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Original contribution

Role of immunohistochemistry for interobserver agreement of Peritoneal Regression Grading Score in peritoneal metastasis☆

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Summary Pressurized intraperitoneal aerosol chemotherapy (PIPAC)−directed therapy is a new treatment option for peritoneal metastasis (PM). The 4-tiered Peritoneal Regression Grading Score (PRGS) has been proposed for assessment of histological treatment response. We aimed to evaluate the effect of immunohistochemistry (IHC) on interobserver agreement of the PRGS. Hematoxylin and eosin (H&E) −stained and IHC-stained slides (n = 662) from 331 peritoneal quadrant biopsies (QBs) taken prior to 99 PIPAC procedures performed on 33 patients were digitalized and uploaded to a web library. Eight raters (five consultants and three residents) assessed the PRGS, and Krippendorff's alpha coefficients (α) were calculated. Results (IHC-PRGS) were compared with data published in 2019, using H&E-stained slides only (H&E-PRGS). Overall, agreement for IHC-PRGS was substantial to almost perfect. Agreement (all raters) regarding single QBs after treatment was substantial for IHC-PRGS (α = 0.69, 95% confidence interval [CI] = 0.66−0.72) and moderate for H&E-PRGS (α = 0.60, 95% CI = 0.56 −0.64). Agreement (all raters) regarding the mean PRGS per QB set after treatment was higher for IHC-PRGS (α = 0.78, 95% CI = 0.73−0.83) than for H&E-PRGS (α = 0.71, 95% CI = 0.64 −0.78). Among residents, agreement was almost perfect for IHC-PRGS and substantial for H&E-PRGS. Agreement (all raters) regarding maximum PRGS per QB set after treatment was substantial for IHC-PRGS (α = 0.61, 95% CI = 0.54−0.68) and moderate for H&E-PRGS (α = 0.60, 95% CI = 0.53−0.66). Among residents, agreement was substantial for IHC-PRGS (α = 0.66, 95% CI = 0.57−0.75) and moderate for H&E-PRGS (α = 0.55, 95% CI = 0.45−0.64). Additional IHC seems to improve the interobserver agreement of PRGS, particularly between less experienced raters.

1. Introduction

Peritoneal lavage cytology and repeated peritoneal quadrant biopsies (QBs) applying the histological 4-tiered Peritoneal Regression Grading Score (PRGS) have been proposed for response assessment of pressurized intraperitoneal aerosol chemotherapy (PIPAC)−directed therapy of peritoneal metastasis (PM) [1,2]. PIPAC is a new treatment option for PM of different origin. The PRGS is currently used for response evaluation of PIPAC at several centers [2−8].

PIPAC aerosolizes chemotherapeutics within the peritoneal cavity during a standard laparoscopy using a capnoperitoneum [9−11]. Currently, PIPAC is an experimental treatment, and randomized, controlled trials are lacking [12−14]. PIPAC seems to be a safe procedure, able to induce objective histological regression, to sustain quality of life, and to result in improved survival [6,15−18]. PIPAC can be used several times, typically every four to six weeks, and is usually performed in the outpatient setting [19]. Hence, it is relevant to assess the histological and cytological response for therapeutic decision-making [20].

In 2016, the PRGS for the histological assessment of response to therapy in PM after PIPAC treatment was proposed by a group of European pathologists [2]. Studies from different countries evaluating the potential clinical value of the PRGS have emerged [5,6,8,21−25], and several clinical trials using the PRGS as primary or secondary outcomes are currently ongoing [3,26]. The PRGS was found to be a useful measure of therapy effect in mouse models of colorectal PM [27,28]. A few studies reported a reduction of the mean PRGS after PIPAC treatment in 67−80% of the patients [4,6,7,18].

A recent study indicated that the maximum PRGS, combined with peritoneal cytology, bears a prognostic value in PM [4]. It is therefore relevant to elucidate methods to improve reproducibility of PRGS [6,18,26,29,30]. A previous study found its reproducibility using H&E-stained slides was substantial regarding estimation of the mean PRGS per QB set and moderate regarding assessment of the maximum PRGS [1]. We aimed to evaluate the effect of IHC on interobserver agreement of the PRGS.

2. Methods

2.1. Study design

This study was approved by the Data Protection Agency of the Region of Southern Denmark (17/30427). Patients were part of the PIPAC-OPC1 (NCT02320448) (n = 27) or PIPAC-OPC2 (EudraCT 2016-003394-18) (n = 6) clinical trials, approved by the Ethics Committee of the Region of Southern Denmark (S-20140211, S-20160100).

All 331 QBs were obtained prior to 99 PIPAC treatment sessions, from 33 patients with PM treated at Odense PIPAC Center (OPC), Odense University Hospital, Denmark, during the course of repeated PIPAC cycles [1]. General information regarding the treatment of the patients in this study has been reported previously [1,6].

Primary tumors were mainly adenocarcinomas, unless otherwise is stated in the following list regarding the origin

of included PMs: colorectum (n = 12 [37%], three mucinous adenocarcinomas), pancreas (n = 4 [12%]), stomach (n = 4 [12%], one poorly cohesive carcinoma), ovary (n = 4 [12%], serous), appendix (n = 4 [12%], three low-grade appendiceal mucinous neoplasms (LAMNs, with low-grade malignant cells in mucin lakes showing a pushing invasive margin) and one mucinous adenocarcinoma), small bowel (n = 2 [6%]), extrahepatic bile ducts (n = 1 [3%]), unknown (n = 1 [3%]), or epithelioid malignant mesothelioma (n = 1 [3%]).

