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► **To cite this version:**

M. La Torre, M. Ferri, M.R. Giovagnoli, N. Sforza, G. Cosenza, et al.. Peritoneal Wash Cytology in Gastric Carcinoma. Prognostic Significance and Therapeutic Consequences. *EJSO - European Journal of Surgical Oncology*, 2010, 36 (10), pp.982. 10.1016/j.ejso.2010.06.007 . hal-00625569

HAL Id: hal-00625569

<https://hal.science/hal-00625569v1>

Submitted on 22 Sep 2011

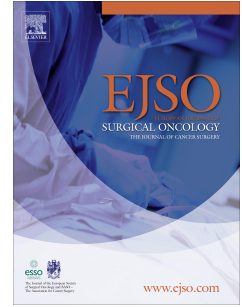
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Accepted Manuscript

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PII: S0748-7983(10)00183-6

DOI: [10.1016/j.ejso.2010.06.007](https://doi.org/10.1016/j.ejso.2010.06.007)

Reference: YEJSO 2995

To appear in: *European Journal of Surgical Oncology*

Received Date: 12 May 2010

Accepted Date: 7 June 2010

Please cite this article as: La Torre M, Ferri M, Giovagnoli MR, Sforza N, Cosenza G, Giarnieri E, Ziparo V. Peritoneal Wash Cytology in Gastric Carcinoma. Prognostic Significance and Therapeutic Consequences, *European Journal of Surgical Oncology* (2010), doi: 10.1016/j.ejso.2010.06.007

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Original Article

**Peritoneal Wash Cytology in Gastric Carcinoma.
Prognostic Significance and Therapeutic Consequences**

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Abstract

Background and Aims: The prognosis of patients with gastric cancer is poor, even following curative resection, and is related primarily to the extent of disease at presentation. In locally advanced gastric tumors, peritoneal lavage cytology (PLC) is a relevant prognostic factor. The Authors present their results of peritoneal washing cytology, evaluating the prognostic value of this technique, and discussing the clinical impact.

Patients and Methods: From July 2003 to May 2008, results of PLC in 64 patients with histologically proven primary gastric adenocarcinomas were analyzed. At laparotomy the abdomen was irrigated with 200 ml of normal saline, and ≥ 50 ml were aspirated and examined by means of cytology and immunocytopathology.

Results: PLC was positive in 7 cases (11%). Overall, 86% of patients with a positive PLC had a pT3/pT4 tumor and 100% with a positive PLC had a N-positive tumor ($p < 0.001$); 71% of patients with a positive PLC had a grade G3/G4 tumor ($p = 0.001$). At a median follow up of 32 months, the cumulative 5-year survival was 28%. The median survival of patients presenting positive PLC (19 months) was significantly lower than that of patients with negative peritoneal cytology (38 months) ($p = 0.0001$). Multivariate analysis identified cytology as a significant predictor of outcome ($p = 0.018$).

Conclusions: Results in the present series demonstrated that patients with a positive peritoneal cytology had advanced disease and poor prognosis, thus indicating that patients with locally advanced gastric cancer should undergo staging laparoscopy and PLC examination in order to select those requiring more aggressive treatment.

Future therapeutic strategies should include PLC examination in preoperative staging, in order to select patients for more aggressive treatment.

Mini-abstract

Positive peritoneal cytology (PC) in gastric cancer is associated with poor prognosis. In locally advanced gastric cancer patients should undergo staging laparoscopy and PC to select those requiring different treatment.

Key Words:

Peritoneal Wash Cytology; Gastric Carcinoma; intraperitoneal free cancer cells

Introduction

Gastric cancer is the third most frequent cause of cancer death in Western countries¹. Prognosis of these patients is poor, with a cumulative 5-year survival rate of 20%, and this is primarily related to the extent of disease at presentation. Even after R0 resections, approximately 50% of patients die from recurrent disease within the first 2 years of follow-up^{2,3}. The most frequent site of recurrence following R0 resection is the peritoneum^{4,5}, probably due to the intraperitoneal presence of free cancer cells shed from the serosal surface of the primary tumor⁶. The majority of patients with intraperitoneal free cancer cells (IFCCs) do not escape postoperative peritoneal recurrence⁷⁻⁹. Clinical studies⁶⁻⁸ have shown that peritoneal cytology findings are an independent prognostic factor in gastric cancer and, indeed, the Japanese Research Society for Gastric Cancer has now included peritoneal cytology as part of the staging process in gastric cancer¹⁰.

