, respectively. Focusing on the SIR markers, Neutrophil to lymphocyte ratio is the one which showed consistently better results, its sensitivity ranging from 59% to 79%. The other biomarkers, most of them dependent from molecular diagnosis techniques, are less accessible not currently obtained in clinical practice and more research is needed regarding their efficacy in clinical context. The studies that combine different indicators obtained better results. This is not limited to biomarkers, tumor characteristics have also been taken into account, increasing significantly the sensitivity to detect PM in GC patients.

Conclusion. Most of these biomarkers are weak predictors. The future should be about the creation and validation of clinical scores that could integrate not only some of these markers, but also tumor characteristics, imaging methods and cytological results. Large-scale multicenter and stronger design studies are needed in this field, in order to produce stronger evidence about the usefulness of these biomarkers.

Keywords

Gastric cancer; biomarkers; predictors; peritoneal metastasis; literature review.

Resumo

Introdução. O Cancro Gástrico é o quinto mais prevalente no mundo e a existência de metástases peritoneais no momento do diagnóstico é um dos principais motivos para a diminuição da sobrevida a médio prazo. Este trabalho pretende rever a literatura existente acerca do papel de biomarcadores como preditores da existência de metástases peritoneais nestes doentes.

Métodos. Esta revisão da literatura foi realizada com base em pesquisas na *PubMed*, identificando publicações relevantes que relacionam metástases peritoneais em doentes com cancro gástrico, com potenciais preditores. Tanto termos livres como *MeSH terms* foram usados. Todos os artigos até ao momento da pesquisa (fevereiro de 2020) foram incluídos. Artigos noutras línguas além de inglês foram excluídos. A ferramenta *Rayyan QCRI* foi utilizada para filtrar num primeiro momento os artigos com base no *abstract*. Um total de 209 estudos foram encontrados, dos quais apenas 37 foram incluídos nesta primeira fase. Adicionalmente, as publicações mais relevantes da lista de referências dos artigos selecionados foram também incluídas. Os trabalhos encontrados foram divididos por categorias tendo em conta o indicador que reportavam: marcadores tumorais, marcadores de resposta inflamatória sistémica, e outros marcadores.

Resultados. Relativamente aos marcadores tumorais, o CA125 mostrou os melhores resultados séricos e o CEA os melhores resultados nos lavados peritoneais com uma sensibilidade entre 38.78% e 79.1% e entre 58% e 84.9%, respetivamente. Dos marcadores de resposta inflamatória sistémica, o rácio de neutrófilos e linfócitos mostra os resultados mais consistentes com uma sensibilidade entre 59% e 79% para prever metástases peritoneais. Os outros biomarcadores têm poucos estudos publicados e são menos acessíveis, não sendo normalmente obtidos na prática clínica. Os estudos que procuraram combinar vários biomarcadores, ou até com outros indicadores, como as características do tumor, mostraram um aumento da sensibilidade para detetar metástases nestes doentes.

Conclusão. A maioria dos biomarcadores são preditores fracos. O futuro deverá passar pela criação de ferramentas que integrem mais do que um marcador ou até outros indicadores como resultados de métodos de imagem, citologia e/ou características tumorais. Estudos com amostras populacionais maiores, multicêntricos e com evidência científica mais forte são necessários nesta área.

Palavras-chave

Cancro gástrico; biomarcadores; preditores; metástases peritoneais; revisão da literatura.

Resumo alargado

Introdução. O Cancro Gástrico é o quinto mais prevalente no mundo e responsável por 8.2% das mortes atribuídas ao cancro. A incidência deste tumor tem variações significativas a nível mundial, sendo mais expressivo em países asiáticos como a Coreia do Sul, Japão e China. Em Portugal a incidência é estimada em 11.1 casos por 100 000, o que, apesar de inferior aos países asiáticos mencionados, é superior aos restantes países da Europa Ocidental e da região do Mediterrâneo. A metastização peritoneal é uma das formas mais frequentes de metastização à distância nestes tumores, e responsável pela diminuição significativa da sobrevida destes doentes. A identificação de metástases peritoneais limita as opções terapêuticas a uma abordagem maioritariamente paliativa. Este trabalho pretende rever a literatura existente acerca do papel de biomarcadores como preditores da existência de metástases peritoneais em doentes com cancro gástrico.

Métodos. Esta revisão da literatura foi realizada com base em pesquisas na *PubMed*, identificando publicações relevantes que relacionam metástases peritoneais, em doentes com cancro gástrico, com potenciais preditores. Foi usada a seguinte chave de pesquisa: (predictors OR predictor OR “predictive factor” OR predictive) AND (“peritoneal metastasis” OR “peritoneal dissemination” OR “peritoneal carcinomatosis” OR “peritoneal seeding” OR “peritoneal involvement”) AND (“neoplasm, stomach” OR “stomach neoplasm” OR “gastric neoplasm” OR “cancer, gastric” OR “gastric cancer” OR “stomach cancer” OR “stomach cancers” OR “gastric tumor” OR “stomach tumor”). Tanto termos livres como *MeSH terms* foram usados. Todos os artigos até ao momento da pesquisa, fevereiro de 2020, foram incluídos. Os artigos noutras línguas além de inglês foram excluídos. A ferramenta *Rayyan QCRI* foi utilizada para filtrar num primeiro momento os artigos com base no *abstract*. Um total de 209 estudos foram encontrados, dos quais apenas 37 foram incluídos nesta primeira fase. Adicionalmente, as publicações mais relevantes da lista de referências dos artigos selecionados foram também incluídas. Os trabalhos encontrados foram divididos por categorias tendo em conta o indicador que reportavam: marcadores tumorais, marcadores de resposta inflamatória sistémica, e outros marcadores (para todos aqueles que não se incluíam num dos outros grupos).

Resultados. Relativamente aos marcadores tumorais, o CA125 mostra os melhores resultados séricos e o CEA os melhores resultados nos lavados peritoneais com uma sensibilidade entre 38.78% e 79.1% e entre 58% e 84.9%, respetivamente. De entre os restantes marcadores, o CA72-4 sérico mostrou uma sensibilidade que varia entre

34.78% e 57%, e o CA19-9 tem uma sensibilidade entre 36.3% e 57%. Dos marcadores de resposta inflamatória sistêmica, o rácio de neutrófilos e linfócitos mostra os resultados mais consistentes com uma sensibilidade entre 59% e 79% para prever a metastização peritoneal. O rácio entre fibrinogénio e linfócitos e o rácio entre Plaquetas e linfócitos apenas mostraram resultados modestos com uma sensibilidade de 65.1% e 69.3%, respetivamente, e especificidades igualmente fracas (65.5% e 51%). Tanto os marcadores tumorais como os marcadores inflamatórios têm como grande vantagem a sua grande acessibilidade e o facto de serem já obtidos com regularidade no processo de estadiamento destes doentes, o que os torna bastante custo-efetivos. Os outros biomarcadores, onde se incluem as metaloproteinases, o tripsinogénio, as telomerasas, entre outros, têm poucos estudos publicados que os relacionem com a previsão da existência de metástases. Adicionalmente, necessitam de técnicas de diagnóstico molecular, como RT-PCR, e não são obtidos por rotina, na prática clínica. Para além da análise individual destes marcadores, houve vários estudos que procuraram obter resultados com modelos preditores que integram vários marcadores em ferramentas ou até a sua utilização combinada com outros indicadores, como as características tumorais do tumor primário. Estes estudos mostraram um aumento da sensibilidade para detetar metástases peritoneais nestes doentes.

Conclusão. A maioria dos biomarcadores são preditores fracos quando usados de forma isolada. O futuro deverá passar pela criação de modelos preditores que integrem mais do que um marcador ou até outros indicadores como resultados dos métodos de imagem, citologia e/ou características tumorais. Os estudos analisados têm várias limitações metodológicas e existe uma clara lacuna na validação destes dados em doentes europeus. Alguns destes marcadores têm um número muito limitado de publicações que os relacionem com a ocorrência de metástases peritoneais em doentes com cancro gástrico. Estudos com amostras populacionais maiores, multicêntricos e com *designs* de estudo mais válidos e consistentes são necessários nesta área.

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Abbreviations List

AFP	Alpha-Fetoprotein
AJJC	American Joint Committee on Cancer
CA125	Carbohydrate Antigen 125
CA19-9	Carbohydrate Antigen 19-9
CA72-4	Carbohydrate Antigen 72-5
CEA	Carcinoembryonic Antigen
CRP	C-reactive Protein
CT	Computed tomography
ESMO	European Society for Medical Oncology
EUS	Endoscopic Ultrasonography
GC	Gastric Cancer
GI	Gastrointestinal
GIST	Gastrointestinal stromal tumor
HIPEC	Hyperthermic intraperitoneal chemotherapy
IUAC	International Union Against Cancer
JGCA	Japanese Gastric Cancer Association
LCR	Lymphocyte/CRP Ratio
MRI	Magnetic Resonance Imaging
NCCN	National Comprehensive Cancer Network
NLR	Neutrophil/Lymphocyte Ratio
PET	Positron Emission Tomography
PM	Peritoneal Metastasis
PLR	Platelet/Lymphocyte Ratio
RCT	Randomized Controlled Trial
RT-PCR	Real-time Reverse transcription - Polymerase Chain Reaction
SAGES	Society of American gastrointestinal and Endoscopic Surgery
SIR	Systemic Inflammatory Response
SL	Staging Laparoscopy
STN	Sialyt Tn Antigens
STX	Sialy Lewis (x) Antigen
TNM	Tumor - Node – Metastasis
UICC	The International Union Against Cancer
US	Ultrasonography
WHO	World Health Organization

Chapter 1

Introduction

Gastric cancer (GC) is the fifth most common type of cancer worldwide, being responsible for 8.2% of all cancer related deaths. The incidence of GC around the world has significant variations, being most expressive in Asian countries like South Korea, Japan and China, with respectively, 39.6, 27.5 and 20.7 cases per 100 000. In Portugal the incidence is estimated in 11.1 per 100 000, however, despite being lower than in East Asia, it is higher than in most of the western and south European countries.(1)

Peritoneal dissemination is a frequent form of GC distant metastasis, especially in advanced stages.(2) Additionally, it is the most common form of relapse of GC after curative surgery and it is responsible for shortened survival of GC patients.(3) Patients presenting peritoneal metastasis will only have indication for palliative treatment, according to the guidelines. (4–6)

Therefore, the effective preoperative prediction of peritoneal metastasis is essential to avoid unnecessary procedures, allowing to choose the best treatment option.(7) Clinical practice guidelines recommend Computed Tomography (CT) for all patients undergoing staging and risk assessment of GC, and laparoscopy, with or without peritoneal washings, to exclude radiologically occult metastasis in patients with advanced and potentially resectable GC.(4) However, laparoscopic exploration is an invasive approach, carrying associated risks.(8)

Nonetheless, there is no consensus regarding the most sensitive imaging modality in detecting peritoneal metastasis(9), and several other methods, including serum tumor markers and systemic inflammatory response (SIR) markers, have been proposed to predict peritoneal involvement in GC patients.(7)

This work aims to review the available literature that approaches biomarkers that can be predictors of the presence of PM in GC patients. It will also try to explore future perspectives regarding the best tools to access peritoneal dissemination in these patients.

