JGH Open Open Access An open access journal of gastroenterology and hepatology

JGHF Journal of Gastroenterology and Hepatology Foundation

doi:10.1002/jgh3.12482

ORIGINAL ARTICLE

Conventional peritoneal cytology lacks the prognostic significance of detecting local or peritoneal recurrence in colorectal cancer: An Egyptian experience

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Key words
colorectal cancer, cytology, peritoneal, recurrence.

Accepted for publication 11 December 2020.

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Declaration of conflict of interest: None

Abstract

Background and Aim: Colorectal cancer (CRC) accounts for over 8% of all deaths each year, with 1.2 million new cases diagnosed annually worldwide. It represents the seventh most common cancer in Egypt. Early detection of peritoneal metastasis is a major challenge in such cases. It helps with the decision of the immediate application of intraperitoneal chemotherapy after resection. Meta-analysis studies reported contrast evidence for a possible prognostic role of intraperitoneal free cancer cells (IPCCs) in peritoneal recurrence and survival after curative resection. In this work, we aim to evaluate the prevalence and impact of detecting free malignant cells in peritoneal fluid on survival and local recurrence and to estimate the incidence of peritoneal carcinomatosis (PC) during follow up.

Methods: Design: This was a prospective cohort study. Settings: From June 2016 to December 2018, samples were collected from 104 patients who underwent abdominal surgery for colorectal cancer in the Egyptian National Cancer Institute. A total of 96 Egyptian CRC patients who underwent curative resection were enrolled. Intraoperative peritoneal lavage was performed to detect IPCC by conventional cytology. Patients with no residual tumor after surgery and no evidence of PC were followed up for a median 14 months. The cumulative 12-month overall survival rate for patients with IPCC was 100% *versus* 86% for patients with negative cytology.

Results: Our results demonstrated that the prevalence of IPCC in the peritoneal lavage was 11.5%. Peritoneal and local recurrence occurred at a higher rate in patients with cytology positive lavage (9.1% *vs* 6.3% and 9.1% *vs* 3.8%, respectively), although this was statistically insignificant. Distant metastasis occurred significantly in patients with positive cytology (45.5% *vs* 8.9%) with P-value <0.001.The conventional cytology technique has a high specificity but less sensitivity.

Conclusions: The presence of IPCC using conventional cytology was not an independent prognostic factor for the development of PC or survival.

Introduction

Rationale. Colorectal cancer (CRC) accounts for over 8% of all deaths annually worldwide and is ranked as the fourth cancer-related cause of death among males and the third for females.[1] It is the seventh most common cancer in Egypt.[2] Each year, almost 1.2 million new cases of CRC are diagnosed worldwide.[1,3] Peritoneal metastases (PMs) are common in advanced-stage CRC patients, representing the second most common metastatic site of CRC following hepatopulmonary metastases, depending on a tumor's primary location.[4]

Recently, the introduction of new chemotherapeutic and targeted biologic agents has improved the prognosis of patients with metastatic CRC.[4] The current multidisciplinary treatment options include cytoreductive surgery (CRS) after assessment of the resectable condition of the primary tumor and hyperthermic intraperitoneal chemotherapy (HIPEC).[5,6]

Early identification of CRC patients at risk of developing peritoneal metastasis is a major challenge. It helps with the decision of immediate application of intraperitoneal chemotherapy after curative resection. This reduces the incidence of peritoneal recurrence and avoids aggressive CRS and HIPEC, which have high mortality and morbidity.[7,8]

Meta-analysis studies argue for the controversial prognostic role of intraperitoneal free cancer cell (IPCC) detection in

peritoneal recurrence and survival after curative resection.[3,7,9–12] Prospective validation is required to identify and compare sensitivities and specificities between the various diagnostic criteria for positive cytology in peritoneal lavage fluid to find the most optimal biomarkers for predicting outcome.[3,7]

Objectives. The principal objectives of this study were to evaluate the impact of detecting free malignant cells in peritoneal fluid on survival and local recurrence and to estimate the incidence of peritoneal carcinomatosis (PC) during follow-up. The secondary objectives were to clarify the prevalence and prognostic significance of IPCC in CRC using conventional cytology.

Table 1 Correlation between the patient characteristics, tumor characteristics, serology tests, and sample volume with the cytology results (n = 96)