2.2. Peritoneal biopsy specimens

The QB procedure was described previously [1]. All patients underwent at least three PIPAC procedures, and peritoneal QBs taken prior to each PIPAC procedure were included. QBs were taken from macroscopically tumor suspect areas in all four abdominal quadrants, if technically possible. QB sites were marked with metal clips to ensure that subsequent biopsies were collected from the same sites.

A total of 662 digitalized H&E- and IHC-stained slides deriving from 331 QBs from 33 patients were included. The exact same QBs were included in our previous study, evaluating the reproducibility of the PRGS when using only H&E-stained slides but presented to the raters in a different order and with different project numbers [1]. The residents participating in the previous study did not participate in the current study, to ensure comparability regarding experience of the rating residents. Hence, three other residents with similar experience at the beginning of the study participated in the current study. The time between the scoring for the present study and the previous study was three years [1]. From all QBs (n = 331), three sections were stained with H&E and one section was IHC stained for EpCAM (mouse monoclonal, clone BS14 [Nordic BioSite ApS], 1:600 [20 min at 32 °C], heat-induced antigen retrieval [HIER] using target retrieval solution-High [pH 9] for 30 min at 97 °C, Omnis platform, Envision detection). For biopsies with malignant mesothelioma, IHC staining for CKAE1/AE3 was used (mouse monoclonal antibody, clone AE1+AE3 [Dako], 1:100 [24 min at 36 °C], HIER using cell conditioning solution 1 [pH 8.5, Ventana Medical Systems] for 32 min at 100 °C, Benchmark Ultra platform).

2.3. Web library

Quantitative evaluation was performed on digitalized H&E- and EpCAM (or CKAE1/AE3 in case of mesothelioma) IHC-stained slides. Slides were scanned using a x20 objective on the NanoZoomer 2.0HT whole-slide scanner (Hamamatsu Photonics, Hamamatsu, Japan) [1]. At the time when this study was performed, the standard scanning objective at our institution (Department of Pathology, Odense University Hospital, Denmark) was x20. This enables digital microscopy of acceptable quality corresponding to a magnification of up to x400 at a conventional optic microscope. The digitalized slides were uploaded to a pseudonymized web library. Each rater received a personalized code to access the web library, and each access to the web library was logged.

2.4. Assessment of the PRGS using additional IHC

The raters (n = 8) were selected so that their level of experience was similar to our previous study: The group of consultants consisted of five raters who have a particular interest in peritoneal pathology. Three raters were residents in pathology with 2, 3, and 5 years of working experience in pathology. All raters assigned a PRGS score to each QB under investigation, using H&E and IHC stained slides (IHC-PRGS). The raters were blinded regarding patient no. and PIPAC no. but were informed regarding the origin of PM.

The findings were compared with the data from our previous study, using a highly similar setting, but only H&E-stained slides without additional IHC (H&E-PRGS). We recalculated some of the raw data from this previous

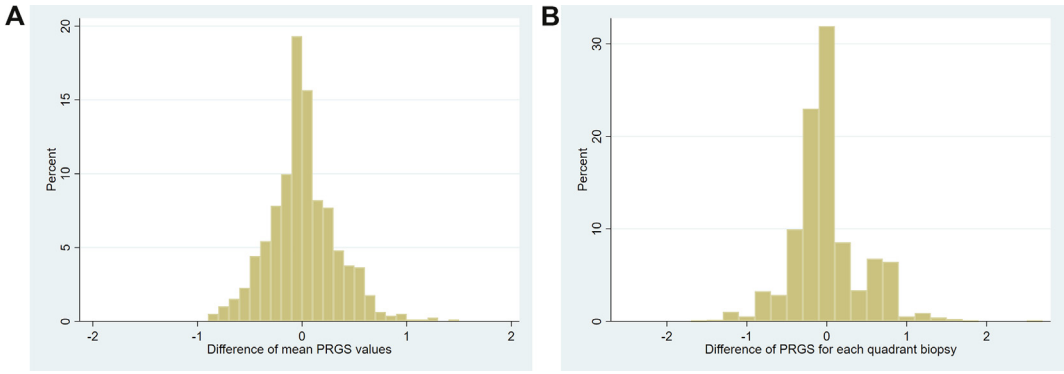

Fig. 1 Interobserver variability among eight raters assessing the Peritoneal Regression Grading Score (PRGS) in peritoneal metastasis (PM) of different origin. (**A**) The difference between the mean PRGS per quadrant biopsy (QB) set from each rater and the average mean PRGS per QB set from all eight raters' scorings (792 plotted values). (**B**) The difference between the PRGS for each QB from each rater and the average PRGS for each QB, calculated from all eight raters' scorings (2644 plotted values).