Chapter 2

Methods

This literature review was performed through searches on PubMed, identifying relevant publications related to peritoneal metastasis in gastric cancer and its predictors. The following search keywords were used in PubMed: (predictors OR predictor OR “predictive factor” OR predictive) AND (“peritoneal metastasis” OR “peritoneal dissemination” OR “peritoneal carcinomatosis” OR “peritoneal seeding” OR “peritoneal involvement”) AND (“neoplasm, stomach” OR “stomach neoplasm” OR “gastric neoplasm” OR “cancer, gastric” OR “gastric cancer” OR “stomach cancer” OR “stomach cancers” OR “gastric tumor” OR “stomach tumor”).

Both free text and MeSH terms were employed. No past date limit was imposed and the upper limit date was February of 2020. Publications in languages other than English were excluded. Rayyan QCRI tool was used to help on the first step of eligible studies selection. A total of 209 studies were found in this first step, and only 37 were included.

Additionally, the most relevant publications in the reference list of the included studies were also searched. In order for these studies to be included, they needed to present clear data that correlates the indicator studied with the detection of peritoneal metastasis in gastric cancer patients.

After the first selection of articles based on their abstract, they were categorized in broad categories considering the indicator they report: Tumor markers, Systemic Inflammatory Response markers and Other Molecular markers (for those who don't fit any of the previous groups). Additional articles were included to contextualize the PM problem and the imaging methods available for their assessment.

In this literature review some methodological limitations were found. Firstly, stomach cancer is especially prevalent in East Asia countries, hence most of the research originated from Japan, South Korea and China. Some of the articles found were written in languages other than English or only had an english abstract, which constituted a limitation for this review. Due to linguistic limitation, those studies were excluded.

Secondly, most of the research in this field was done on Asian patients. Although it is possible to extrapolate some of this data to non-Asian patients, there is a clear lack of validation for some of these results in the context of other populations, like the European population, where Portugal is included.

Lastly, another limitation of this work is the study design of the publications searched. Most of the articles are single-center retrospective studies, so there may be some bias. Furthermore, the samples are in most of cases not sufficiently large and have a low proportion of PM cases in the studied universe. Not a single one randomized controlled trial (RCT) was included. Therefore, it is clear that multicenter, large-scale and stronger design studies are needed in this field, in order to produce stronger evidence.

Chapter 3

General approach to gastric cancer and peritoneal metastasis

Epidemiology

GC is one of the most common types of cancer worldwide, ranking fifth in all cancer diagnosis, which represents more than 1 000 000 cases in 2018, and third in cancer deaths, accounting for nearly 783 000 cases. In general, it is twice more prevalent in men than in women.(1)

The incidence rate of GC around the world has significant variations, being most expressive in East Asian countries like South Korea, Japan and China, having, respectively, 39.6, 27.5 and 20.7 cases per 100 000.(1) This represents almost half of GC cases at a global scale, and justifies that some of these countries (eg, Japan and South Korea) have nationwide GC screening programs.(10) This also accounts for the fact that patients in Asian countries are more often diagnosed with GC at an earlier stage than in non-Asian countries.(4)

In Europe, GC has higher incidence rates in Eastern Europe countries (eg, Ukraine and Belarus) with 17.1 and 7.5 cases per 100 000, for males and females respectively, followed by far by other European regions. Northern Europe, United States and Africa have the lowest rates. In Portugal the incidence is estimated in 11.1 per 100 000, however, despite being lower than in East Europe and East Asian countries, it is higher than in most of the western and south European countries.(1)

Gastric adenocarcinoma represents the most common primary malignant gastric neoplasm (95%), and it is the main focus of this work. Gastric lymphomas, gastrointestinal stromal tumors (GISTs) and neuroendocrine tumors make for the remaining cases.(11)

Risk Factors

GC is a multifactorial entity.(11) Strong differences between world regions could suggest ethnicity as a possible risk factor, however, some studies identified that generations of

migrants acquire the risk rate of the new environment. This supports that environmental factors have a role of major importance in GC etiology, including diet and behavior. Nonetheless, despite most of GCs being sporadic, nearly 10% have associated family history.(12)

Diets rich in salt, pickled or smoked food, high in nitrates and nitrites have been associated with increased risk of GC.(11) The prolonged consumption of these foods leads to atrophic gastritis, creating an achlorhydric change in stomach, which promotes the generation of N-nitroso compounds, known carcinogens.(12) In the opposite direction, diets high in fruits and vegetables (specially containing vitamin A and C) have been proposed to have a protective effect, lowering the risk.(11)

The *Helicobacter pylori* infection is a major risk factor for GC development(10), promoting changes in the gastric mucosa which predispose the cells to dysplasia. Furthermore, people with blood group A phenotype are at special risk because it is a promoter of *H. pylori* chronic infection.(12)

Additionally, pernicious anemia, tobacco, previous gastric surgery with bile reflux, hypertrophic gastropathy, gastric polyps, low socioeconomic status and obesity are postulated to increase the risk of distal GC.(12)

The risk factors can variate based on the tumor's topographic location. So, while distal GCs (non-cardia GCs) are more often associated with the already mentioned risk factors, the proximal GCs (cardia GCs) are closer to the esophageal adenocarcinoma, from an epidemiological point of view and associated risk factors, being more common in non-Asian countries. (1,4)

Clinical Considerations

Dysplasia of the gastric mucosa is accepted as the GC precursor, and it can follow an intestinal metaplasia, or arise directly from the gastric epithelium. To categorize it, several classification systems were developed based on different tumor features, being the most commonly used the Lauren Classification and the World Health Organization (WHO) system.(11,12) The first one, divides the GC in two main categories, based on histologic features: intestinal type (less aggressive, more distal and common) and diffuse type (more aggressive, more proximal, associated with younger patients and family clusters).(12)

The clinical manifestations are, most of the time, very unspecific: weight loss, anorexia and early satiety. Other symptoms include abdominal pain, nausea or vomiting. Acute Gastrointestinal (GI) bleeding is rare, but chronic occult blood loss accompanied by iron deficiency anemia is common.(11) This lack of early signs contributes to the diagnostic delay, and consequently, most of patients present in advanced stages of GC at the time of diagnosis.(12)

The TNM (Tumor-Node-Metastasis) system created by AJCC/IUAC is the most used staging system worldwide. Clinical practice guidelines of ESMO recommend, for initial staging and risk assessment, a complete physical exam, an analytic panel, endoscopy and CT (thorax and abdomen) for all patients. Endoscopic Ultrasound (EUS) could bring additional information of the T and N stage, especially in proximal GC. PET-CT can also be used, in conjunction with CT, to detect metastatic disease.(4) Similar orientations are given by NCCN guidelines from USA.(13) In patients with potentially resectable advanced GC, staging laparoscopy, with or without peritoneal washings, is recommended by ESMO to exclude radiologically occult metastasis.(4)

Surgical resection is the only potentially curative treatment for GC.(11) Therefore, the effective staging is important to choose the best treatment option and avoid unnecessary procedures.(7) Patients presenting peritoneal metastasis will only have indication for palliative treatment, according to the guidelines.(4–6)

Peritoneal Metastasis in Gastric Cancer

Gastrointestinal and gynecological tumors have potential for peritoneal dissemination, defined as peritoneal metastasis through positive cytology or histologic diagnosis.(14) This condition is associated with poor prognosis and decrease in overall survival in patients with GC.(15) Some studies suggest that it is already present in 5-20% of patients undergoing a surgery with curative intent.(16)

Peritoneal metastasis are associated with end-stage invasive GC and the most accepted theory for this occurring is the exfoliation of tumor cells directly into the peritoneum when GC penetrates the serosa.(15) In early stage GC, the peritoneal dissemination is rare, and other routes through lymphatic spread are suggested.(14) After an intended curative surgery, 50% of recurrences occur in the peritoneum. This is especially hard to detect in early stages.(17)

As expected, tumor characteristics are very significantly correlated with the existence of PM: higher pathological T stage ($p < 0.0001$), higher pathological N stage ($p < 0.0001$), lymphatic invasion ($p < 0.0001$), venous invasion ($p < 0.0001$) and higher pathological TNM stage ($p < 0.0001$). The more invasive the tumor is, the more probable is the existence of metastasis in the peritoneum.(18)

Currently, ESMO guidelines (4) recommend staging laparoscopy (with or without peritoneal washings for malignant cells) for patients with potentially resectable GC in all stages IB-III to exclude occult metastatic disease involving the peritoneum. The benefit showed to be greater in patients with T3/T4 disease [III,B]. The recommendations for staging laparoscopy are not totally consensual between the various societies. In USA, the SAGES only recommends this approach to patients with T3 or T4 GC without evidence of lymph node or distant metastasis on preoperative imaging. The Japanese Gastric Cancer Association (JGCA) recommend SL to all patients with clinical stages II-III prior to neoadjuvant treatment.(19) Despite that, there is no doubt that SL is a useful tool to complement the preoperative imaging studies.