Variable	Cytology (–) (n = 85), n (%)	Cytology (+) (n = 11), n (%)	P-value
Patient characteristics			
Age			0.538
≤55.5	38 (86.4)	6 (13.6)	
>55.5	47 (90.4)	5 (9.6)	
Gender			
Male	41 (89.1)	5 (10.9)	0.862
Female	44 (88)	6 (12)	
Site of cancer			
Colon	55 (87.3)	8 (12.7)	0.598
Rectum	30 (90.9)	3 (9.1)	
Performance status			
PS1	65 (86.7)	10 (13.3)	—
PS2	19 (95)	1 (5)	
PS3	1 (100)	0 (0)	
Tumor characteristics			
Tumor stage (n = 91)			
T2	12 (100)	0 (0)	0.122
T3	64 (87.7)	9 (12.3)	
T4	6 (100)	0 (0)	
Lymph node (n = 91)			
N0	45 (95.7)	2 (4.3)	0.128
N1	25 (83.3)	5 (16.7)	
N2	12 (85.7)	2 (14.3)	
Stage (n = 91)			
I and II	45 (95.7)	2 (4.3)	0.08
III	37 (84.1)	7 (15.9)	
Histological type			
Mucinous adenocarcinoma	13 (15.3)	6 (54.5)	<0.001
Signet ring	5 (5.9)	3 (27.3)	
Well-differentiated	67 (78.8)	2 (18.2)	
Serological tests and sample volume			
CEA			
≤3 (n = 61)	32 (94.1)	2 (5.9)	0.22
>3 (n = 61)	22 (81.5)	5 (18.5)	
CA 19–9			
≤11 (n = 61)	29 (93.5)	2 (6.5)	0.25
>11(n = 61)	25 (83.3)	5 (16.7)	
Sample volume			
≤(120 mL)	48 (84.2)	9 (15.8)	0.107
>(120 mL)	37 (94.9)	2 (5.1)	

Methods

Study design. This was a prospective cohort study.

Settings. From June 2016 to December 2018, samples were collected with a continuous follow-up period until June 2019 in the National Cancer Institute (NCI).

Patients. Samples were collected from 104 patients who underwent abdominal surgery for CRC. Preoperative staging workup was performed, including abdominopelvic computed tomography (CT), chest CT, and magnetic resonance imaging (MRI) pelvis for patients with cancer of the rectum. The patients were followed up for the 3 years of the study, with a median of 14 months.

Variables. For the detection of recurrence during the follow-up period, physical examinations and CEA and CA19-9 checks were carried out every 3 months, and CT chest, abdomen, and pelvis scans and MRI pelvis (for patients with cancer of the rectum) were performed every 6 months for the first 2 years after operation. Colonoscopy was performed once in the first year, and if the patient was disease free, it was to be repeated after 3 years. Peritoneal and any systemic recurrences were diagnosed based on radiologic, laboratory, and/or pathologic evidence of cancer recurrence.

Table 2 Correlation between tumor lymphovascular invasion, tumor perforation, patient management, recurrence and metastases, and post-operative status with the cytology results (n = 96)

Character	Cytology (–) (n = 85)	Cytology (+) (n = 11)	P-value
Tumor-related factors			
Lymphovascular invasion (n = 96)	5 (5.9)	3 (27.3)	0.016
Perforated Tumor (n = 96)	6 (7.1)	1 (9.1)	0.807
Management			
Resection (n = 96)			
Curative	82 (96.5)	9 (81.8)	0.099
Irresectable	3 (3.5)	2 (18.2)	
Composite resection	7 (8.2)	2 (18.2)	0.287
Adjuvant CTH (n = 91)			
Yes	48 (60)	9 (81.8)	0.161
No	32 (40)	2 (18.2)	
Neoadjuvant CTH			
Yes	24 (28.2)	4 (36.4)	0.577
No	61 (71.8)	7 (63.6)	
Recurrence and spread			
Peritoneal recurrence (n = 90)	5 (6.3)	1 (9.1)	0.731
Local recurrence (n = 90)	3 (3.8)	1 (9.1)	—
Distant metastasis (n = 90)	7 (8.9)	5 (45.5)	<0.001
Postoperative status			
Medical comorbidity (n = 96)	24 (28.2)	2 (18.2)	0.480
Postoperative complications (n = 96)	15 (17.6)	0 (0)	0.129

Data sources/measurement

Procedures. Peritoneal lavage was performed immediately after laparotomy and just before manipulation of the tumor. About 200 mL of physiologic saline solution (37°C) was instilled into the abdominal cavity around the tumor, through paracolic gutters, with the patient in a supine position. Then, the patient was put in the Trendelenburg position. After gentle stirring,[2,3] 50-ml syringes were used to drain the fluids collected in the Douglas pouch after putting the patient in the anti-Trendelenburg position.

Samples and cytology. All samples were processed using both conventional cytological preparation and the BD SurePath™ liquid-based cytology (LBC) technique (Becton, Dickinson Co., New York, NY, USA). All collected fluids were sent to the cytology unit unfixed and were labeled with the patient's name and hospital number. The fluids were evaluated for physical characteristics.

For conventional cytological staining, a minimum of 20 mL of fluid was centrifuged, and a minimum of four smears was prepared from the sediments and fixed immediately in 95% ethyl alcohol. The slides were stained using the Giemsa and Papanicolaou methods.