In this report findings are presented related to a series of 64 patients who underwent laparotomy and PLC for potentially resectable gastric carcinoma, in order to determine the prevalence of positive cytology and to analyze the prognostic significance of IFCCs.

Materials and Methods

A review was made of PLC data from 64 consecutive patients (14 female, 50 male, mean age 64.5 years, range 29-84) undergoing laparotomy for gastric carcinoma, between July 2003 and May 2008, at the S. Andrea Hospital - University of Rome.

Overall, 62 patients underwent R0 tumour resection by means of D 1.5 gastrectomy (D1 gastrectomy including lymphectomy of celiac trunk) . Two patients were considered not resectable at laparotomy on account of local extension of the tumor, without evidence of peritoneal carcinomatosis.

TNM pathological staging (UICC 1997) revealed 33% Stage I, 23% Stage II, 14% Stage III and 30% non-metastatic Stage IV tumors. Non-metastatic stage IV patients presented pT4 tumors reaching the mesocolon, duodenum or left hepatic lobe, all of whom submitted to a R0 resection.

Tumor grading was as follows: G1 7%, G2 33%, G3 51%, G4 9%.

Of 64 patients, 27 (42%) underwent postoperative chemotherapy.

All patients were followed for at least one year or until death. At the time of the present evaluation, the median follow-up time of surviving patients was 32 months (range 12 - 56).

Peritoneal washing

Upon entering the abdominal cavity, prior to manipulating the tumor, 200 ml of warm normal saline were introduced and manually dispersed in the Douglas cavity, para-colic gutters and in the right and left subphrenic cavity. At least 50 ml of fluid was subsequently recovered, after gentle stirring, from several regions of the abdominal cavity. The fluid was then centrifuged for 5 min at 1500 rpm. The sediment was smeared onto one or more glass slides and stained using the Papanicolau's method. All cytological examinations were performed by experienced cytopathologists. Cytological findings were classified as positive, negative or suspicious. The following cell characteristics were used to determine the presence of malignant cells: presence of aggregate, size, shape, type of cytoplasm, cytoplasmic vacuoli, mainly nuclear abnormalities, nuclear chromatin, nuclear-cytoplasmic ratio, mitotic figures, and nucleolar prominence.

When necessary, the glass slide containing the nucleated cell layer was analyzed at immunocytochemistry (ICC) using the CEA antigen antibodies (monoclonal CEA clone 11-7 Dako®). The glass slide was decolorized using 95% alcohol and 1% of chlorhydric acid, and then treated with CEA antibodies. ICC was performed in 20 suspicious cases.

Statistics

Statistical analyses were performed using MedCalc for Windows, version 10.2.0.0 (MedCalc Software, MariaKerke, Belgium). Differences in distribution were calculated using the chi-square

test or Fisher's exact test depending on the number of cases in each subgroup. Survival was estimated using Kaplan-Meier's method, and differences were assessed by means of the log-rank test^{11,12}. Overall survival was defined as the time interval between surgery and death, regardless of the cause.

$p < 0.1$ was used as the cut-off value for statistical significance in the variable selection in the multivariate modelling in order not to overlook any potentially important predictors. Only those variables significant at univariate analysis were included in the model. Statistical significance remained conventionally defined as $p < 0.05$ in all other cases.

Results

Of the 64 tumors, 7 (11%) showed a positive cytology.

PLC was significantly related to the pathological findings (Table I). Overall 86% of patients with a positive PLC had a pT3/pT4 tumor and 100% of the patients with a positive PLC had a N-positive tumor ($p<0.001$); in 71% of patients with a positive PLC, the tumor grade was G3/G4 ($p=0.001$). These results indicate that the rate of positive peritoneal wash samples increases proportionally when the tumor invades the deeper layers of the gastric wall or the lymph nodes, and when the tumor has lost differentiation.