Cytological examination of the peritoneal lavage is one of the measures to eliminate the suspicion of peritoneal dissemination before or at the time of curative surgery, therefore trying to eliminate the risk of recurrence.(20) Since the 7th edition of the TNM AJCC staging system, positive peritoneal lavage cytology is considered an indicator of M1 disease.(6) However, some reports have suggested low sensitivity. Even when patients showed negative results on peritoneal lavage cytology, some still suffered from peritoneal recurrence.(20)

This classification system is relevant to select the best treatment possible. In resectable GC for patients with stage IB or more is recommended to do neo- and adjuvant chemotherapy [I,A], while in patients with metastatic disease (including PM) is recommended to do only palliative chemotherapy [I,A], eventually re-assessing the potential benefit from surgery, including metastasectomy/peritonectomy.(4)

Additionally, some studies, most of them conducted in Asian countries, have demonstrated the potential benefit of adjuvant hyperthermic intraperitoneal chemotherapy (HIPEC) in resected GC patients which are high-risk for PM or with PM with a carcinomatosis index < 7 . In other studies it was considered higher carcinomatosis index for peritonectomy and HIPEC. Nonetheless, these results have not been yet

validated in non-Asian patients and European guidelines continue not to recommend this technique in regular clinical practice.(4)

In western countries, where Portugal is included, the reference is the TNM classification system from AJCC/UICC, now in its 8th edition. This staging system includes all types of distant metastasis, such as those in peritoneum, lung and liver in the M-category. The most evident difference, regarding peritoneal metastasis, from other relevant classifications like the Japanese classification of gastric carcinoma by JGCA - which also integrates the TNM system, is that PM has its own independent category that classifies PM in three groups: P0 (no PM), P1 (PM positive) and Px (PM unknown). Worthy of note is that in the 1st edition of Japanese Classification of Gastric Carcinoma, the PM was classified differently, in three main groups based on their degree: P1 (metastasis to the adjacent peritoneum but not distant peritoneum), P2 (few metastasis to the distant peritoneum) and P3 (numerous metastasis to the distant peritoneum). Nonetheless, this classification was abandoned because there was no significant difference in prognosis between any two positive-PM groups (P1, P2 and P3).(21)

These considerations reinforce the need of useful tools that are able to detect the existence of PM with high sensitivity, in order to allow the best clinical decision for each individual patient.

Chapter 4

Imaging methods

Background

The accurate pre-operative assessment of tumor invasion depth, lymph node metastasis and other distant metastasis (including PM) is a fundamental step to choose the best therapeutic approach in GC patients.(22) Today, there is no single gold standard imaging method for staging GC, and several of them could be used complementarily depending on the situation, providing supplementary information to the clinical decision. Besides that, from all the conventional methods available, CT, EUS and 18F-fluoro-2-deoxyglucose positron emission tomography (18FDG-PET) are the most systematically used in clinical practice to stage GC.(23)

While focusing in assessing the pre-operative status of PM, imaging methods have two significant positive effects: avoiding unnecessary laparoscopy/laparotomy and evaluating the potential effectiveness of neoadjuvant protocols in absence of histopathological confirmation.(9) Nonetheless, the early detection of early PM by any of these imaging techniques is still very limited.(24)

Wang and Chen(9), in 2011, conducted a systematic review and meta-analysis on imaging assessment of hepatic and peritoneal metastasis in gastric cancer, with the objective of overviewing the sensitivity and specificity of different imaging methods. This work concluded that EUS is the most sensitive imaging method with 34% sensitivity, followed by CT with 33% of sensitivity. But, when considering the overall sensitivity and specificity, CT is the best technique.

Table 4.1 - Pooled sensitivity and specificity of conventional imaging methods for detection of PM in GC patients (according to Wang and Chen systematic review and meta-analysis(9)).

Imaging method	Sensitivity	Specificity	Diagnostic Odds Ratio	Comments
Computed Tomography (CT)	33%	99%	66.18	The best technique, considering the overall sensitivity and specificity.
US	9%	99%	10.63	-
EUS	34%	96%	13.07	The most sensitive imaging method.
¹⁸ F-FDG-PET	28%	97%	12.49	-
MRI	*	*	*	Wang and Chen(9), in 2011, didn't found any study that would fit their inclusion criteria and relate MRI to PM. Stronger evidence is needed to assure the sensitivity and specificity of MRI in PM detection.

*Data not reported.

However, caution should be used in analyzing these and other data reported on the imaging techniques. Some of the studies have low methodological quality and even the CT studies (the imaging method with more studies available) have a considerable variability in the conditions applied, which contributes to a very wide range of results reported. Nonetheless, it is clear that all the imaging methods have limitations inherent to its low sensitivity in PM detection, which affects their diagnostic capacity.

Computed tomography (CT)

CT has been the imaging modality of choice for PM diagnosis, because it is widely available at most medical centers and it is less expensive than other imaging modalities.(25) Abdominal CT can demonstrate not only the stomach wall and the adjacent tissue, but also the distant metastasis. (9)

The conventional CT scanning has some limitations. The thickness of the sections is one of the most significant. Some of the seeds in the peritoneum could have just a few millimeters so there is a risk of some lesions passing undetected if the thickness of the section used exceeds the size of the lesion. Hence, the thinner the sections applied, the more accurate CT is in detecting PM. (25)

Wang and Chen(9), in their systematic review and meta-analysis, reported a sensitivity of 33% and a specificity of 99% in detecting PM, which is only fairly good. However, the variability between studies is considerable: firstly, in the number of radiologists that analyze the images; secondly, in the section thickness that is used, including some studies that do not report this data; thirdly, in the number of patients taken into account and also the cancer stage. Besides that, it is clear that CT didn't have consistently high sensitivity to detect PM.

More recently, a few works tried to overcome some limitations of conventional CT, using radiomics analysis(26) or complex score tools based on CT findings(27), showing promising results.

Ultrasonography (US)

US is capable of detecting superficial peritoneal metastasis, as small as 2 to 3 mm, in the presence of ascites. However, deeper seeds are difficult, or even impossible to visualize, due to the interference of other structures.(28) This justifies the very low pooled sensitivity (only 9% in the systematic review and meta-analysis of Wang and Chen(9)) of this method to detect PM and the reason most studies exclude US from M-category assessment. Moreover, it is an operator dependent technique, which is a great disadvantage.(9)

Endoscopic Ultrasonography (EUS)

EUS has been established as the modality of choice for T staging of GC with a pooled accuracy of 75%. Nonetheless, EUS has a limited capacity to evaluate N and M-categories, not being designed to look at distant metastasis.(23) Wang and Chen(9), in their systematic review and meta-analysis about imaging assessment of PM, reported a sensitivity of 34% and a specificity 96% to detect PM.

Adding to this, it has an inherent limitation of being operator dependent, although it overcomes other great limitations of abdominal US, based on the advantage of placing the transponder close to the lesions.(9)

18FDG - Positron Emission Tomography (18F-FDG PET)

18F-fluoro-2-deoxyglucose – PET is becoming more relevant in M-staging in GC, however for PM alone the sensitivity is limited.(23) Compared to CT, PET has the advantage of providing functional information and today it is not only useful in the staging process but also relevant in the follow-up of GC patients.(9) Wang and Chen(9), in their systematic review and meta-analysis about imaging assessment of PM, reported a sensitivity of 28% and a specificity 97%.

Several limitations of PET scanning are known and could be associated with lower diagnostic performance to detect PM. Firstly, its low spatial resolution, which may cause small seeded peritoneal nodules to be missed. The cell differentiation of tumors influences the radioisotope uptake, so the histological type of GC can influence the sensitivity to detect PM. Poorly differentiated adenocarcinomas and signet ring cell (diffuse) or mucinous carcinomas showed less uptake of 18F-FDG. Also, the metastasis in peritoneum do not necessarily have the same differentiation grade of the primary tumors. Thirdly, the interobserver variability. Fourthly, PET cannot detect ascites, which is a PM related finding that is easily detected by other imaging techniques, like CT.(25)

Despite the lower diagnostic performance when comparing with CT (which makes it difficult to base the surveillance of PM in GC patients on PET only), this imaging method could be useful in equivocal cases in CT.(25) Also, ESMO Clinical Practice Guidelines(4) also support that PET, if available, may improve detection of occult metastasis in some cases. In the future more attention will perhaps be paid to FDG-PET/CT, which showed better accuracy than either of this methods alone(13) and new PET tracers in detection of PM.(9)

Magnetic Resonance Imaging (MRI)

The role of MRI in GC assessment has been limited. Although most of the evidence produced reports to T and N evaluation the few studies related to PM have recognized its possible value in PM detection.(28)

One of the great advantages of MRI over CT in the evaluation of systemic disease is the significantly greater soft tissue contrast resolution and avoiding the need for contrast.(9) Some studies have reported a similar diagnostic performance between 18F-FDG PET and MRI for detection of peritoneal seeding.(29) Stronger evidence is needed to ascertain the sensitivity of MRI and some researchers have also reported that diffusion-weighted MRI (DW-MRI) has higher sensitivity to detect PM, comparable to CT.(9)

Final Considerations

The diagnosis of PM remains a challenge for conventional imaging methods, especially because of its variability in appearance, the size and the location of the lesions in the peritoneal cavity. Also, none of these techniques showed highly predictive value on PM diagnosis, justifying the need of more than one imaging technique in M-category assessment or even other staging tools like laparoscopy to complement the preoperative imaging studies.

Chapter 5

Tumor markers

Background

Tumor markers, especially the ones measured in serum, have been shown to correlate with clinical status of patients with GC. Most of them are used on current clinical practice as prognostic tools, assessing the efficacy of the response to chemotherapy treatments and identifying recurrences of the tumor, for example. This supplementary evidence takes an even greater importance when talking about peritoneal recurrence, where tumor markers are often the only tool, because peritoneal lesions are usually too small, making their detection by imaging methods difficult. (30)

Furthermore, the peritoneal recurrence often occurs after a GC resection surgery with curative intent, which indicates that possibly there were undetected PM in the peritoneal cavity at the time of surgery.(31) Taking this into account, tumor markers have possible predictive value to assess the true spread of GC at the time of its management, allowing to choose the best treatment option for these patients.