For BD SurePath™ LBC, samples were processed following the manufacturer's protocol for material preservation and slide preparation. Briefly, the fluid was centrifuged, and the sediment was transferred to 10 mL of CytoRich® (New York, NY, USA) ethanol-based preservative mixed with hemolytic solution. A CyRinge, a syringe-like device, was inserted into the CytoRich® vials to disaggregate any larger cell fragments. The

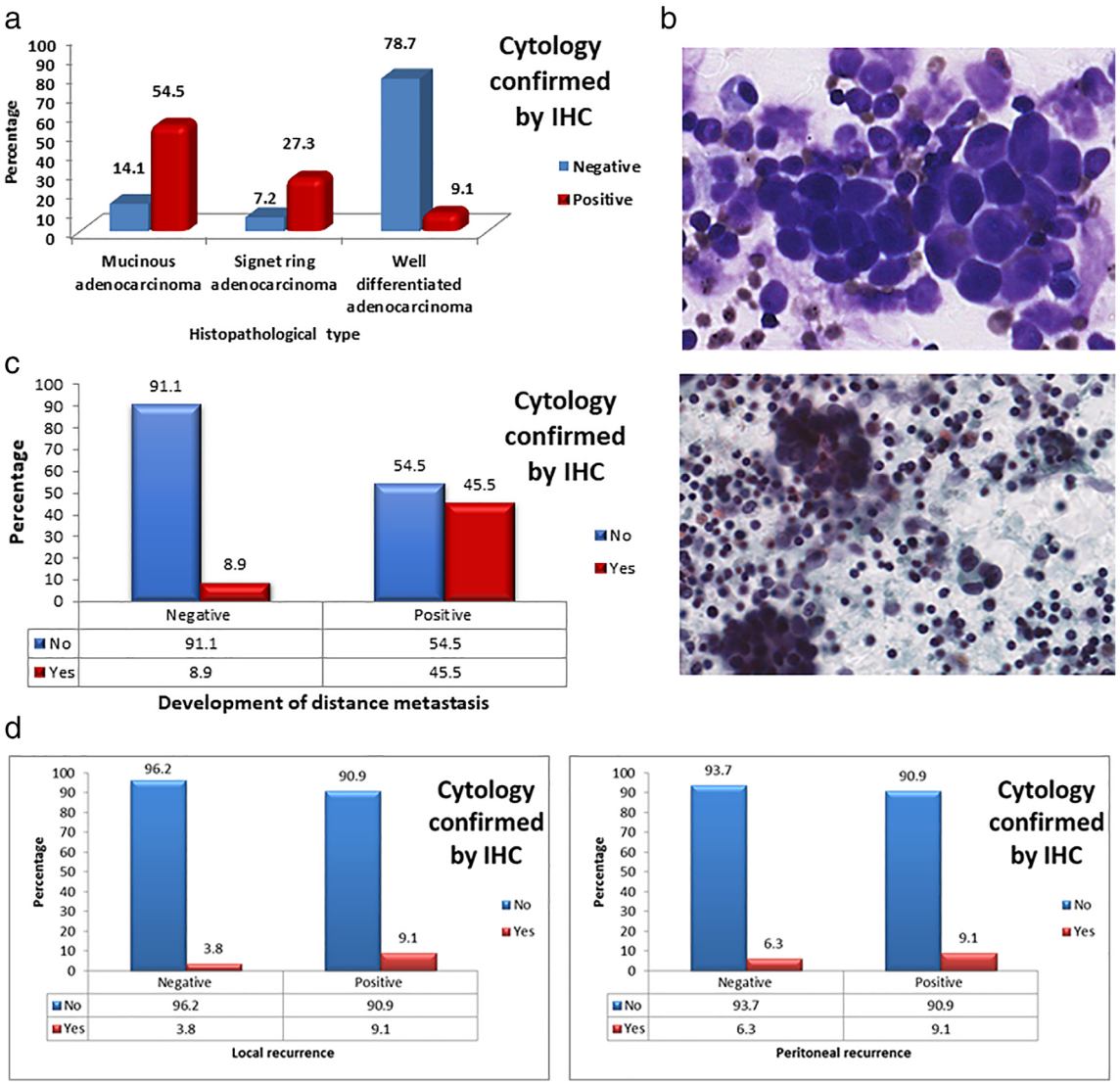

Figure 1 (a) Graph showing the relation between tumor histologic type and positive peritoneal cytology (*P*-value <0.001). (b) Giemsa-stained smear (diff-quick stain) and SurePath LBC smear (Papanicolaou stain) featuring metastatic adenocarcinoma of primary colorectal origin [original magnification x400]. Graph showing high incidence of positive peritoneal cytology in cases with distant metastases (*P*-value <0.001) (c) and patients with peritoneal and local recurrence (d).

samples in CyRinge (New York, NY, USA) were poured into a centrifuge tube filled with 4 mL of a density gradient reagent, a polysaccharide solution that acts to trap small particulates and debris. Sample processing was completed using the PrepStain™ slide processor and stained with Papanicolaou stain. The remaining material was stored in the Preservcyt™ (New York, NY, USA) solution for further use of a second LBC slide if necessary or the preparation of cell block for application of immunocytochemistry (ICC) in case it is needed.