At a median follow-up of 32 months, the cumulative 5-year survival rate was 28%. The median overall survival in patients with a positive PLC was significantly lower than that in patients with a negative PLC (19 vs 38 months; $p=0.0001$) (Figure 1). In the univariate analysis, the extent of the tumor (pT), patient sex and lymph node involvement were also found to be significant predictors of survival, while the tumor grading and age of the patients were not. When these variables were included in a multivariate model, only cytology and the extent of the tumor remained significant independent predictors of survival (Table II).

In a subgroup analysis of pT3/pT4 tumors, patients with a negative PLC lived longer than those with a positive PLC (27 months vs 18 months, $p=0.06$) (Figure 2). Moreover, as far as concerns patients with lymph node involvement, those with a positive PLC had a worse prognosis (19.5 months vs 36 months $p=0.001$) (Figure 3).

Discussion

Although the surgical treatment (i.e., gastric resection and lymphadenectomy) for gastric carcinoma is well established, patients with advanced disease still have a poor prognosis. Peritoneal metastasis is the most frequent cause of death, with a mean survival of only 6 months following peritoneal recurrence¹³. In patients with serosa involvement, peritoneal recurrence reaches 50% even if curative resections are performed^{14,15}. Peritoneal recurrence develops from peritoneal free cancer cells originating from the primary lesion or metastatic lymph nodes¹⁶⁻¹⁸. It has been shown that patients with a gastric tumor involving the serosa or the lymph nodes have a high probability of producing peritoneal free cancer cells and developing peritoneal recurrence or carcinomatosis⁵⁻¹⁰.

Data in the literature show a significant difference, in terms of survival, between patients with a positive peritoneal cytology and those with negative findings, thus indicating that positive peritoneal cytology is an important prognostic factor. In fact, since 1998, the Japanese Gastric Cancer Association (JGCA) has suggested that the presence of free cancer cells in the peritoneal cavity, should be considered as an independent prognostic marker in patients with gastric cancer¹⁰, and cytology-positive patients are classified as Stage IV in the gastric cancer classification of the Union International Control Cancer (UICC)¹⁹.

In the present study, the median overall survival in patients with positive PLC was significantly lower than that in patients with negative PLC (19 vs 38 months; $p=0.008$), and cytology was a significant prognostic marker both at the univariate and multivariate analysis.

PLC is widely accepted as the gold standard for diagnosis of IFCCs. Cytology is easy and safe to perform, it takes approximately 15 min for a cytopathologist to analyze the patient's slides, and the estimated cost for this procedure is \$ 60²⁰. The rate of detection of IFCC in the literature ranges from 14%-47%, depending upon the cohort of patients studied²¹. When only potentially curative resections are included, the rate of IFCC varies from 4.4%-11%, and ranges from 22%-30% in gastric carcinoma involving the serosa²²⁻²⁴. In our experience, cytology was positive in 11% of the entire population studied and in 25% of patients with a pT3/pT4 tumor.

In agreement with Koga et al.⁶, we found positive peritoneal cytology in 1/30 (2.5%) pT2 tumors. This finding may depend upon one of 2 conditions, namely: the histological examination of the depth of invasion may not always be performed at a spot where the cancer infiltration is deepest, resulting in pT downstaging or, the cancer cells could be shed through the lymphatics, from the metastatic lymph nodes or through the lymphatic canals via the omentum²⁰. In our series, in fact, the patient with positive cytology and a pT2 tumor presented a N1 status at the final histopathological examination.

Although IFCCs are detected in a considerable number of gastric cancer patients, the probability of peritoneal recurrence far exceeds the rate of IFCC detected^{14,15}. The use of real-time RT-PCR (real-time quantitative reverse transcriptase-polymerase chain reaction) has been reported to increase the sensibility of IFCC detection. In a recent study, CEA mRNA RT-PCR was reported to detect peritoneal free gastric cancer cells at a positive rate 30.0% higher than cytology alone²⁵. PCR sensitivity is determined primarily by the expression level of the marker gene but also by the corresponding background level of the peritoneal washing. Background level expression requires cut-off strategies especially for samples, such as peritoneal washing, rich in debris and various other different epithelial (frequently reactive mesothelial) and inflammatory cells influencing cDNA quality¹⁶. Furthermore, the expression of CEA, in disseminated tumor cells, might differ from the tissue resident cells or marker concentration may vary within the tissue due to intratumoral heterogeneity. Therefore, many different variables can be a problem in order to standardize the RT-PCR technique for routine diagnostic activity.