A systematic review published by the Japanese Gastric Cancer Association in 2013(32), reported a positive rate in GC patients for different tumor markers in serum: CEA (16%-68%), CA19-9 (14%-68%) and CA72-4 (16% - 70%). In these three markers, studies showed a strong correlation with clinical stage, having as higher levels as advanced is the stage. Additionally, other serum markers have been recognized and studied in relation to gastric cancer, like CA125, Alpha-fetoprotein (AFP) and Sialyl Tn antigens (STN). Nonetheless, not all of these have demonstrated predictive value on PM, and the sensitivity of each single indicator is low.(33)

Despite the relevance given to serum markers, some works also propose the preoperative assay of peritoneal washings as a reliable method to detect early stages of peritoneal dissemination. The analysis of some tumor markers in peritoneal lavage is then postulated to be capable of improving the accuracy of PM prediction.(24)

Carcinoembryonic antigen - CEA

CEA is the most commonly used tumor marker for GC.(24) Although the levels in serum have proved useful to predict recurrence in colon cancer, its usefulness in GC remains unclear.(31) Most of the studies that report data on the relation between serum levels of CEA (sCEA) and peritoneal involvement did not show a significant correlation in GC patients, and the few published works that found some statistically significant results between sCEA value and peritoneal involvement, describe low predictive value in comparison with other tumor markers also studied.

The sensitivity of sCEA in distinguishing patients with and without PM that is reported in these studies is also very low. Lai *et al*(34) findings put CEA in last place amongst the four most commonly used serum tumor markers, with the worst sensitivity (23%) and diagnostic accuracy (44.38%). Emoto *et al*(30) reported only 18.6% of sensitivity in PM prediction and Hwang *et al*(35), 31.8% of sensitivity in PM with a specificity above 85%. These results support that serum CEA doesn't have the profile to be a good predictor of PM.

Table 5.1 - Reported sensitivity and specificity of serum CEA (sCEA) for prediction of PM in GC.

Reference	n	n (PM)*	Cut-off value	Sensitivity	Specificity	Comments
Huang <i>et al</i> , 2019.(33)	344	86	>5 ng/ml	**	**	Low predictive value in comparison to other tumor markers. (p=0.031)
Hasbahceci <i>et al</i> , 2018.(36)	67	21	>0.5 ng/ml	**	**	sCEA is not significantly associated with peritoneal carcinomatosis
Ohi <i>et al</i> , 2015.(18)	493	44	>5 ng/ml	**	**	Not significantly associated with PM in GC. (p=0.6692)
Lai <i>et al</i> , 2014.(34)	215	46	>5 ng/ml	23.91%	89.35%	sCEA has predictive ability for PM in GC. (p<0.05)
Emoto <i>et al</i> , 2012.(30)	102	102	>5 ng/ml	18.6%	n = n(PM)	Does not correlate with the degree of PM. (p = 0.14)
Ucar <i>et al</i> , 2008.(37)	95	**	>5 ng/ml	**	**	Didn't found association between CEA positivity and PM.
Çetin <i>et al</i> , 2005.(31)	70	19	>10 ng/ml	**	**	sCEA didn't have predictive value for PM (p=0.36)

Hwang <i>et al</i> , 2004.(35)	768	88	>5 ng/ml	31.8%	86.7%	sCEA did not add significant predictive information. (p=0.471)
Fujimura <i>et al</i> , 2002.(38)	39	18	>5 ng/ml	**	**	No statistically significant relationship with PM was demonstrated.
Nakata <i>et al</i> , 1998.(39)	384	33	>6.5 ng/ml	**	**	Low predictive ability in comparison to other tumor markers. (p=0.0375)

*n(PM) = Presence of peritoneal metastasis (such as macroscopic peritoneal dissemination and/or positive peritoneal cytology) detected during staging laparoscopy/laparotomy or surgical procedure.

**Data not reported.

In contrast with the poor results found on sCEA, peritoneal lavage levels of CEA (pCEA) were significantly higher in GC patients with PM. Several studies report some correlation with PM in GC patients. Kanetaka *et al*(20) was a prospective study that analyzed the pCEA values of 597 patients who were submitted to laparotomy, 35 of them with detected PM. A significant relation was demonstrated between pCEA and PM (p<0.001), with 84.9% sensitivity above the cut-off of 100ng/g of protein. With regards to the comparison between sCEA and pCEA, the latter showed to be a better biomarker of clinical utility in GC.

Yamamoto *et al*(24), from 2004, is one of the several studies that didn't found any significant correlation between CEA levels in serum and peritoneal lavage fluid. This study also concluded that pCEA has an overall good sensitivity and specificity (75.8% and 90.8%, respectively) for diagnosis of PM in GC, and showed better results than CA125 and CA19-9 levels in peritoneal lavage. Furthermore, this paper claims that pCEA sensitivity is higher than cytology examination itself, proposing that patients with high CEA levels in peritoneum lavage could be good candidates for adjuvant chemotherapy due to the high risk of occult PM.

Posteriorly, Yamamoto and his colleagues presented another paper(17) combining pCEA and pCA72-4, also showing good results, with a sensitivity of 83.5% and specificity of 89.3% to pCEA alone. Another interesting work to explore was developed by Çetin *et al*(31), which compares sCEA and pCEA and only found statistically significant correlation in peritoneum lavage values.

More recently, Hasbahceci *et al*(36) also didn't find any significant association between sCEA and peritoneal carcinomatosis. On the other hand, pCEA levels were significantly correlated.

Table 5.2 - Reported sensitivity and specificity of peritoneal lavage CEA (pCEA) for prediction of PM in GC.

Reference	n	n (PM)*	Cut-off value	Sensitivity	Specificity	Comments
Hasbahceci <i>et al</i> , 2018.(36)	67	21	>0.5 ng/ml	**	**	pCEA is significantly associated with peritoneal carcinomatosis
Yamamoto <i>et al</i> , 2014.(17)	193	**	>0.5 ng/ml	83.5%	89.3%	pCEA is an independent factor predicting peritoneal dissemination. (p<0.0001)
Kanetaka <i>et al</i> , 2013.(20)	597	35***	>100 ng/g of protein	84.9%	22.8%	pCEA is significantly correlated with PM in GC. (p<0.001)
Li <i>et al</i> , 2005	61	**	>0.5 ng/ml	**	**	pCEA is correlated with PM in GC. (p<0.05)
Çetin <i>et al</i> , 2005.(31)	70	19	>10 ng/g of protein	58%	72%	pCEA is correlated with PM in GC. (p=0.026)
Yamamoto <i>et al</i> , 2004.(24)	22 9	33	>0.5 ng/ml	75.8%	90.8%	pCEA is a significant factor for the prediction of PM. (p<0.0001)
Fujimura <i>et al</i> , 2002.(38)	39	18	>5 ng/ml	**	**	Statistically significant correlation with PM. (p <0.034)

*n(PM) = Presence of peritoneal metastasis (such as macroscopic peritoneal dissemination and/or positive peritoneal cytology) detected during staging laparoscopy/laparotomy or surgical procedure.

**Data not reported.

*** Kanetaha *et al*, considered presence of PM only by the macroscopic findings detected during laparotomy. Positive peritoneal lavage cytology had a different category.

Moreover, the Reverse transcriptase-polymerase chain reaction (RT-PCR) was found to allow diagnosis of micro-metastasis based on tissue-specific mRNA expression by tumor cells, with good sensitivity.(40)

Nakanishi *et al*,(40) in 1997, proposed this method as more sensitive than cytology to detect PM. Other studies also focused on CEA mRNA detected by RT-PCR in the peritoneal lavage. Kodera *et al*(41) reported a correlation between these values and

peritoneal metastasis. Both univariate analysis and multivariate analysis showed the value of this technique in diagnosing PM as the endpoint, with a sensitivity and a specificity of 77% and 94%, respectively. This study includes a follow-up, hence it assumes that the patients who showed PM had free cancer cells in peritoneum at the time of their first approach. A previous study also by Kodera *et al*(42), reported similar results with a more extensive pool of GC patients.

Nonetheless, the parallel between these results and pCEA needs a prudent interpretation. CEA mRNA fragments originate from the cancer cells, which points to the existence of viable cancer cells in peritoneum; on the other hand, pCEA is postulated to also enter the peritoneum through systemic circulation.(20) Other issue to take into account is that sometimes non-cancerous cells could express the target mRNA resulting in false-positives when applying RT-PCR techniques to detect CEA mRNA.(41,42)

The results showed by these studies pointed to a higher usefulness of CEA levels measurement in the peritoneal lavage. This could be associated to the fact that the peritoneal cavity is a more restrict environment, so the CEA produced by tumor cells that disseminate in this space could reach higher concentrations there, with no correspondence with serum levels released by the rest of the tumor mass into the blood circulation.(31)

Carbohydrate antigen 19-9 - CA19-9

CA19-9, also known as sialyl Lewis(a), is a tumor marker greatly associated with gastrointestinal cancers (mainly pancreatic cancer, colon cancer and GC). It is a glycoprotein that exists in serum assuming a mucin form, and various studies found its correlation with tumor size and metastasis.(33) It has also been reported that serum CA19-9 is a useful marker in the early detection of GC recurrence after surgery.(24)

In GC patients with PM, Lai *et al*(34) reported the best sensitivity using CA19-9 serum tumor marker (at 36.96%), higher than CEA, CA125 and CA72-4. Additionally, Hwang *et al*(35) demonstrated that preoperative serum CA19-9 levels may be useful in predicting PM in patients with GC, reporting 37.5% sensitivity. Better results were presented by Ohi *et al*(18), claiming that the risk of PM at laparotomy was significantly higher in patients with higher levels of CA19-9 (>37 U/ml), having a sensitivity of 54.55% and a specificity of 74.39% to detect PM.

Table 5.3 - Reported sensitivity and specificity of serum CA19-9 for prediction of PM in GC.

Reference	n	n (PM)*	Cut-off value	Sensitivity	Specificity	Comments
Huang <i>et al</i> , 2019.(33)	344	86	>27.315 U/ml	57%	79.8%	<0.0001
Hasbahceci <i>et al</i> , 2018.(36)	67	21	>37 U/ml	**	**	sCA19-9 is significantly associated with peritoneal carcinomatosis
Ohi <i>et al</i> , 2015.(18)	493	44	>24 U/ml	54.55%	74.39%	0.0006
Lai <i>et al</i> , 2014.(34)	215	46	>37 U/ml	36.96%	79.88%	<0.05
Emoto <i>et al</i> , 2012.(30)	102	102	>37 U/ml	36.3%	n = n(PM)	<0.05
Ucar <i>et al</i> , 2008.(37)	95	**	>35 U/ml	**	**	CA19-9 is associated with PM. (p=0.01)
Hwang <i>et al</i> , 2004.(35)	768	88	>37 U/ml	37.5%	95%	< 0.0001
Fujimura <i>et al</i> , 2002.(38)	39	18	>37 U/ml	**	**	No statistically significant relationship with PM was demonstrated.