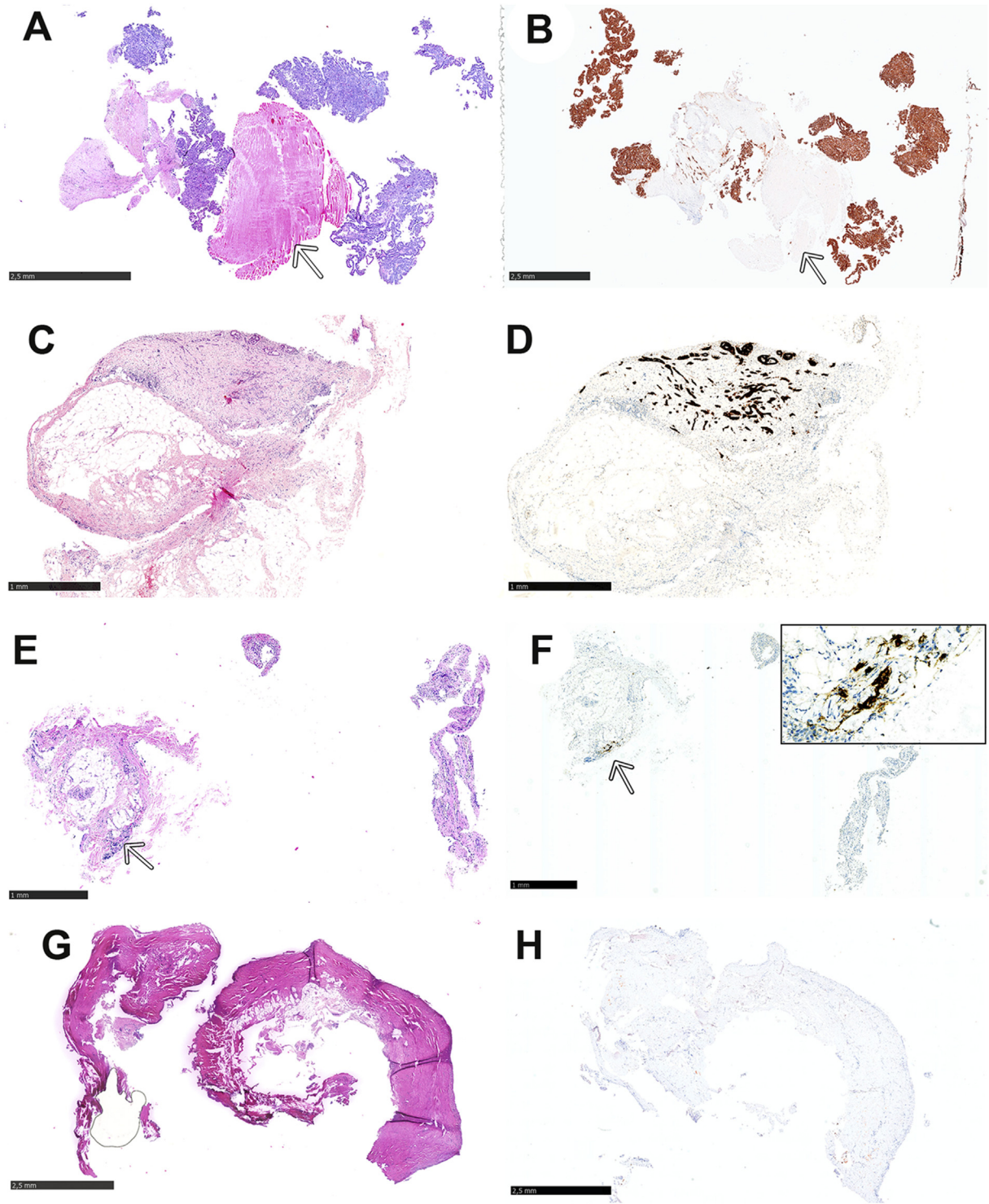

Fig. 2 Histological examples of peritoneal quadrant biopsy (QB) specimens where there was high agreement between the participating raters. (**A-B**) Peritoneal metastasis (PM) from serous adenocarcinoma, PRGS 4, close to striated muscle (arrow). Seven raters scored PRGS 4, and one rater scored PRGS 2 (A, H&E. B, EpCAM immunostaining). (**C-D**) Peritoneal metastasis (PM) from colorectal adenocarcinoma, PRGS 3. Seven raters scored PRGS 3, and one rater scored PRGS 2 (C, H&E. D, EpCAM immunostaining). (**E-F**) PM from poorly cohesive gastric adenocarcinoma, PRGS 2. The arrow indicates a small group of malignant cells. All raters (n = 8) scored PRGS 2 (E, H&E. F, EpCAM immunostaining). (**G-H**) PM from small bowel adenocarcinoma, PRGS score 1. All raters (n = 8) scored PRGS 1 (G, H&E. H, EpCAM immunostaining).