Immunocytochemistry. The remaining cell pellets after centrifugation of lavage were fixed in formalin and embedded in paraffin for tissue block sections. Immunohistochemistry was performed on 5-μm-thick sections. The sections were mounted on positively charged glass slides, and the paraffin was removed; the sections were rehydrated in a graded ethanol series and then subjected to antigen retrieval at 100°C for 3 min in preheated sodium citrate buffer (pH 6.0). Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide. The primary antibody, anticalretinin FLEX, monoclonal mouse anti-Human calretinin, Clone DAK-Calretinin Ready to use or anti-CDX2 FLEX, monoclonal mouse anti-Human CDX2, or Clone DAK-CDX2 Ready to use (*Dako*, *Denmark*), was applied, and samples were then incubated for 90 min at room temperature. After washing, the secondary antibody was applied, and samples were further incubated for 30 min at room temperature. Finally, the slides were incubated with the mixed solution of 3–3′-diaminobenzidine tetrahydrochloride (DAB) and horseradish peroxidase substrate buffer for color development and were then washed with deionized water. The sections were counterstained with Mayer hematoxylin and then dehydrated, cleared, and mounted. Positive and negative control slides for each marker were included in each run. In addition, as a negative control, a section was processed as described but with the primary antibody omitted.

Cytological interpretation. All slides were reviewed under a light microscope and diagnosed by an experienced cytopathologist with a specialization in gastrointestinal oncology. Fluids were cytologically diagnosed as "negative for malignancy," "suspicious for malignancy," or "positive for malignancy." Both results with positive and suspicious malignant cells by conventional cytology were further subjected to ICC for confirmation.

Bias. Patients' performance was assessed before surgery using the Eastern Cooperative Oncology Group (ECOG) scale. Patients with severe medical comorbidities contraindicated for major surgery under general anesthesia were excluded from this study. The pathologic staging of cancer was assessed postoperatively according to the eighth edition of the American Joint Committee on Cancer (AJCC) TNM grading system. The Institutional Review Board and ethical committee of National Cancer Institute approved this study, and written informed consent for tissue collection was obtained from all patients.

Study size. Ninety-six patients had no evidence of distant metastasis at preoperative staging workup. Six patients with primary peritoneal carcinomatosis were discovered intraoperatively. Two patients had distant metastasis.

Quantitative variables. Data were analyzed using IBM SPSS advanced statistics, version 24 (SPSS Inc., Chicago, IL, USA). Numerical data were described as median and range or mean and standard deviation as appropriate, while qualitative data were described as number and percentage. Disease-free survival (DFS) was calculated from date of complete remission till date of relapse, metastasis, death, or last follow-up. Overall survival (OS) was calculated from date of diagnosis till date of death or last follow-up.

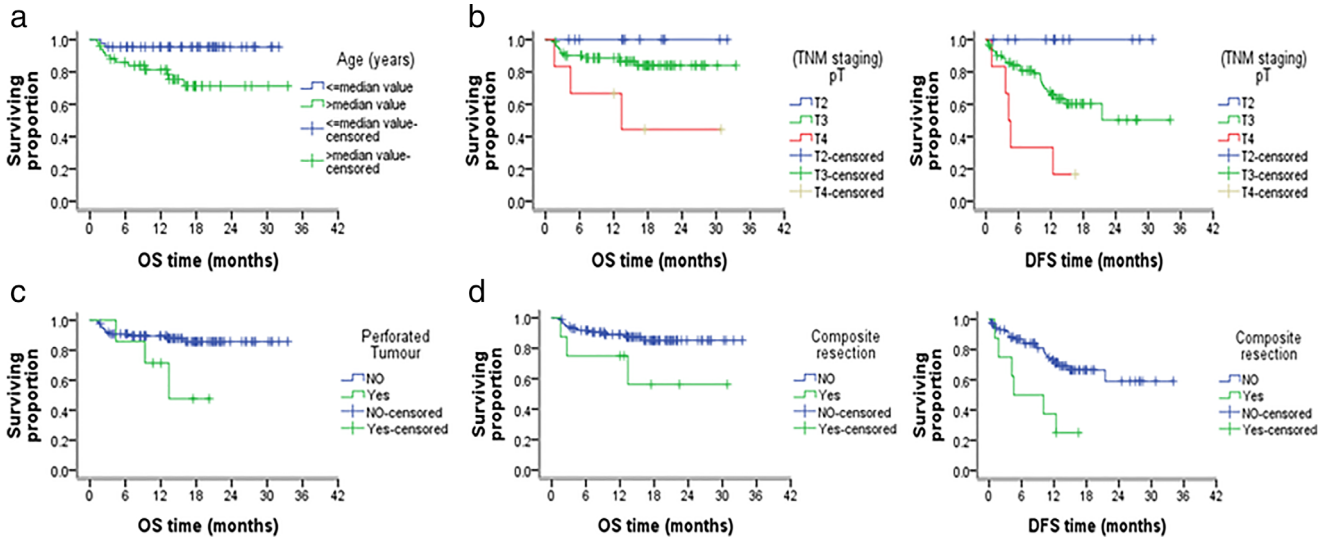

Figure 2 (a) Graph showing the relation between patients' age and OS (*P*-value 0.009). (b) Graphs showing relation between tumor stage (pT) and OS (*P*-value 0.015) and DFS (*P*-value 0.001). Lower OS was encountered at a higher rate with perforated tumors (*P*-value 0.042) as shown in the graph (c). Composite resection was associated with a considerable deleterious effect on survival outcome and DFS (*P*-value 0.054 and 0.003, respectively) (d).