*n(PM) = Presence of peritoneal metastasis (such as macroscopic peritoneal dissemination and/or positive peritoneal cytology) detected during staging laparoscopy/laparotomy or surgical procedure.

**Data not reported.

In the opposite direction, Fujimura *et al*(38), analyzed clinical data from a small pool of GC patients (39 patients, 18 of them with diagnosed PM) and didn't find a significant correlation between serum levels of CA19-9 and PM. The same study presented similar results with peritoneal lavage CA19-9 (pCA19-9), and didn't find correlation between serum and peritoneal fluid values.

Yamamoto *et al*(24) and Hasbahceci *et al*(36) showed similar views about the possible use of peritoneal levels of CA19-9 (pCA19-9) and, in their results, this marker didn't prove to be a significant factor for the prediction of peritoneal dissemination.

Carbohydrate antigen 125 - CA 125

Carbohydrate antigen 125 (CA125) is a tumor marker mostly used in patients with ovarian cancer, that has been recognized as potentially useful in GC.(35) CA 125 is found in mesothelial cells of the peritoneum, pleura and pericardium, as well as in the fallopian tubes, endometrium and endocervix in the female reproductive system, which suggests that dissemination of GC to the peritoneum can cause a diffuse insult to mesothelial cells, and as inflammation could be a local effect of PM, it may affect the levels of CA 125.(39)

Furthermore, it was reported that the production of CA125 by gastrointestinal tumors is infrequent, which further supports the thesis that elevated levels in GC were not a result of increased tumor cell volume but mainly reflected the severity of induced peritonitis. Also, in ovarian cancer, the studies suggest a relation between ascites and the levels of CA125 at the time of diagnosis.(30)

The half-life of this tumor marker in serum is approximately 5 days, and this is especially important because some studies have reported a decrease in specificity of CA125 during the first 2 months after a surgical procedure, probably due to the inflammation induced in the peritoneum and serosa and the healing process.(39)

Several studies put CA125 in a privileged position, between other tumor markers, in terms of possible clinical value in the prediction of PM in GC patients.(33) Nakata *et al*(39) was the first study to report the value of CA125 in the diagnosis of PM in GC and concluded that the sensitivity, specificity and accuracy of serum CA125 were 39.4%, 95.7% and 90.8%, respectively.

Hwang *et al*(35), in 2004, showed a sensitivity, specificity and accuracy of 38.3%, 98.4% and 91.5%, respectively, and the highest odd ratio to predict PM amongst the several studied indicators. This work used the 1st edition of the Japanese classification of gastric carcinoma by the Japanese Research Society for Gastric cancer, which classified PM in three different groups (P1, P2 and P3) and correlated significantly the rise in levels of serum CA125 with the degree of peritoneal dissemination. More recently, Emoto *et al*(30), in a study contemplating only PM positive patients, concluded that serum CA125 was significantly positively correlated with PM, with a diagnostic sensitivity of 46.1%, the higher between all studied markers, and it also positively correlated CA125 with the degree of PM based on the same Japanese classification. No significant correlation was observed between the other markers in study (CEA, CA72-4, CA19-9). Similar results were obtain in relation to the presence of ascites. It's important to notice that this

classification was abandoned because there was no significant difference in prognosis between any PM groups (P1, P2 and P3).(21)

The highest sensitivity reported was in Huang *et al*(33), with 79%, plus 84.9% and 82% of specificity and accuracy, respectively, being the most important marker in the proposed algorithm.

Table 5.4 - Reported sensitivity and specificity of serum CA125 for prediction of PM in GC.

Reference	<i>n</i>	<i>n</i> (PM)*	Cut-off value	Sensitivity	Specificity	Comments
Huang <i>et al</i> , 2019.(33)	344	86	>17.3 U/ml	79.1%	84.9%	<0.0001
Lai <i>et al</i> , 2014.(34)	215	46	>35 U/ml	34.78%	85.80%	<0.05
Emoto <i>et al</i> , 2012.(30)	102	102	>30 U/ml	46.1%	<i>n</i> = <i>n</i> (PM)	Significantly positively correlated with PM (<0.05)
Hwang <i>et al</i> , 2004.(35)	768	88	>35 U/ml	38.2%	98.4%	< 0.0001
Fujimura <i>et al</i> , 2002.(38)	39	18	>35 U/ml	55%	100%	Statistically significant relation with PM was demonstrated. (p<0.001)
Nakata <i>et al</i> , 1998.(39)	384	33	>35 U/ml	39.4%	95.7%	Statistically significant correlation with PM. (p<0.0001)

**n*(PM) = Presence of peritoneal metastasis (such as macroscopic peritoneal dissemination and/or positive peritoneal cytology) detected during staging laparoscopy/laparotomy or surgical procedure.

**Data not reported.

Other studies also compared serum and peritoneal lavage values of CA125. Fujimura *et al*(38) analyzed clinical data from 39 patients with GC, 18 of them diagnosed with PM and reported a sensitivity, specificity and accuracy of serum CA125 of 55%, 100% and 76%, respectively. This study also determined the value of CA125 in peritoneal lavage (pCEA), and didn't find correlation between serum and peritoneal fluid values, further concluding that sCA125 is a stronger predictor than pCA125.

Fujimura *et al*(38) results overlap those of Yamamoto *et al*(24), 2004, and no correlation between sCA125 and pCA125 was found. The latter study also concluded that pCA125 is

a significant factor for PM prediction with a sensitivity and specificity of 42.4% and 93.9%.

Carbohydrate antigen 72-4 - CA72-4

CA72-4 is a glycoprotein that can be found in a variety of cancers, and some published works have reported a great specificity for the diagnosis of GC. Also, several studies consistently reported higher positive rate of CA72-4 in the serum of GC patients with PM, when comparing to serum CEA.(33) Additionally, this marker has been associated with poor prognosis and advanced stages of GC and other gastrointestinal tumors.(30)

Emoto *et al*(30), reported the value of CA72-4 in the diagnosis of PM and concluded that the sensitivity of this marker to detect PM is 44.97%, considering this indicator, together with CA125, it's the most valuable single marker in predicting PM in GC. Lai *et al*(34), a work with more than 300 patients with GC, 86 of them PM positive, reported 34.78% of sensitivity, 82.25% of specificity and 72.09% of diagnostic accuracy. More recently, Huang *et al*(33) found a sensitivity, specificity and accuracy of 57%, plus 86.4% and 71.7%, respectively.

Table 5.5 - Reported sensitivity and specificity of serum CA72-4 for prediction of PM in GC.

Reference	n	n (PM)*	Cut-off value	Sensitivity	Specificity	Comments
Huang <i>et al</i> , 2019.(33)	344	86	>7.25 U/ml	57%	86.4%	<0.0001
Lai <i>et al</i> , 2014.(34)	215	46	>5 U/ml	34.78%	82.25%	<0.05
Emoto <i>et al</i> , 2012.(30)	102	102	>4 U/ml	44.97%	n = n(PM)	<0.05
Ucar <i>et al</i> , 2008.(37)	95	**	>6 U/ml	**	**	CA72-4 is associated with PM. (p=0.03)

*n(PM) = Presence of peritoneal metastasis (such as macroscopic peritoneal dissemination and/or positive peritoneal cytology) detected during staging laparoscopy/laparotomy or surgical procedure.

**Data not reported.

From 2014, Yamamoto *et al*(17) established a cut-off of 1.3 U/ml in peritoneal lavage (pCA72-4) and found a correlation between high pCA72-4 and peritoneal dissemination. The research showed a sensitivity of 84.4% and specificity of 77% to pCA72-4 alone. When combined to pCEA, these two markers showed a good accuracy in PM prediction.

Sialy Lewis (x) and Sialyl Tn antigens - STN

Less studied than other tumor markers in GC, STN and SLX have reported as possible PM predictors by some published works. Nakata *et al*(39) reported that a value of STN above the cut-off 45 U/ml showed the best sensitivity to detect PM in GC amongst the serum tumor markers studied (CA125, CEA, CA19-9 and STN), with a value above 50%. Other studies also concluded that both SLX and STN are associated with peritoneal dissemination.(43)

Final considerations

There is no specific tumor-associated antigens in GC. However, CEA and CA19-9 are known to be elevated in the serum of patients with advanced GC, being the most widely used.(35) Nonetheless, other tumor markers like CA 125 and CA72-4 have been recognized as potentially useful in advanced stage GC. In any case, the power of any tumor markers, in terms of decision-making, remains unclear.(30)

There have been many conflicting reports in regards to the association of levels of serum and peritoneal tumor markers and PM in patients with gastric cancer, before and after the curative surgery.(36) The available data supports that only some of the serum tumor markers have been proved to have consistently predictive value to detect PM, identified as CA19-9, CA125, CA72-4 and STN. This conclusion is further supported by the systematic review conducted by Japanese Gastric Cancer Association Task Force in 2012(32), which reported clinical significance related to PM in CA19-9, CA125, CA72-4 and STN. Apart from that, it claims that there is no evidence that supports the relation between serum CEA and PM and also a lack of evidence in relation to AFP.

In contrast with the results found on serum, peritoneal lavage levels of CEA (pCEA) were significantly higher in GC patients with PM, and correlation with PM in GC patients was established. The same happens with pCA125 and pCA72-4, although with worst results than the correspondent serum values.

Nonetheless, the results of a single tumor marker in predicting PM in GC are only fairly good, with low sensitivity reported, which makes its potential in clinical application limited.(34) Therefore, several studies analyzed the results of these markers combined. Emoto *et al*(30) showed that combined use of CEA, CA19-9, CA72-4, and CA125 may improve sensitivity of those markers in detecting PM in GC the four markers have a combined sensitivity of 78.4%. In pairs, CA72-4 plus CA125 showed the best results with 68% sensibility and CEA plus CA19-9 the lowest sensitivity with 44.1%.