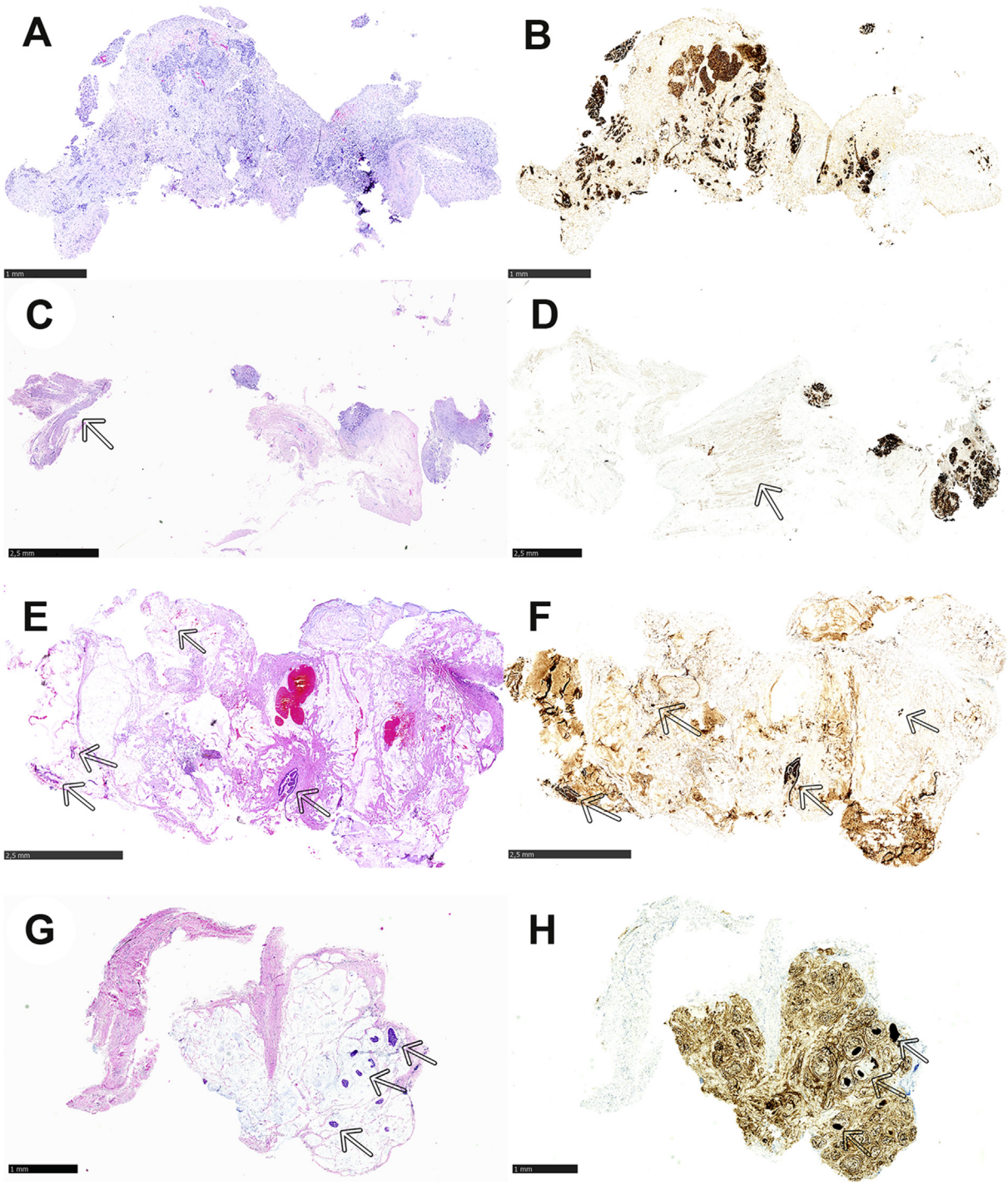

Fig. 3 Histological examples of peritoneal quadrant biopsy (QB) specimens where there was low agreement between the participating raters. (**A-B**) Peritoneal metastasis (PM) from serous adenocarcinoma. One rater scored PRGS 2, three raters scored PRGS 3, and four raters scored PRGS 4 (A, H&E. B, EpCAM immunostaining). (**C-D**) Peritoneal metastasis (PM) from pancreatic ductal adenocarcinoma. One rater scored PRGS 2, four raters scored PRGS 3, and three raters scored PRGS 4. The arrow indicates areas with smooth muscle (C, H&E. D, EpCAM immunostaining). (**E-F**) PM from low-grade appendiceal mucinous neoplasm (LAMN). Three raters scored PRGS 2, three raters scored PRGS 3, and two raters scored PRGS 4. Several foci of malignant cells are present (arrows) (E, H&E. F, EpCAM immunostaining). (**G-H**) PM from colorectal mucinous adenocarcinoma. Four raters scored PRGS 2, two raters scored PRGS 3, and two raters scored PRGS 4. A few foci with groups of malignant cells are present (arrows) (G, H&E. H, EpCAM immunostaining).

Table 1 Interobserver agreement of the Peritoneal Regression Grading Score (PRGS) for scoring each quadrant biopsy, when using immunohistochemistry (IHC) in addition to H&E-stained slides (IHC-PRGS), compared to H&E only (H&E-PRGS)[a].

Time point	n	α value	95% CI
Baseline (PIPAC 1)			
IHC-PRGS	107	0.65	0.59−0.72
H&E-PRGS[a]	106	0.66	0.59−0.73
After treatment (PIPAC 2 & 3)			
IHC-PRGS	224	0.69	0.66−0.72
H&E-PRGS[a]	225	0.60	0.56−0.64

Abbreviations: CI, confidence interval; H&E, hematoxylin and eosin; PIPAC, pressurized intraperitoneal aerosol chemotherapy.