Statistical methods. Chi-square (Fisher's exact) test was used to examine the relation between qualitative variables as appropriate. Survival analysis was carried out using the Kaplan–Meier method. Comparison between two survival curves was performed using the log-rank test. Multivariate analysis was performed by Cox regression model to test for the independent prognostic effect of statistically significant variables on the univariate level by calculating the hazard ratio and its 95% confidence interval. Bonferroni correction of *P*-value was carried out to avoid hyperinflation of type 1 error, which arises from multiple testing. A *P*-value less than or equal to 0.05 was considered statistically significant. All tests were two-tailed.

Results

Patients. A total of 104 patients underwent abdominal surgery for CRC, of which 96 patients (46 male and 50 female) had no evidence of distant metastasis. Six[6] patients were excluded due to primary peritoneal carcinomatosis discovered intraoperatively and two patients due to distant metastasis. Patients with severe medical comorbidities contraindicated for major surgery under general anesthesia were excluded from the study.

Descriptive data. Patients were diagnosed with stages I, II, and III CRC and had no evidence of distant metastasis. One patient died within 3 months of surgery. Five patients were irresectable. CA and CEA tests were available only for 60 patients. The patients were followed up during the 3 years of the study, with a median of 14 months.

Outcome data. The median age was 55.5 years with a range of 19–81 years; 63 patients had colon cancer, and 33 patients had rectal cancer. Five patients were irresectable. Tumor staging according to the TNM was as follows: 12 patients (13.2%) were pT2, 73 (80.2%) were pT3, and 6 (6.6%) were pT4. For lymph nodes, it was as follows: 47 patients (51.6%) were N0, 30 (33%)