Huang *et al*(33) combined four serum tumor markers (CA125, CA19-9, CA72-4) and FLR (fibrinogen/lymphocyte ratio) and created a classification tree program with an accuracy, sensitivity and specificity of 77.4%, 94% and 89.5% respectively. Additionally, this study proposes a decision algorithm to assess the risk of PM that also showed good results with an accuracy, sensitivity and specificity of 91%, 89.5% and 79.5%, respectively.

In a similar way, Lai *et al*(34) concluded that the combined use of CEA, CA19-9, CA72-4, and CA125 with LOX (Lysyl Oxidase), another biomarker, could increase the capacity to predict PM. The results looked promising with more than 90% sensitivity, but with lower specificity and diagnostic accuracy (only 20.59% and 29.3%, respectively).

Combining independent predictors of PM is not limited to biomarkers. Tumor characteristics have also been taken into account in the design of more complex predictor models. In studies like Ohi *et al*(18), the investigators concluded that the combination of preoperative tumor features (including tumor histopathology and morphology) associated with preoperative serum markers (including serum tumor markers and SIR markers) increased significantly the sensitivity (84.09%) and specificity (82.63%) in detecting PM in GC patients.

Furthermore, molecular diagnosis of cancer micro-metastasis with the RT-PCR method in peritoneum lavage fluids have been taken into account, especially with CEA mRNA. Nonetheless, the publications available still very limited, because these techniques require trained technicians, specialized equipment and good quality control in handling the genetic material. Also molecular diagnosis is far more expensive than immunohistochemistry techniques. Although the results are promising, all of these conditions, limit their use in current clinical practice in a more generalized way.(44)

Finally, most of the studies conducted on serum tumor markers have some limitations. Firstly, the majority of them are single-center retrospective cohort studies, which potentialize the existence of bias. Secondly, the samples are, in most of the cases, not sufficiently large and have low proportion of PM cases in the studied universe. Therefore, multicenter large-scale prospective randomized controlled trials are necessary, in order to obtain stronger evidence.

Chapter 6

Systemic Inflammatory Response Markers

Background

Increasing attention has been paid to the relation between tumorigenesis and body inflammatory status.(7) It is proposed that cancer promotes a local disturbance, caused by tissue damage, which induces a systemic inflammatory response (SIR). This response, although an attempt to protect the host, is used by the tumor to further progression, playing a key role in the cancer microenvironment, promoting tumor growth and metastasis. Furthermore, inflammatory factors inhibit apoptosis, promote angiogenesis and induce DNA damage. This non-ending cycle is the reason why cancers are described as “wounds that don’t heal”.(16)

Inflammation indices, such as C-reactive Protein (CRP), neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), serum albumin and others, have been studied as prognostic factors in different types of cancers, including GC, and associated with poor outcomes.(45,46) Most of these parameters are easily obtained from the routine preoperative exams in GC patients.(4) Nonetheless, few studies have directly related SIR markers with the occurrence of peritoneal metastasis, and most of them are related to NLR.(7,16,18,45,46)

Neutrophil to Lymphocyte Ratio (NLR)

NLR is calculated by dividing absolute neutrophils count by the absolute lymphocytes count, which is easily obtained using one of the most simple and reliable tests: the complete blood cell count (CBC).(46) Elevation of this index can occur as a result of neutrophilia, lymphopenia or both, and suggests a systemic inflammatory state.

Even if the whole process is not yet known in detail, the inflammatory status promoted by tumors rises neutrophils count. Due to inflammatory signals generated by tumor microenvironment (like IL-1, TNF- α and IL-8), neutrophils are able to localize and concentrate in tumor spots, locally promoting a pro-cancer environment by secreting VEGF, MMP-9 and reactive oxygen species. This provides favorable conditions to

angiogenesis and tumor growth, including metastasis. Some of these conditions could also be associated with the inhibition of active T cells.(47)

In the opposite direction, lymphocytes are essential to inhibit the tumor progression, especially through cytolytic effect, being proposed that lower lymphocyte count reflects that the system is unable to perform anti-tumoral activity.(16) So, whether as a result of neutrophilia or lymphopenia, elevated NLR suggests an inability to suppress the tumor progression (47), and it is possible to postulate that it may be closely related with peritoneal metastasis (PM).

A Systematic review and meta-analysis conducted by Bowen *et al*(47) suggested that NLR greater than cut-off values indicates reduced overall survival in GI cancers, regardless of geographic location or cancer stage. Despite this work being about GI cancer prognostic and including other GI cancers besides GC, we can extrapolate information to support the hypothesis of NLR as a predictor of PM in GC, and it also provides a reference of a median cut-off value to consider NLR in GC: $NLR > 3.0$.

Five research works^{7,15-18}, with publication dates ranging from 2014 to 2019, establish NLR as a direct predictor of PM. In all of them, the *p value* obtained by univariate analysis and/or multivariate analysis provides evidence to establish NLR as an independent predicting factor of PM. The cut-off values proposed in these studies oscillated between $NLR > 1.95$ in Chen *et al*(7), and $NLR > 3.5$ in Nakamura *et al*(45).

Table 6.1 - Reported cut-off values of NLR for prediction of PM in GC.

Reference	<i>n</i> (total)	<i>n</i> (PM)*	Clinical stage**	Cut-off value	Comments
Nakamura <i>et al</i> , 2019.(45)	35	16	Stage II/III/IV: 7/16/12	NLR > 3.5	Independent predictor of PM during staging laparoscopy. (p < 0.0001)
Chen <i>et al</i> , 2017.(7)	1080	101	***	NLR > 1.95	Independent predictive value of PM. (p = 0.011)
Grenader <i>et al</i> , 2015.(46) ****	117	25	Stage II/III: 22%/78%	NLR > 3.28	Independent predicting factor for the discovery of peritoneal and/or metastatic disease. (p = 0.005)
Ohi <i>et al</i> , 2015.(18)	493	44	Stage I/II/III/IV: 264/79/78/72	NLR > 2.64	Independent preoperative predictor of PM. (p = 0.0002)
Nakayama <i>et al</i> , 2014.(16)	359	58	Stage I/II/III/IV: 35/104/163/58	NLR > 2.37	Independent predictive value of PM. (p = 0.001)

**n*(PM) = Presence of peritoneal metastasis (such as macroscopic peritoneal dissemination and/or positive peritoneal cytology) detected during staging laparoscopy/laparotomy or surgical procedure.

** According to the 7th edition of AJJC/UICC TNM classification

*** Data not reported.

**** Study includes gastric and esophageal cancers.

The sensitivity of NLR reported in these studies varies between 59.09% and 75%, and specificity between 40.4% and 89%. Ohi *et al*(18) and Nakayama *et al*(16), which show the more consistent results and have more close cut-off values (NLR > 2.64 and NLR > 2.37), report 59.09 % and 63.8%, respectively, for sensitivity and 69.71% and 67.4% respectively, for specificity. The lowest specificity reported in Chen *et al*(7), (40%) could be explained by the low cut-off point (NLR > 1.95) applied to a universe of more than a thousand patients with a wide range of GC clinical stages.

Table 6.2 - Reported sensitivity and specificity of NLR for prediction of PM in GC.

Reference	Variables	n	Sensitivity	Specificity	AUC*	OR (95% CI)	P
Nakamura <i>et al</i> , 2019.(45)	NLR > 3.5	**	75%	89%	0.86	25.5	<0.0001
Chen <i>et al</i> , 2017.(7)	NLR > 1.95	662	74.3%	40.4%	0.576	*	0.005
Grenader <i>et al</i> , 2015.(46)	NLR > 3.28	33	**	**	**	3.9	0.005
Ohi <i>et al</i> , 2015.(18)	NLR > 2.64	**	59.09%	69.71%	0.621	3.2895	0.0002
Nakayama <i>et al</i> , 2014.(16)	NLR > 2.37	134	63.8%	67.4%	0.677	3.71	<0.001

*AUC = Area under the curve in the ROC curve (Receiving Operating Characteristic curve)

**Data not reported.

There are some limitations to consider in these studies. All of them are retrospective Cohort Studies and their nature accounts for high likelihood of confounding variables. None of them are a multicenter study, reporting only data from a single institution. The proportion of advanced stage GC (more associated with PM) is not consistent between studies and could influence the best cut-off reported for NLR as well as the variation between sensitivity and specificity reported in different studies. One of the studies, Grenader *et al*(46), also includes patients with esophageal cancer, which confers an important risk of bias.

Other SIR markers

Other inflammatory signals have been associated with PM in GC with a big range of statistical significance (considering significant a *p value* < 0.05).

Table 6.3 - Reported cut-off values of other SIR markers (excluding NLR) for prediction of PM in GC.

Reference	n (total)	n (PM)*	Clinical stage	Cut-off value	Comments
Huang <i>et al</i> , 2019.(33)	344	86	****	FLR > 2.555	Independent risk factor for GC with PM. (p < 0.0001)
Okugawa <i>et al</i> , 2019(48)	551	48	Stage** I/II/III/IV: 296/86/87/82	LCR < ****	Low preoperative LCR was significantly associated with PM (p = 0.006)
Nakamura <i>et al</i> , 2019.(45)	35	16	Stage** II/III/IV: 7/16/12	Serum Albumin < 3.5 g/dl Total Protein < 6.5 g/dl	Significantly correlated with positive PM. (p = 0.04) Significantly correlated with positive PM. (p = 0.03)
Chen <i>et al</i> , 2017.(7)	1080	101	****	PLR > 131	Independent predictive value of PM. (p = 0.001)
Wei <i>et al</i> , 2016.(49)	298	25	****	CCL22 > 987.50 pg/ml	Elevated levels of CCL22 are associated with PM. (p < 0.001)
Wang <i>et al</i> , 2016.(50)	105	22	****	CCL5 > 67.5 pg/ml	Elevated levels of CCL5 predict occult PM. (p = 0.0002)
Ohi <i>et al</i> , 2015.(18)	493	44	Stage** I/II/III/IV: 264/79/78/72	Serum Albumin < 3.8 g/dl Lymphocyte count $\leq 1110 \times 10^3$ /ml	Significantly discriminates GC patients with PM from those without PM. (p = 0.002) Independent preoperative predictor of PM. (p = 0.0004)
Nakayama <i>et al</i> , 2014.(16)	359	58	Stage** I/II/III/IV: 35/104/163/58	Serum Albumin < 3.5 g/dl CRP < 1mg/dl	Significantly related to the presence of PM. (p = 0.014) Significantly related to the presence of PM. (p = 0.022)
Kim <i>et al</i> , 2009.(51)	115	6	Stage*** I/II/III/IV: 43/23/30/19	IL-6 > 6.77 pg/ml	Levels of IL-6 are associated with PM. (p = 0.012)

*n(PM) = Presence of peritoneal metastasis (such as macroscopic peritoneal dissemination and/or positive peritoneal cytology) detected during staging laparoscopy/laparotomy or surgical procedure.