The status and viability of free cancer cells considerably influence the potential of the cells for generating metastases. Moreover, not all cancer cells have metastatic potential in the peritoneum. Thus the presence of intact, well preserved cancer cells, as demonstrated by means of the cytological examination, may be of greater prognostic value than free CEA mRNA in gastric cancer patients.

Although general consensus exists regarding the prognostic significance of IFCC in locally advanced gastric cancer, the clinical implications remain unclear since it does not alter the subsequent treatment in the majority of cases. New therapeutic strategies would require peritoneal cytology as part of the preoperative staging. Bryan et al. reported that conventional staging failed to detect incurable disease in 10 patients, representing 11% of the entire series, inasmuch as 7 of these patients were IFCC positive and could have been more appropriately managed if laparoscopic peritoneal lavage cytology had been used²⁶. In a series of 100 patients with locally advanced gastric cancer, Nakagawa et al.²⁷ reported that staging laparoscopy with peritoneal lavage cytology allowed upstaging of 44 patients (44%), thus demonstrating unsuspected IFCC or peritoneal deposits. They also reported that 11/18 patients (61.1%) with positive cytology who received neoadjuvant chemotherapy, had no free cancer cells at surgery. Patients with positive cytology may also benefit from intraoperative hyperthermic chemotherapy. Several studies have demonstrated the efficacy of this treatment with improvements both in the survival rate and a decrease in the incidence of peritoneal recurrence²⁸⁻³⁰ of patients with gastric cancer and serosal involvement. These data indicate that patients with positive cytology, at staging laparoscopy, could possibly be

redirected to intraoperative peritoneal hyperthermic chemotherapy or neoadjuvant chemotherapy. Patients presenting progressive disease, during neoadjuvant treatment, may be spared laparotomy. A prospective study is now mandatory in order to assess the efficacy of this alternative treatment in prolonging survival in patients with locally advanced disease.

In conclusion, the results of the present study demonstrate that positive peritoneal cytology, in gastric cancer, is associated with advanced disease and poor prognosis. Future studies should include staging laparoscopy with peritoneal fluid cytology in locally advanced tumors, in order to select those patients with IFCCs for more aggressive therapy.

ACCEPTED MANUSCRIPT

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Figure Legend

Fig. 1. Overall survival related to cytology, $p=0.0001$

Fig. 2. Survival in patients with a pT3/pT4 tumor according to cytology, $p=0.06$

Fig. 3. Survival in patients with a pN+ tumor according to cytology, $p=0.001$

Table Legend

Table I. Correlation between the results of cytology and pathology.

Table II. Multivariate model considering cytology, sex, lymph node involvement (pN) and extent of the tumor (pT). b: coefficient beta; SE: standard error; Exp(b): expected beta value; CI: confidence interval.

Table I.

	Cytology positive	Cytology negative	p
No. patients	7	57	
Depth of invasion			0.001
pT1	0	9	
pT2	1	30	
pT3	3	12	
pT4	3	6	
Metastatic nodes			0.001
pN+	7	36	
pN-	0	21	
Histopathology			0.001
G1/G2	2	24	
G3/G4	5	33	

Table II

Covariate	b	SE	Exp(b)	95% CI of Exp(b)	p
Cytology	1.2378	0.5613	3.4482	1.1542 to 10.3016	0.02742
Sex	-0.4615	0.6712	0.6303	0.1703 to 2.3336	0.4917
pT	1.0330	0.5867	2.8096	0.8949 to 8.8208	0.07828
pN	-0.02576	0.6438	0.9746	0.2777 to 3.4199	0.9681