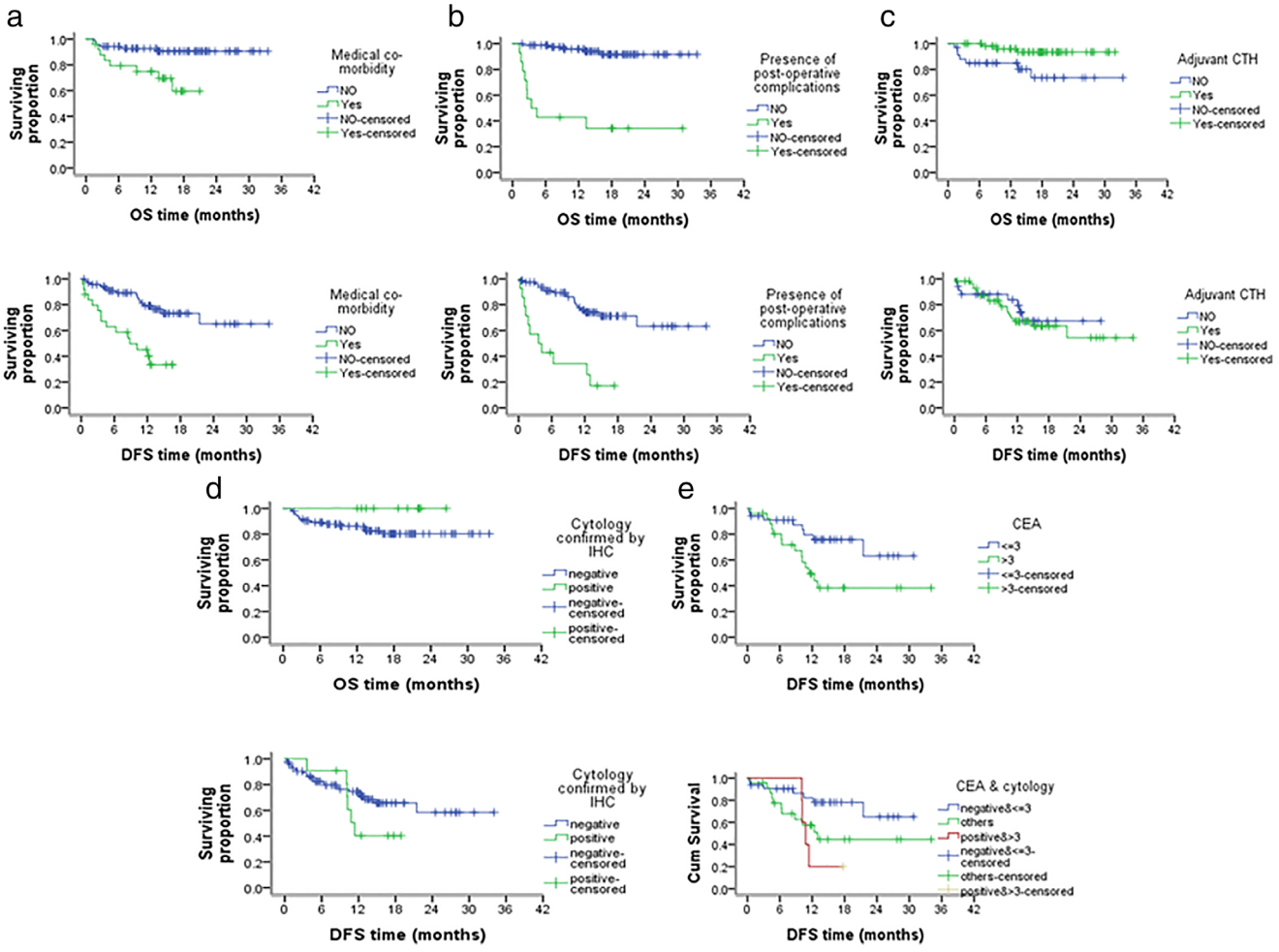

Figure 3 Medical comorbidity is an ominous sign for both OS and DFS graphs (a). The presence of postoperative complications is an independent poor prognostic factor regarding OS and DFS graphs (b), while adjuvant CTH has increased survival significantly (*P*-value 0.015) graphs (c). Graphs (d) show irrelevance between cytology results and OS or DFS. Serum CEA showed an adverse prognostic value in DFS prediction either alone or combined with cytology results (*P*-values of 0.019 and 0.039, respectively) (e).

were N1, and 14 (15.4%) were N2. The cancer stage was as follows: 12 patients (13.2%) were stage I, 35 (38.4%) were stage II, and 44 (48.4%) were stage III. The prevalent pathology was well-differentiated adenocarcinoma encountered in 69 (71.9%) patients in comparison to 19 patients (19.8%) who had mucinous carcinoma and 8 patients (8.3%) who had signet ring carcinoma (Table S1).

Main results. The prevalence of positive malignant cells in the peritoneal lavage fluid of the enrolled patients was 11.5%. Most patients were found to be positive by conventional cytology, with only two patients having suspicious malignant cells found on conventional cytology and were confirmed positive by ICC. Peritoneal recurrence occurred in six patients (6.7%), and local recurrence occurred in four patients (4.4%). Twelve patients developed distant metastasis (13.3%), seven patients (58.3%) developed liver deposits, two patients (16.7%) developed pulmonary metastasis, and three patients (25%) developed nodal metastasis (Table S1).

Studying the predictive factors that are assumed related to positive cytology results demonstrated that all factors are not statistically significantly, except for the histological type of cancer (Table 1), presence of lymphovascular invasion (LVI), and distant metastases (Table 2). Positive cytology was associated with mucinous and signet ring cancers more than well-differentiated carcinoma subtypes (54.5% and 27.3% *vs* 18.2%) (*P*-value <0.001) (Fig. 1a,b). Multivariate analysis revealed that distant metastasis and LVI are independent prognostic factors with regard to cytology results. LVI was detected in patients with positive cytology more than in those with negative cytology (27.3% *vs* 5.6%) (*P*-value 0.016). Distant metastasis had a higher prevalence rate in those with positive peritoneal wash rather than negative cases (45.5% *vs* 8.9%) (*P*-value <0.001) (Fig. 1c). Of patients with tumor perforation, 9.1% had positive peritoneal cytology *versus* 7.1% with negative cytology results and a *P*-value of 0.807. Positive cytology result did not show any significant relation with peritoneal and local recurrence as 9.1% of cases were cytology positive *versus* 6.3% and 3.8% who were negative for peritoneal and local recurrence, respectively, with a *P*-value of 0.731 for peritoneal recurrence (Fig. 1d).

Other analyses. Univariate analysis showed a 2-year overall survival (OS) for patients 55 years old or younger reach 95%, while older patients have only 71% (*P*-value 0.009) (Fig. 2a). Stage T3 tumors have better survival than T4, with 84% *versus* 44%, respectively, in 2 years (*P*-value 0.015). The former tumor stage also had better DFS than the latter, 68% *versus* 33%, respectively, in the first 12 months (*P*-value 0.001) (Fig. 2b). Lower OS was encountered more often with perforated tumors (*P*-value 0.042) (Fig. 2c).

Composite resection was associated with a considerable deleterious effect on survival outcome and DFS (*P*-value 0.054 and 0.003, respectively) (Fig. 2d), while adjuvant chemotherapy (CTH) increased the survival significantly (*P*-value 0.015). Medical comorbidity, low performance status, and postoperative complications are ominous signs for both OS and DFS (Fig. 3a). Multivariant analysis results found that only adjuvant CTH and presence of postoperative complications were independent prognostic factors with regard to OS and DFS. (Fig. 3b,c).