** According to the 7th edition of AJCC/UICC TNM classification

*** According to the 6th edition of AJCC/UICC TNM classification

**** Data not reported.

Platelets and fibrinogen

Related to the inflammatory response promoted by tumors, coagulation factors, like fibrinogen, play an important regulator role of SIR to cancer, stabilizing the tumor extracellular matrix, a key step for further progression and invasion. Also, previous studies have postulated that fibrinogen may mediate the recruitment of leukocytes, promoting the release of pro-inflammatory cytokines, like IL-6 or TNF- α .(52) Platelets, in their interaction with fibrinogen, protect tumor cells from being killed by the host's immune response. Additionally, activated platelets could secrete growth factors that feed the tumor.(7)

In spite of the absence of previous studies showing direct links between hyperfibrinogenemia and PM, a meta-analysis by Cheng *et al*(52), has shown evidence that correlates high preoperative values of fibrinogen with diminished overall survival, and more aggressive clinicopathological features, including distant metastasis, yet not specifying PM.

Based on this coagulation role, two studies(7,33) have shown evidence that support fibrinogen/lymphocyte ratio (FLR) and platelet/lymphocyte ratio (PLR) as predictors of PM in GC. FLR is calculated by dividing serum fibrinogen by the absolute lymphocytes count, hence, elevation of this ratio can be a reflex of hyperfibrinogenemia, lymphopenia or both. The same rationale is applied to PLR, calculated by dividing platelets count by the absolute lymphocytes count.

Huang *et al*(33) reported a cut-off value for FLR above 2.555, with a sensitivity and specificity around 65% for risk assessment of GC with PM. Despite the weak results, this study proposes a decision algorithm to assess the risk of PM, based on a classification tree that integrates serum tumor markers (CA125, CA19-9, CA72-4) and FLR. This algorithm showed better results with an accuracy, sensitivity and specificity of 91%, 89.5% and 79.5% respectively.

Related to PLR, Chen *et al*(7), proposed 131 as the best cut-off value to predict PM. This study recognizes PLR as an independent indicator but also a predictor instrument that integrates indicators like invasion depth, lymphatic invasion and pathological type, presenting more reliable results. The analysis done reported a sensitivity and specificity of 69.3% and 51% respectively, for PLR. This study is also interesting because it compares directly PLR and NLR, pointing to a better performance of PLR. Attention is needed when looking at this data, in fact, it's possible to identify some issues that could

negatively influence NLR results, such as the low cut-off used when compared to the reported in other studies. The predicting system proposed, which includes tumor characteristics and PLR as inflammatory index, showed a good performance with 84% of sensitivity and 82.63% reported, suggesting that this score system has a potential diagnostic value for PM.

Table 6.4 - Reported sensitivity and specificity of FLR and PLR for prediction of PM in GC.

Reference	Variables	n	Sensitivity	Specificity	AUC*	p**
Huang <i>et al</i> , 2019.(33)	FLR > 2.555	***	65.1%	65.5%	0.653	< 0.0001
Chen <i>et al</i> , 2017.(7)	PLR > 131	***	69.3%	51%	0.599	< 0.001

*AUC = Area under the curve in the ROC curve (Receiving Operating Characteristic curve).

** p value for distinguishing GC cases with or without peritoneal metastasis.

***Data not reported.

Both platelets and fibrinogen have the advantage of being inexpensive and non-invasive markers, easy to obtain in regular clinical practice. The studies that reported predictive value to detect PM in GC patients of these markers also recognized that they are weak predictors independently, and could be more useful integrating models with other predictors, in order for them to be used as effective assessment tools to predict PM.

C-reactive Protein

C-reactive protein (CRP) is an acute reactive protein synthesized by the liver and one of the most sensitive indicators of inflammation, its secretion appears to be influenced directly by cytokines - IL-6, IL-1 and TNF. In several chronic inflammatory diseases, like coronary disease, CRP has been already correlated with disease severity. The same applies to some cancers, some more studied than others.(53)

A systematic review of literature conducted by Shrotriya *et al*(53) showed evidence that high CRP is, at least, related with high mortality in solid tumors, particularly in GI cancers. Nonetheless, few studies directly correlate the absolute CRP value with PM. Nakayama *et al*(16), reported that a value over 1.0 mg/dl cut-off point as a significant relation with PM (p = 0.0022). Different results are shown by Kim *et al*(51), in a work based on IL-6 and CRP value, which didn't find any statistically significant difference

between patients with and without PM ($p = 0.061$). In any of these cases, CRP, as individual marker, didn't show promise as a good predictor of PM in GC.

In other study, Okugawa *et al*(48) studied the prognostic value of lymphocyte/C-reactive protein ratio (LCR). LCR is calculated by dividing absolute lymphocytes count by serum CRP value. They reported that low preoperative LCR was significantly associated with PM in GC ($p = 0.006$). Unfortunately, no ROC curve analysis was performed to get the best cut-off for PM prediction.

Moreover, some studies have proposed C-reactive protein/albumin ratio (CAR) as having prognostic value in several cancers. In fact, an updated meta-analysis by Xu *et al*(54) showed its association with poor overall survival in GC. There is an evident lack of studies correlating CAR with PM in GC, and this could be a potential object of study in the future.

Serum Albumin

Albumin is the most common plasma protein, accounting for $\frac{1}{2}$ of total plasma proteins.(54) A systematic review and meta-analysis, conducted in 2010, associated higher levels of serum albumin to a better prognosis in GI tumors in general.(55) Nonetheless, few studies directly correlated serum albumin to PM in GC and the ones who have done this, showed weak statistical significance, comparing to other SIR markers.

Nakayama *et al*(16), found that low serum albumin (<3.5 g/dl) is correlated with the presence of PM. Similar results are appointed by Nakamura *et al*(45) and Ohi *et al*(18), proposing that a decrease in albumin is significantly correlated with macroscopic peritoneal dissemination and/or positive peritoneal cytology (p value = 0.04 and p value = 0.002, respectively). Moreover, Nakamura *et al*(45) also reported significant results on total protein value (<6.5 g/dl) and their predictive capacity over PM ($p = 0.03$).

Cytokines

Cytokines are key players in the inflammation process. Between them, special attention has been paid to interleukin-6 (IL-6) due to its role in angiogenesis and invasion promotion. Kim *et al*(51) studied serum IL-6 values, and found that the values were significantly higher in patients with peritoneal seeding, with a significant difference

between patients with and without PM ($p= 0.012$). In this study the cut-off established was 6.77pg/ml.

Besides that, it's important to take into account that IL-6 is implicated in other conditions characterized by chronic inflammation, including viral and bacterial infections and autoimmune diseases, lacking specificity.(51)

Chemokines

Other inflammatory markers, that are not part of routine analytics, have been also studied. Two Chinese studies (49,50), by Wang *et al* and Wei *et al*, reported that elevated serum levels of some chemokines, CCL5 and CCL22, respectively, are predictors of PM in GC patients. Chemokines are inflammatory mediators that interact with the respective receptors in the tumor microenvironment, to promote tumor growth and metastasis by several mechanisms. They are capable of recruiting leukocytes (specially neutrophils and T-reg lymphocytes) that further support the tumor development. CCL22 plays a key role in recruiting T-reg cells that downregulate T cell mediated immune response, which favors cancer progression.(49) CCL5 has been more studied in relation to breast and lung cancer, where it showed to enhance cell migration from the primary tumors.(50) In any of these cases, further investigation is required on the role of chemokines in GC and in other cancers in general.

The sensitivity reported for chemokines, above cut-off values, for distinguish GC cases with or without PM is 80% for both CCL22 and CCL5. The specificity is 71% for CCL22 and 69% for CCL5. Despite this fairly good results it is important to consider that these are not currently measured markers and lack some of the greatest advantages seen in other SIR markers: low cost, to be widely available, easy and quick to get results from and to be already routinely done in GC patients during staging assessment.

Table 6.5 - Reported sensitivity and specificity of some chemokines for prediction of PM in GC.

Reference	Variables	<i>n</i>	Sensitivity	Specificity	AUC*	<i>p</i> **
Wei <i>et al</i> , 2016.(49)	CCL22 > 987.50 pg/ml	***	80%	71%	0.83	<0.05
Wang <i>et al</i> , 2016.(50)	CCL5 > 67.5 pg/ml	***	80%	69%	0.795	0.0002

*AUC = Area under the curve in the ROC curve (Receiving Operating Characteristic curve).

** *p* value to distinguish GC cases with or without peritoneal metastasis.

***Data not reported.

Final considerations

Based on the available evidence, it is possible to postulate that decreased immune response of the host against the tumor may be closely related with PM. However, until today, few studies were invested in trying to correlate SIR markers with PM in GC, with most of them focusing only on prognostic value and risk of recurrence after GC surgery. The data available also indicates that most of these potential markers, as individual indicators, have low accuracy and sensitivity to predict PM in GC. So, as some of these studies have determined, SIR markers could be more useful in combination with other predictors, creating tools that show a more effective assessment of PM in GC. Furthermore, the fact that most of these markers are also elevated in a great variety of conditions characterized by chronic inflammation, can contribute for some lack of specificity.

Performing a single marker evaluation, NLR proved to be the most promising marker individually, combining good results with the advantage of being very cost-effective to obtain in regular clinical practice. The other markers probably need more research to find out if they show consistently good results. Accessibility and low cost are good qualities for PM predictors. In nowadays clinical practice most of them are already measured, so the information they can give is readily available. This also could potentiate their integration in clinical score systems, helping the clinicians to make treatment choices.

Most of the studies conducted on SIR markers have the same limitations. Firstly, they are single-center retrospective studies, so there may be some bias. Secondly, the samples are, in most of cases, not sufficiently large and have low proportion of PM cases in the studied universe. Therefore, multicenter large-scale prospective randomized controlled trials are necessary, in order to obtain stronger evidence.