[a] For comparison, data from our previously published study, obtained using H&E-stained slides without IHC (H&E-PRGS), are also shown [1], with permission from the publisher (John Wiley & Sons). The given coefficients are based on a single-rater, absolute-agreement, 2-way random-effects model.

Table 2 Interobserver agreement for rating the mean Peritoneal Regression Grading Score (PRGS) per quadrant biopsy (QB) set, when using immunohistochemistry (IHC) in addition to H&E-stained slides (IHC-PRGS), compared to H&E only (H&E-PRGS)[a].

Time point	n	α value	95% CI
Baseline (PIPAC 1)			
IHC-PRGS	33	0.76	0.66−0.86
H&E-PRGS[a]	33	0.74	0.65−0.84
After treatment (PIPAC 2 & 3)			
IHC-PRGS	66	0.78	0.73−0.83
H&E-PRGS[a]	66	0.71	0.64−0.78

Abbreviations: CI, confidence interval; H&E, hematoxylin and eosin; PIPAC, pressurized intraperitoneal aerosol chemotherapy.

[a] For comparison, data from our previously published study, obtained using H&E-stained slides without IHC (H&E-PRGS), are also shown [1], with permission from the publisher (John Wiley & Sons). The given coefficients are based on a single rater, absolute-agreement, 2-way random-effects model.

Table 3 Interobserver agreement for rating the maximum Peritoneal Regression Grading Score (PRGS) per quadrant biopsy (QB) set, when using immunohistochemistry in addition to H&E-stained slides (IHC-PRGS), compared to H&E only (H&E-PRGS)[a].

Time point	n	α value	95% CI
Baseline (PIPAC 1)			
IHC-PRGS	33	0.59	0.43−0.75
H&E-PRGS[a]	33	0.59	0.43−0.76
After treatment (PIPAC 2 & 3)			
IHC-PRGS	66	0.61	0.54−0.68
H&E-PRGS[a]	66	0.60	0.53−0.66

Abbreviations: CI, confidence interval; H&E, hematoxylin and eosin; PIPAC, pressurized intraperitoneal aerosol chemotherapy.

[a] For comparison, data from our previously published study, obtained only using H&E-stained slides without IHC (H&E-PRGS), are also shown [1], with permission from the publisher (John Wiley & Sons). The given coefficients are based on a single rater, absolute-agreement, 2-way random-effects model.

study intraclass correlation coefficients [ICCs] and α values for HE-PRGS after treatment (combination of QBs taken prior to PIPAC 2 and 3) [1].

2.5. Peritoneal Regression Grading Score

The PRGS is a four-tiered scoring system, based on the relative amounts of residual tumor and the extent of regressive features [2]. The most important histological features of regression are regressive fibrosis, accompanied by varied numbers of inflammatory cells. Typically, regressive fibrosis is characterized by reduced numbers, scanty, or absence of cancer cells. Also acellular mucin pools are considered features of regression. However, acellular mucin pools in patients with LAMNs should not be considered a sign of regression [31−33]. Accumulation of foamy macrophages and multinucleated giant cells as well as elastosis were also considered signs of regression [1,2]. Different types of necrosis should be distinguished: One type (usual necrosis) is *dirty* appearing necrosis observed in different types of untreated cancer and should not be considered a sign of response [1,2,34]; the other type (infarct-like necrosis) is characterized by larger areas of hypocellular necrosis often with cholesterol clefts, surrounded by regressive fibrosis, and should be considered a sign of regression [1,2,34].

PRGS 1 corresponds to a complete regression with the absence of tumor cells. PRGS 2 is defined as a major histological response, meaning that regressive features are predominant over residual tumor cells. PRGS 3 is defined as a minor histological response with predominance of residual tumor cells over regressive features. PRGS 4 is defined as the absence of regressive features, consisting of residual tumor only [2].

All observers assessed a PRGS for each quadrant biopsy (IHC-PRGS). Mean and maximum PRGSs, based on the individual scores from the QBs of a given biopsy set, were calculated.

2.6. Statistics

Interobserver agreement was calculated as described previously [1]. In short, for evaluating the interobserver agreement between multiple raters, Krippendorff's alpha (α) using ordinal data was calculated. Krippendorff's α takes coefficients ranging from 0 (or <0 in extreme cases) to 1. An α coefficient of 0 is indicative of no agreement,

Table 4 Interobserver agreement between groups (consultants vs. residents) for scoring the mean PRGS per quadrant biopsy (QB) set, when using immunohistochemistry in addition to H&E-stained slides (IHC-PRGS), compared to H&E only (H&E-PRGS)[a].

Groups of raters	Number of raters	N	α value	95% CI
Baseline (PIPAC 1)				
Consultants				
IHC-PRGS	5	33	0.71	0.59−0.82
H&E-PRGS[a]	5	33	0.72	0.61−0.83
Residents				
IHC-PRGS	3	33	0.83	0.74−0.92
H&E-PRGS[a]	3	33	0.79	0.68−0.90
After treatment (PIPAC 2 & 3)				
Consultants				
IHC-PRGS	5	66	0.72	0.66−0.79
H&E-PRGS[a]	5	66	0.69	0.61−0.77
Residents				
IHC-PRGS	3	66	0.84	0.79−0.90
H&E-PRGS[a]	3	66	0.65	0.56−0.75

Abbreviations: CI, confidence interval; H&E, hematoxylin and eosin; PIPAC, pressurized intraperitoneal aerosol chemotherapy; PRGS, Peritoneal Regression Grading Score.