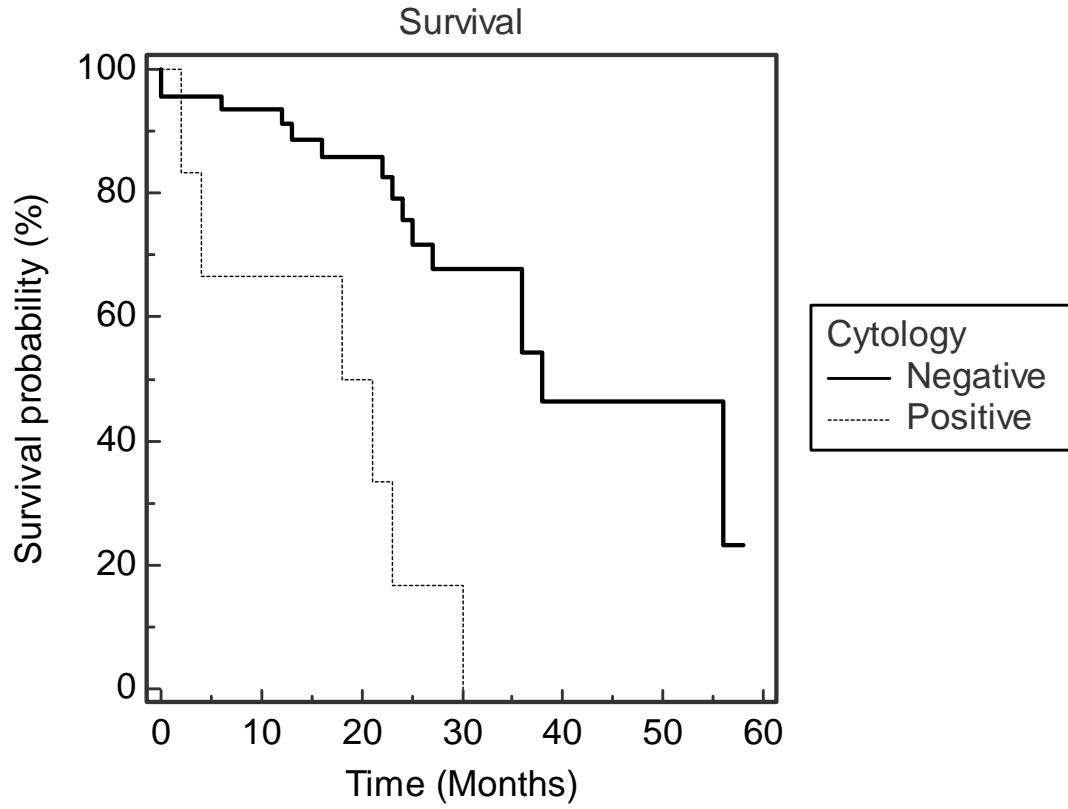


Figure 1.