Peritoneal recurrence and local recurrence did not have any significant effect on OS. The cumulative DFS at 12 months was 40% in patients with positive cytology *versus* 75% in negative ones (*P*-value 0.195) (Fig. 3d). Interestingly, serum CEA showed adverse prognostic value in DFS prediction either alone or combined with cytology results, with a *P*-value of 0.019 and 0.039, respectively (Fig. 3e).

Discussion

Key results. CRC represents 3.47% of male cancers and 3% of female cancers among Egyptian patients.[2] Up to 50% of high-risk patients may show peritoneal carcinomatosis on second-look laparotomy when unrecognized on clinical and imaging examination.[13]

It is a major concern whether an aggressive preventive surgical strategy, such as peritoneal cytoreductive measures (CRS), is indicated during the first surgical intervention. These measures include omentectomy, adnexectomy, appendectomy, and round ligament resection, regardless of whether they are macroscopically involved.[14] In addition, the early use of intraoperative HIPEC or targeted therapies is a reasonable alternative treatment modality for the prevention of peritoneal recurrence.[15] A clinical practice of repeated administration of intraperitoneal chemotherapy is used to provide high local concentrations and prolonged exposure to drugs to allow better penetration into small tumor nodules and lower drug impedance.[16] Extensive intraperitoneal lavage is another prophylactic strategy suggested for patients with positive IPCC.[17] Using either single or combined locoregional approaches, we endeavor to eradicate microscopic undetectable peritoneal deposits and prevent peritoneal spread.

Multicenter studies reported that IPCCs are associated with peritoneal recurrence and worse survival.[3,7] However, other authors argue for the prognostic role of free IPCCs on survival and rejected its predictive significance for peritoneal recurrence after a curative resection.[10,12] Despite the current debate of the actual significance of positive peritoneal fluid cytology, some authors still considered it evidence of the local aggressiveness of the disease during surgery. It may help in making the decision of whether to apply any of the previous treatment strategies immediately after resection of the tumor.[7,17] Either side of this argument should be supported by multi-institutional studies to determine the prevalence of IPCC and to evaluate the real prognostic impact of peritoneal lavage cytology. This study represents an Egyptian experience to document the value of positive IPCC and to estimate the incidence of peritoneal and local recurrence during follow-up, as well as to evaluate the impact on survival.

Limitation. The conventional cytology technique represents the most popular method for examining peritoneal lavage for IPCC due to its simplicity, with no need for a complex technique, plus it is relatively inexpensive.[18] It has a high specificity[19] but low sensitivity.[20] ICC is a subjective test and depends on the strength of cellular staining, and PCR-based methods require the preservation of RNA and have inherent problems as they cannot delineate cancerous cells from nonmalignant cells or cellular debris.[21,22] In this study, the LBC technique was also used, supplemented with immunostaining in equivocal cases. This technique has a higher accuracy than conventional method.

It can eliminate disturbing background in conventional cytology, such as large numbers of blood cells and inflammatory cells in the peritoneal lavage fluid, which may obscure malignant cells.

Interpretation. The prevalence rate of positive cytology in the peritoneal lavage fluid revealed by this study was 11.5%, which is within the range reported from previous studies from 2.1% to 52% in patients with CRC.[3,11,17] This range may be a result of the heterogeneity of the techniques used to detect malignant cells in peritoneal lavage fluid.

CRC in Egypt is commonly diagnosed in older people with a mean age of about 53 years, which is a decade younger than the corresponding age in the United States.[23,24] An increasing incidence of young adult patients diagnosed with CRC was noted, with an incidence of 29–31%.[25,26] The results of this study showed that 45.8% of the patients were younger than 55.5 years (44 patients). Positive cytology samples were more common in those patients (13.6%). Although our results were not statistically significant, we agree with Lemmens et al.[27] to still consider those populations as being at high risk of developing recurrence or distant metastasis. This is also supported by results from those patients excluded from the study due to intraoperative peritoneal metastases (IPM) and liver metastasis. Patients with IPM had a median age of 35 years, while those who had intraoperative liver metastasis had a median age of 45 years (Table S2).

It is worth noting that univariate analysis showed a decreased overall 2-year survival rate in cytology-positive patients—only 20% for those with negative IPCC. This is probably due to the influence of other factors (Table S3), mainly age, as nearly half of the patients were younger than 55.5 years. Increasing age is associated with fewer colon cancer-related deaths but showed a concomitant increase in the proportion of cardiovascular disease-related deaths.[28] Despite this, the ≤50-year-old cohort was supposed to be associated with worse OS due to higher proportions of pathologic stage according to Gabriel et al.;[24] however, on the other hand, this age group has significantly better performance and surveillance and fewer medical comorbidities.