[a] For comparison, data from our previously published study, obtained using H&E-stained slides without IHC (H&E-PRGS), are also shown [1], with permission from publisher (John Wiley & Sons). The given coefficients are based on a single rater, absolute-agreement, 2-way random-effects model.

Table 5 Interobserver agreement between groups (consultants vs. residents) for scoring the maximum PRGS per quadrant biopsy (QB) set, when using immunohistochemistry in addition to H&E-stained slides (IHC-PRGS), compared to H&E only (H&E-PRGS).[a]

Groups of raters	Number of raters	n	α value	95% CI
Baseline (PIPAC 1)				
Consultants				
IHC-PRGS	5	33	0.53	0.36−0.69
H&E-PRGS[a]	5	33	0.54	0.36−0.72
Residents				
IHC-PRGS	3	33	0.61	0.41−0.80
H&E-PRGS[a]	3	33	0.67	0.49−0.86
After treatment (PIPAC 2 & 3)				
Consultants				
IHC-PRGS	5	33	0.56	0.48−0.65
H&E-PRGS[a]	5	66	0.60	0.53−0.68
Residents				
IHC-PRGS	3	33	0.66	0.57−0.75
H&E-PRGS[a]	3	66	0.55	0.45−0.64

Abbreviations: CI, confidence interval; H&E, hematoxylin and eosin; PIPAC, pressurized intraperitoneal aerosol chemotherapy; PRGS, Peritoneal Regression Grading Score.

[a] For comparison, data from our previously published study, obtained only using H&E-stained slides without IHC (H&E-PRGS), are also shown [1], with permission from publisher (John Wiley & Sons). The given coefficients are based on a single rater, absolute-agreement, 2-way random-effects model.

and a coefficient of 1 represents perfect agreement. Coefficients below 0 indicate poor/systematic disagreement, between 0 and 0.2 slight agreement, between 0.21 and 0.40 fair, between 0.41 and 0.60 moderate, between 0.61 and 0.80 substantial, and between 0.81 and 1.0 almost perfect agreement [1]. ICCs were reported with 95% confidence intervals (CIs), as described previously [1]. Statistical analyses were performed using Stata, version 16 (StataCorp LLC, College Station, Texas), with the addition of *kappaetc* (Daniel Klein, INCHER-Kassel, University of Kassel, Germany) to calculate α values.

3. Results

3.1. Overall findings

A total of 662 slides from 331 QBs taken from 33 patients were prepared for evaluation. There were 106, 112, and 113 QBs from PIPAC 1, 2, and 3, respectively. All but 4 QBs were scored by all raters. Altogether, 2644 ratings were performed. The combined PRGSs from all raters at the different time points (*i.e.*, PIPAC treatments) demonstrated increasing frequency of lower PRGSs from baseline (prior to PIPAC 1) to QBs obtained prior to PIPAC 3 ($p < 0.001$): At baseline (PIPAC 1), PIPAC 2, and PIPAC 3, PRGS 1, 2, 3, and 4 were used with the following frequencies: 228/326/216/85, 401/323/142/29, and 408/307/143/36.

The difference between the mean PRGS per QB set from each rater and average mean PRGS per QB set from all eight raters' scorings is illustrated in Fig. 1A. Fig. 1B shows the difference between the PRGS for each QB from each rater and the average PRGS for each QB calculated from all eight raters' scores. These differences were normally distributed, and the majority of scores did not differ by more than 0.5 PRGS from the mean values.

Histological examples of cases where there was high agreement are given in Fig. 2. High agreement was for example found for a QB showing PM from serous adenocarcinoma growing in solid masses without histological features of response, corresponding to PRGS 4 (Fig. 2A and B). High agreement was also found for a QB showing PM from colorectal adenocarcinoma with PRGS 3 (Fig. 2C−D), a QB showing PM from poorly cohesive gastric adenocarcinoma with PRGS 2 (Fig. 2E−F), and a QB with complete histological response (PRGS 1) in a PM from small bowel adenocarcinoma (Fig. 2G and H).

Fig. 3 illustrates examples of cases with low agreement. Low agreement was for example found for a QB showing PM from serous adenocarcinoma growing in rather small groups and thin trabeculae (Fig. 3A and B) and in a QB from pancreatic ductal adenocarcinoma growing in areas

with fibrosis and smooth muscle (Fig. 3C and D). Also in a QB with PM from LAMN with mucin lakes containing low-grade malignant cells (Fig. 3E and F) and in a QB with PM from colorectal mucinous adenocarcinoma (Fig. 3G and H), interobserver agreement was low.