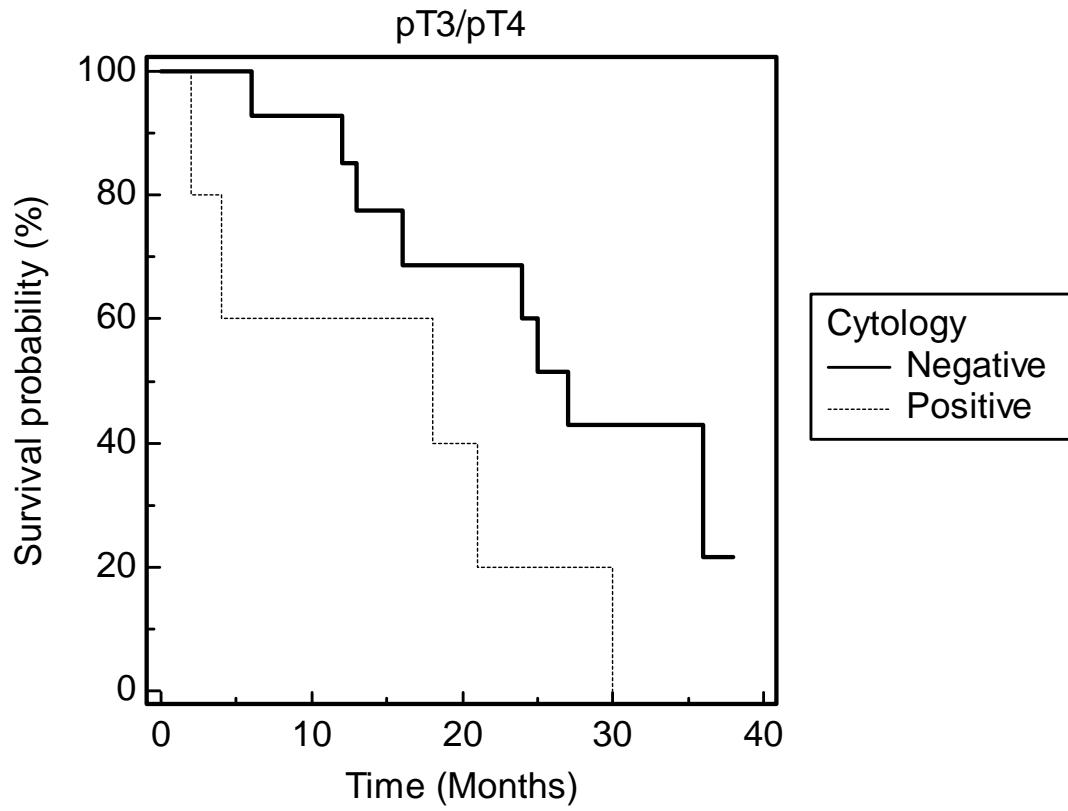


Figure 2.

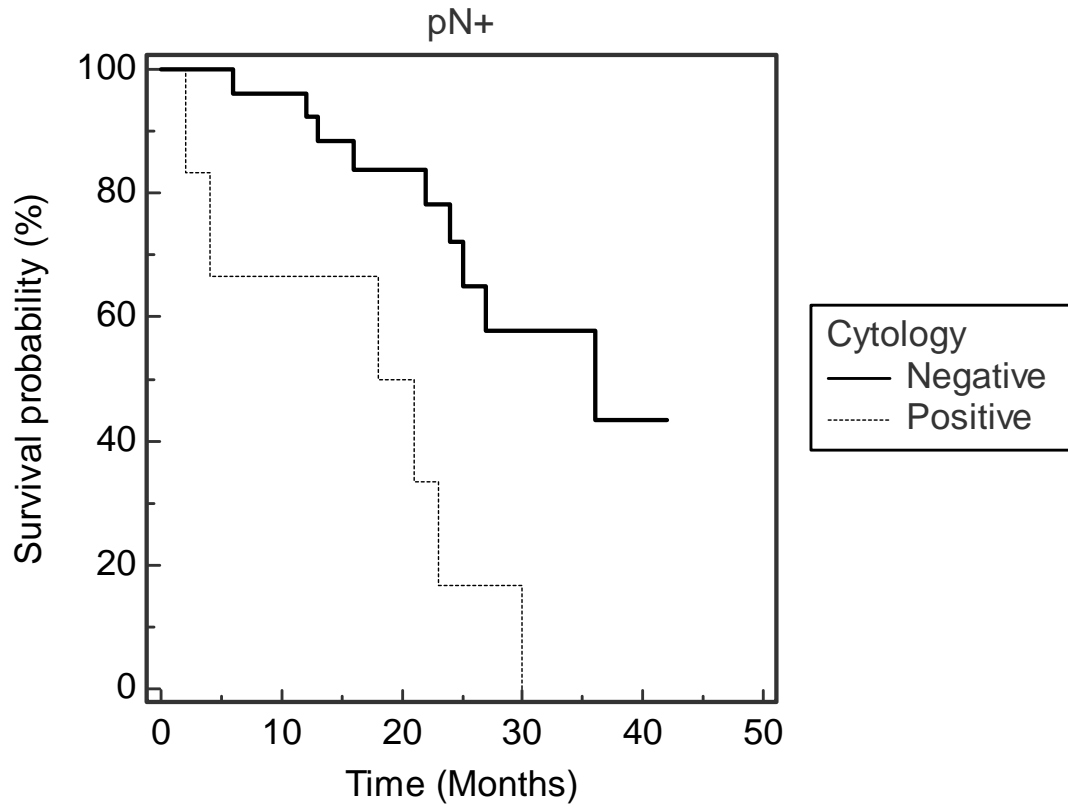


Figure 3.