Interestingly, serum CEA showed a significant prognostic value in DFS prediction either alone or combined with cytology results (Table S4). This is in agreement with the study by Kanellos et al., where patients with combined positive cytology and high peritoneal CEA level were at a higher risk of recurrence.[29]

Patients with colon cancer have a higher prevalence of positive cytological peritoneal samples than those with rectal cancer (12.7% *vs* 9.1%). It can be explained by the fact that organs which have omental appendages have a higher incidence of implants due to more fluid resorption. In addition, there is a higher incidence of the mucinous subtype of CRC that affects the proximal colon more than rectum or distal colon,[30] which in turn is associated with higher free IPCCs.[31,32] The study revealed that the histologic variant of primary tumors of colorectal origin, especially the mucinous subtype and signet ring, are associated with a higher incidence of free IPCCs, with *P*-value <0.001. This may explain their preferential tendency to metastasize to the peritoneum. Moreover, the most enciered histologic type in patients with IPM was mucinous and signet ring adenocarcinomas. The samples from female patients showed slightly higher positive cytology more than male patients (12% *vs* 10.9%). This agrees with the fact that the mucinous subtype of CRC has higher ratios in women compared to nonmucinous colorectal adenocarcinoma.[33]

The presence of lymphovascular invasion was noted, with a higher rate observed in cases with positive malignant cells in the peritoneal fluid cytology with a incidence of 27.3% *versus* 5.9% in patients with negative cytology (*P*-value 0.016). Likewise, distant metastasis was reported with an incidence of 45.5% in positive cytology cases *versus* 8.9% in negative ones (*P*-value <0.001). This is in agreement with the logic that metastatic tumor cells migrate toward the closest microvessel within the capillary network within the peritoneal cavity and start dividing there.[34] The most common mechanisms of metastasis in large-bowel cancer are lymphatic spread to regional lymph nodes and hematogenous spread to the liver via the portal vein. Undetected lymphatic or hematogenous micrometastases are thought to be responsible. These can present prior to surgical resection or occur iatrogenically by shedding of tumor cells intraoperatively during tumor excision.

The data showed high association of positive peritoneal lavage with tumor perforation (9.1% *vs* 7.1%), although figures were statistically insignificant. However, our result showed significant lower OS in this subgroup of patients; this is coherent with the high risk defined in the literature.[35] A significant factor affecting the prognosis is the pathologic TNM stage, especially T-stage. The more advanced stages of disease which is the depth of bowel wall invasion (pT3/4), the higher incidence of tumor-positive peritoneal cytology.[14,36] Kanellos et al. reported a significant association detected between cytology and tumor stage.[29] This is consistent with the study findings as all positive cytology results were obtained from patients with T3. It was also found that positive cytology results were obtained at a higher rate in stage III patients than those with stage II disease (15.9% *vs* 4.3%); however, this difference was not statistically significant.

Generalizability. The prognostic value of detecting IPCC for peritoneal and local recurrence after curative resection is still controversial.[3,7,9-12,17] This study showed a higher incidence of peritoneal and local recurrence in the presence of malignant peritoneal lavage cytology over negative cytology; however, neither has reached statistical significance. An analysis of OS showed no dependence on the result of peritoneal cytology. The result is supported by one side of the current debate, which suggests the lack of a predictive value for peritoneal recurrence and survival.[10,12,29,37]

It worth noting that, when studying the patients excluded from the study because of IPM and distant metastasis, unexpectedly, only 33.3% of those with IPM had positive malignant cytology in the peritoneal wash, while 50% of those with intraoperative liver metastasis showed positive cytology (Table S2).

We suggest that the peritoneal lavage result during surgery will not reflect accurate prognostic significance for local or peritoneal recurrence. An analysis of the generally low prevalence of positive cytology could be explained by the entrapment of free intraperitoneal malignant cells by fibrosis and adhesions developed due to cancer-induced fibrosis[38] or if postoperative adhesion has developed in the dissection plane.[7] This can result in false negative cytology, as well as hinder intraperitoneal chemotherapy drugs from reaching tumor cells. Moreover, the theory that only cancer stem cells, and not any viable cancer cells, are able to reproduce the cancer[39,40] gives a further reason against

the reliability and effectiveness of searching for free peritoneal cancer cells in CRC.

Conclusion

Our results demonstrate that the presence of IPCC observed when using conventional cytology was not an independent prognostic factor for the development of PC or for survival. Finally, we discussed and summarized the possible rationales of the low prevalence of IPCC and the inaccurate results of using conventional cytology to evaluate local recurrence in case of CRC.

Acknowledgment

All procedures performed in this work, including operations settings, equipment, laboratory kits, and drugs, were all obtained through the National Cancer Institute, Cairo University, Egypt.

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Supporting information

Additional supporting information may be found in the online version of this article at the publisher's website:

Table S1. Clinicopathological data of patients without metastasis ($n = 96$).
Table S2. Clinicopathological data of patients with intraoperative peritoneal metastasis ($n = 6$) and patients with intraoperative liver metastasis ($n = 2$).
Table S3. Univariate analysis of prognostic factors in overall survival (OS) ($n = 95$*).
Table S4. Univariate analysis of prognostic factors in disease-free survival (DFS) ($n = 95$*).