3.2. PRGS of each quadrant biopsy

α Values of IHC-PRGS and H&E-PRGS for each QB (n = 324) at baseline (prior to PIPAC 1) and after localized treatment (prior to PIPAC 2 and 3) are given in Table 1. Agreement (all raters, n = 8) regarding single QBs after treatment was substantial for IHC-PRGS (α 0.69, 95% CI = 0.66−0.72) and moderate for H&E-PRGS (α 0.60, 95% CI = 0.56−0.64) (Table 1). ICCs for scoring each QB regarding IHC-PRGS and H&E-PRGS at baseline (prior to PIPAC 1) were 0.70 (95% CI = 0.63−0.77) and 0.70 (0.63−0.76) (data not shown). ICCs for scoring each QB regarding IHC-PRGS and H&E-PRGS after treatment (PIPAC 2&3) were 0.73 (95% CI = 0.69−0.78) and 0.64 (0.58−0.69) (data not shown).

3.3. Mean PRGS per quadrant biopsy set

In Table 2, α values for mean IHC-PRGS and mean HE-PRGS for each QB set are shown. In QBs taken after localized treatment (PIPAC 2&3), the α value (all raters) for mean IHC-PRGS was higher than for H&E-PRGS (α = 0.78 (95% CI = 0.73−0.83) vs. 0.71 (95% CI = 0.64−0.78)). ICCs for mean IHC-PRGS and mean H&E-PRGS at baseline (prior to PIPAC 1) were 0.78 (95% CI = 0.66−0.87) and 0.76 (0.65−0.85) (data not shown). ICCs for mean IHC-PRGS and mean H&E-PRGS after treatment (PIPAC 2&3) were 0.77 (95% CI = 0.68−0.84) and 0.70 (0.61−0.78) (data not shown).

3.4. Maximum PRGS per QB set

Table 3 summarizes α values regarding the maximum PRGS per QB set. Agreement (all raters) for maximum PRGS per QB set after treatment was substantial for IHC-PRGS (α = 0.61, 95% CI = 0.54−0.68) and moderate for H&E-PRGS (α = 0.60, 95% CI = 0.53−0.66) (Table 3). ICCs for maximum IHC-PRGS and maximum H&E-PRGS at baseline (prior to PIPAC 1) were 0.66 (95% CI = 0.52−0.79) and 0.65 (0.52−0.77) (data not shown). ICCs for maximum IHC-PRGS and maximum H&E-PRGS after treatment (PIPAC 2&3) were 0.67 (95% CI = 0.57−0.76) and 0.65 (0.55−0.75) (data not shown).

3.5. Agreement dependent on rater experience

α Values for agreement between groups (consultants vs. residents) regarding the mean PRGS per QB set at baseline (prior to PIPAC 1) and after localized treatment (prior to PIPAC 2 and 3) are shown in Table 4. Agreement among residents (n = 3) for baseline QBs was almost perfect for IHC-PRGS (α = 0.83, 95% CI = 0.74−0.92) and substantial for HE-PRGS (α = 0.79, 95% CI = 0.68−0.90). Agreement among residents for QBs obtained after localized treatment (prior to PIPAC 2 and 3) was almost perfect for IHC-PRGS (α = 0.84, 95% CI = 0.79−0.90) and moderate for HE-PRGS (α = 0.65, 95% CI = 0.65−0.75) (Table 4).

ICCs for consultants regarding mean IHC-PRGS and mean H&E-PRGS at baseline (prior to PIPAC 1) were 0.73 (95% CI = 0.54−0.85) and 0.73 (0.59−0.84) (data not shown). ICCs for residents regarding mean IHC-PRGS and mean H&E-PRGS at baseline (prior to PIPAC 1) were 0.82 (95% CI = 0.71−0.90) and 0.81 (0.69−0.90) (data not shown). ICCs for consultants regarding mean IHC-PRGS and mean H&E-PRGS after treatment (PIPAC 2&3) were 0.71 (95% CI = 0.58−0.80) and 0.70 (0.59−0.79) (data not shown). ICCs for residents regarding mean IHC-PRGS and mean H&E-PRGS after treatment (PIPAC 2&3) were 0.86 (95% CI = 0.80−0.90) and 0.69 (0.54−0.80) (data not shown).

Table 5 summarizes agreement between groups (consultants vs. residents) regarding the maximum PRGS per QB set at baseline (prior to PIPAC 1) and after localized treatment (prior to PIPAC 2 and 3). For residents (n = 3), agreement was substantial (α = 0.66, 95% CI = 0.57−0.75) for IHC-PRGS and moderate (α = 0.55, 95% CI = 0.45−0.64) for H&E-PRGS. ICCs for consultants regarding maximum IHC-PRGS and H&E-PRGS at baseline (prior to PIPAC 1) were 0.61 (95% CI = 0.39−0.77) and 0.60 (0.44−0.75) (data not shown). ICCs for residents regarding maximum IHC-PRGS and H&E-PRGS at baseline (prior to PIPAC 1) were 0.68 (95% CI = 0.51−0.81) and 0.71 (0.56−0.83) (data not shown). ICCs for consultants regarding maximum IHC-PRGS and H&E-PRGS after treatment (PIPAC 2&3) were 0.62 (95% CI = 0.47−0.74) and 0.66 (0.54−0.76) (data not shown). ICCs for residents regarding maximum IHC-PRGS and H&E-PRGS after treatment (PIPAC 2&3) were 0.72 (95% CI = 0.61−0.81) and 0.62 (0.48−0.74) (data not shown).