Chapter 7

Other Molecular Markers

Background

The development of PM is a multistep process counting on the contribution of multiple molecular mechanisms. The understanding of these molecular events in each step of peritoneal dissemination is important to identify potential molecular markers that could be of use in clinical practice, improving prognosis and the early detection of this condition.(56) The panel of potential molecular markers is vast, however, the number of works published regarding this particular association with PM in GC is limited.

Metalloproteinases - MMPs

There are more than 17 Metalloproteinases (MMPs), of which MMP-7 was reported to be produced mainly by tumor cells, not stromal cells, and expressed in peritoneal dissemination from gastric tumors. MMPs play an important role in invasion due to the destruction of extracellular matrix.(57)

Yonemura *et al*(58), reported that RT-PCR assay for MMP-7 mRNA, preoperatively, could be a useful tool in combination with cytological examination, to detect occult PM. The results showed also that this molecule is not expressed by fibroblasts, mesothelial cells or normal gastric mucosa, being specific from the GC cells. MMP-7 alone had a sensitivity of 33%, a specificity of 88% and an accuracy of 70%. In combination with cytology, the sensitivity improves to 62%.

Trypsinogen

Trypsin is a serine protease produced by the exocrine pancreas, that acts like a digestive enzyme. The expression of trypsinogen-1 protein was reported in pancreatic adenocarcinomas and GC.(57) It is postulated that trypsin's proteolytic activity and it's inappropriate activation could enhance the peritoneal dissemination and infiltration process of the gastric tumor.(59)

Taking this into account, Fujimura *et al*(59), in 1998, published a paper reporting the potential use of this marker to detect occult PM in GC patients. In this work, they used

RT-PCR for trypsinogen-1 in both primary tumors and peritoneal lavage, of eight tumors known to have showed immunoreactivity to trypsinogen protein. In the three cases with detected PM by laparotomy or cytological examination, they obtain positive PCR results in the peritoneal lavage. Also they reported a case, where laparotomy and cytology were negative, with positive trypsinogen-1 by PCR that showed peritoneal recurrence in short term follow-up.

Nonetheless, the number of patients involved in this study is very limited. Validation of these results by other studies is needed.

Telomerase

Telomerase is an enzyme (ribonucleoprotein polymerase) responsible for maintaining the length of telomeric regions. Telomerase is inactive in most of normal somatic cells. Tumors use this activity to proliferate and multiply without restrictions.

Some studies have tried to determine the clinical significance of telomerase activity in GC, and its relation with PM in GC patients. Hu *et al*(60) showed some good results using a protocol of telomeric repeat amplification with ELISA, concluding that this method is more sensible than cytology and pCA125 immunoassay to detect peritoneal seeding. Nonetheless, telomerase has showed some lack of specificity comparing to the other techniques in this research, with a positive rate in peritoneal washes around 14% in cases of GC with no PM, comparing to the 7.1% of pCA125 and 0% of cytological examination.

Da *et al*(61) also used a TRAP-ELISA technique to evaluate the efficacy of telomerase activity in the peritoneal washes. The positive rate obtained in peritoneal fluid was 41.7%, against only 25% in the case of cytology, in all cases of GC studied. Taking into account the cases with detected PM, telomerase activity was detected in all, being more sensitive than cytological examination. These findings overlap the previous studies, showing that this technique could be useful combined with cytology, the gold standard.

The detection of telomerase activity could be a useful tool to detect early PM in patients with GC, being worthy of further investigation.

E-cadherin

Also known as cadherin 1, E-cadherin is an adhesion molecule that plays a key role in establishing epithelial architecture. Therefore, dysregulation of this molecule contributes to tumor invasion and progression.(56) In previous studies, Fujimura *et al*(59) reported the usefulness of E-cadherin's mRNA expression in epithelial cells, collected from intraperitoneal fluid, in the early diagnosis of PM.

Legumain

Legumain is a lysosomal protease, more accurately, a cysteine endopeptidase that was found to be highly expressed in several cancers, including GC. This protease is expressed both intracellularly and on the cell's surface, and tumors with higher levels of legumain showed enhanced invasive and metastatic properties.(62)

Guo *et al*(62) reported that high levels of legumain in primary gastric tumors showed a significant statistical correlation with some clinicopathological features, including PM ($p=0.002$). More recently, in 2019, Wang *et al*(63) associated legumain to diffuse type GC, concluding that legumain could be an important predictor of PM in these cases, being significantly associated ($p=0.0003$).

Chapter 8

Conclusions

Gastric Cancer (GC) is a worldwide problem, but more prevalent in East Asian countries.(1) This compelled some of these countries, like Japan and South Korea, to adopt population screening programs, with good results.(10) In fact, today, patients in Asian countries are more often diagnosed with GC at an earlier stage than in European countries.(4) In spite of this, probably the lower incidence rates in some European countries, like Portugal, do not justify the same approach, but something needs to be done in order to diagnose GC patients in early stages, giving them more treatment options and better chances of surviving.

One of the main factors for the decrease in overall survival and poor prognosis in patients with GC is the occurrence of peritoneal metastasis (PM). The five-year survival rate in these patients is lower than 3%.(15) The presence of free peritoneal tumor cells and metastasis in peritoneum increase with the stage of the primary tumor. The selection of these patients is relevant in order to make the best clinical decision and choose the best treatment. Currently, patients with potentially resectable GC in all stages IB-III have indication to go under laparoscopy (with or without peritoneal washings for malignant cells) to exclude occult metastatic disease involving the peritoneum(4).

One of the problems is that neither of the currently performed imaging methods or cytological examination have strong and consistent sensitivity rates in PM diagnosis, and the earlier the stage, the less sensitive they become. There is no single gold standard imaging method for staging GC and detect peritoneal involvement.(23) Despite that, from all the conventional methods available, CT is the most used in clinical practice to detect PM, but only has a sensitivity of 33%.(9) Cytological examination, on the other hand, has proved to be more accurate and sensitive, with some reports concluding that its sensitivity could be over 70%.(3) But even when patients showed negative results on peritoneal lavage cytology, some still suffered from peritoneal recurrence.(20) This reinforces the need for useful tools that are able to detect the existence of PM with higher sensitivity.

In this work consisted in a thorough review of the studies that aimed to determine the the sensitivity and specificity of several biomarkers that are able to predict PM with more accuracy. The biomarkers researched fit in three categories: Tumor markers, Systemic

Inflammatory Response markers, and other markers who don't fit any of the previous groups. Nonetheless, none of these indicators showed strong sensitivity to detect PM at an individual level.

Regarding the tumor markers, CA125 showed the best results in serum (sCA125) and CEA was the most useful measure in the peritoneal lavages (pCEA). But if we look to this results in detail, the sensitivity reported is only fairly good, between 38.78% and 79.1% for sCA125 and 58% to 84.9% for CEA. Jointly, these and the other tumor markers have high specificity, probably due to the fact that they are, as the main name states, "tumor" markers, hence it is less probable for them to be elevated due to other conditions not related to the primary cancer.

Focusing on the Systemic Inflammatory Response (SIR) markers, Neutrophil to lymphocyte ratio (NLR) is the most studied single marker and the one who showed consistently better results. The sensitivity in research works ranges from 59% to 79%. Other SIR markers, like PLR have also showed promising results. Common to all of these markers, and probably the greatest handicap when comparing to tumor markers, is the lower specificity. This is a reflex of the fact that several other condition, not directly related to the tumor, can influence these values more often than in the tumor markers case.

Moreover, both serum tumor markers and SIR markers, especially the latter, are easy and very cost-effective to obtain. In fact, most of them are already measured in current clinical practice or easy to calculate in the case of ratios. The same doesn't apply to other biomarkers, most of them dependent from molecular diagnosis techniques. Nonetheless, these approaches, using real-time reverse transcription-polymerase chain reaction (RT-PCR), have made it possible to increase the sensitivity for detecting micro metastasis in the peritoneal cavity, even when applied to tumor makers like CEA mRNA. The most relevant limitation is their cost, the few published works about their use in PM detection context, and the need for most advanced techniques, when comparing to immunohistochemistry.

Furthermore, it became clear from the studies that combine different indicators that they obtain better results. For example, Huang *et al*(33), combined serum tumor markers (CA125, CA19-9, CA72-4) and FLR and created a classification tree program and a decision algorithm with increased accuracy, sensitivity and specificity to assess the risk of PM. In a similar way, Lai *et al*(34) concluded that the combined use of CEA, CA19-9, CA72-4, and CA125 with LOX (Lysyl Oxidase), another biomarker, could increase the

capacity to predict PM with more than 90% sensitivity. Combining independent predictors of PM is not limited to biomarkers. Tumor characteristics have also been taken into account in the design of more complex formulas. In studies like Ohi *et al*(18), the investigators concluded that the combination of preoperative tumor features (including tumor histopathology and morphology) with preoperative serum markers (including serum tumor markers and SIR markers) increased significantly the sensitivity (84.09%) to detect PM in GC patients.(63)

As any conclusion in a literature review, the limitations reflect the nature of the studies included. The most significant ones are: the lack of research in non-Asian patients and, as a consequence, the lack of validation of some of these results in the context of other populations; additionally, the study design of the publications found was mostly based in cohort studies and single-centers. Finally, some of these biomarkers have few works published on them or, in some cases, only one relating them with PM prediction specifically in GC patients.

Based on all of this, in the future, large-scale multicenter and stronger design studies are needed in this field, in order to produce stronger evidence about the usefulness of these biomarkers. Also, the results showed that the future should be about the creation and validation of clinical scores that could integrate not only some of these markers, but also tumor characteristics, imaging methods and cytological results. The usefulness of these assessment tools could be significant in a particular group of patients, in the stages IB-III, helping to rule out the possibility of missing peritoneal seedings that will form the peritoneal recurrence ground, after a supposedly curative surgery.

Eventually, more research will be done on the molecular diagnosis field and even if now we are currently still distant from an organized process of assessing PM in GC patients using these tools, they eventually could become cost-effective, if validated, in selected patients. The future perspective is good, and biomarkers will certainly become more relevant in this field, providing supplementary but relevant information to assist the clinical decisions.

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