3.6. Influence of training on agreement

We also compared the agreement for the first 33% and last 67% of the scorings regarding PIPAC 1 and PIPAC 2 & 3. For baseline QBs (prior to PIPAC 1), the α value decreased from 0.74 (95% CI = 0.65−0.82) to 0.60 (95% CI = 0.52−0.69) (data not shown). For QBs taken after localized treatment (PIPAC 2 and 3), agreement did not deteriorate over time (α values of 0.68 [95% CI = 0.63−0.74] and 0.69 [95% CI = 0.65−0.73]) (data not shown).

4. Discussion

In this study, we evaluated the interobserver agreement of the PRGS in PM when using IHC, in addition to standard H&E slides, comparing our results (IHC-PRGS) with data from our previously published study, using H&E-stained slides only (H&E-PRGS) [1]. Overall, agreement for IHC-PRGS was substantial to almost perfect for most calculated variables. Agreement (all raters) regarding single QBs after treatment was substantial for IHC-PRGS and only moderate for H&E-PRGS. Agreement (all raters) regarding the mean PRGS per QB set after treatment was higher for IHC-PRGS than for H&E-PRGS. For residents, agreement was almost perfect for IHC-PRGS and only substantial for H&E-PRGS. Agreement (all raters and residents) regarding the maximum PRGS per QB set after treatment was substantial for IHC-PRGS and only moderate for H&E-PRGS.

A total of 662 microscopic slides obtained from 33 patients with PM taken at three different time points (prior to PIPAC treatment no. 1, 2, and 3) were evaluated. In QBs taken after localized treatment (prior to PIPAC 2 and 3), where regressive changes are often encountered, the agreement was generally higher for IHC-PRGS than for HE-PRGS [1]. In agreement with our previous study, we found a slightly higher agreement among residents (n = 3) than among consultants (n = 5) [1]. This was particularly true for QBs taken after localized treatment, where regressive changes were more frequently found [1]. This may indicate that less experienced raters use the proposed PRGS criteria more strictly and categorically, particularly when having access to IHC. Hence, it may be suggested that the use of IHC for PRGS assessment should be considered particularly during the start-up phase of a new PIPAC treatment program at a given institution, when the involved pathologists are less familiar with its use. We are not aware of other published studies examining the effect of IHC on the reproducibility of the PRGS.

The fact that we could use a setting highly similar to the previous study evaluating the reproducibility of the PRGS without IHC should be considered a strength [1]. Likewise, we used the exact same cases, and the raters had a highly similar level of experience. For these reasons, there were some cases where the IHC slide did not add any information—for example, when no tumor cells were present in the deeper sections used for IHC. We chose to not exclude such cases (n = 5), to avoid introducing selection bias, as this is also the case in clinical pathology, where biopsy material may not be sufficient for additional stains, reflecting the *real-life* situation. The fact that we used QBs with PM of different origin makes our findings more generally applicable. Still, future studies should evaluate possible differences in reproducibility of the PRGS, dependent on the origin of PM. For example, we included only one poorly cohesive gastric carcinoma. It may be hypothesized that the value of IHC in such tumors would be higher. Also, LAMNs are challenging to judge. To our knowledge, there is at present no international consensus whether a case with abundant mucin and rare strips of epithelium represents a reflection of response to therapy, or simply the usual growth pattern of the tumor. PRGS 4 was relatively rarely used, even in baseline biopsies (PIPAC 1), prior to treatment. The reasons for this are unknown, but it may be hypothesized that desmoplastic stroma sometimes was interpreted as regressive stroma or that there may have been a partial effect of systemic chemotherapy given prior to PIPAC-directed treatment. Future studies should aim to define precise criteria for the differentiation of desmoplastic from regressive fibrosis. It is tempting to speculate whether IHC or special stains for stromal markers may aid in this task. The number of raters in the residents group was lower than in the consultants group, which may have influenced the calculated agreement in the subgroup analyses. However, for the sake of comparability, we had to use the same number of raters as in our previous study [1].

As the PIPAC treatment for PM of various origin is worldwide increasingly used, the PRGS for assessment of histological response is gaining relevance for therapeutic decision-making [1,2,20]. In the absence of reliable and reproducible noninvasive response evaluation methods, histological and/or cytological evaluation of QB and peritoneal fluids seem to be an option for assessment of local treatment response. Future studies on homogeneous cohorts (*e.g.*, PM from colorectal cancer) shall determine whether PRGS response evaluation correlates significantly with survival data.

5. Conclusions

In conclusion, we found that IHC in addition to H&E improved the reproducibility of the PRGS in QBs after PIPAC treatment, particularly between less experienced raters. The role of IHC for scoring the maximum PRGS per QB set seems, however, to be limited, as only a slight improvement was found. Large prospective studies addressing the prognostic and predictive role of the PRGS, alone and in combination with other modalities, such as peritoneal cytology, are needed.

CRediT author roles

Sönke Detlefsen: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Writing − original draft; Writing − review & editing.

Tobias Windedal: Data curation; Project administration; Software; Writing − review & editing.

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Daniel Neureiter: Data curation; Methodology; Writing − review & editing.

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Malene Theilmann Thinesen: Data curation; Methodology; Writing − review & editing.

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