Improving diagnostic accuracy of identifying gastric cancer patients with peritoneal metastases: tumor-guided cell-free DNA analysis of peritoneal fluid

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BRIEF COMMUNICATION

Improving diagnostic accuracy of identifying gastric cancer patients with peritoneal metastases: tumor-guided cell-free DNA analysis of peritoneal fluid

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Detection of peritoneal dissemination (PD) in gastric cancer (GC) patients remains challenging. The feasibility of tumor-guided cell-free DNA (cfDNA) detection in prospectively collected peritoneal fluid (ascites and peritoneal lavage) was investigated and compared to conventional cytology in 28 patients. Besides conventional cytology, next generation sequencing was performed on primary tumor DNA and cell-free DNA from peritoneal fluid. Patients were retrospectively grouped into: a *positive group* (with PD) and a *negative group* (without PD). Detectable mutations were found in the primary tumor of 68% ($n = 19$). Sensitivity of PD detection by tumor-guided cfDNA analysis was 91%, compared to 64% by conventional cytology. Within the positive group ($n = 11$), tumor-guided cfDNA was detected in all patients with ascites samples (4/4, 100%) and in 86% (6/7) of the lavage samples, opposed to 4/4 (100%) patients with ascites and 43% (3/7) with lavage by conventional cytology. Within the negative group ($n = 8$), conventional cytology was negative for all samples. In two patients, tumor-guided cfDNA was detected in peritoneal lavage fluid. Interestingly, these 2 patients developed PD within 6 months, suggesting a prognostic value of tumor-guided cfDNA detection. This study showed that tumor-guided cfDNA detection in peritoneal fluids of GC patients is feasible and superior to conventional cytology in detecting PD.

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INTRODUCTION

The prognosis of patients with locally advanced gastric adenocarcinoma is poor with a five-year overall survival estimated at 45% [1]. The poor outcomes are explained by the high rate of peritoneal metastases [2]. Identification of patients who are at increased risk of developing peritoneal metastases remains challenging.

Staging laparoscopy has been found to be the most accurate technique to detect limited peritoneal dissemination (PD) and is therefore the golden standard in locally advanced gastric cancer to diagnose or exclude peritoneal metastases [3]. Cytological analysis of peritoneal fluid is advised to detect microscopic PD. Tumor-positive peritoneal cytology at staging laparoscopy is considered to be prognostically unfavorable [4]. Whereas Japanese [5] and the USA guidelines [6] classify tumor-positive peritoneal cytology as metastatic disease, the European Society of Medical Oncology guideline does not specify the stage of disease in these patients [7]. These discrepancies contribute to the worldwide debate regarding current therapeutic implications of tumor-positive cytology. The swift towards individualized treatments and alternative treatment strategies fuels this discussion. Patients with (a high risk of) peritoneal metastases could potentially benefit from intraperitoneal therapy or treatment with curative intent, if no micro- or macroscopic metastases are found at restaging after induction chemotherapy [8–11]. Tailoring treatment is therefore heavily dependent on the accuracy of detecting occult peritoneal disease. Conventional cytology however, is known for its low sensitivity [12]. There is a need for a more accurate method in detecting PD to improve patient selection in gastric cancer treatment.

A relatively new technique to detect microscopic dissemination of tumor cells is cell-free tumor DNA analysis [13]. Within gastric adenocarcinomas, cell-free tumor DNA is proven detectable in plasma and has been shown predictive for disease recurrence and prognosis [14]. However, plasma-derived cell-free tumor DNA was undetectable in gastric cancer patients with peritoneal metastases [14]. However, cell-free tumor DNA was successfully extracted from peritoneal fluid of KRAS or BRAF mutated colorectal cancer patients with peritoneal metastases, achieving a sensitivity of 100% [15]. Whether PD in patients with gastric adenocarcinoma can be

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diagnosed by cell-free tumor DNA analysis in peritoneal fluid is currently unknown. Therefore, we designed a proof-of-principle study to investigate the feasibility of tumor-guided cell-free DNA detection from *peritoneal fluid* in patients with gastric cancer. Its diagnostic accuracy is determined and compared to conventional peritoneal cytology.

RESULTS

Clinical and tumor characteristics

In total, 28 patients were included. The peritoneal fluid samples were derived during staging laparoscopy ($n = 26$) or by ultrasound guided paracentesis ($n = 2$) (Fig. 1). Median age of the patients at diagnosis was 62.5 (interquartile range [IQR] 54.5–74.8) years and 71% of the patients was male. All study patients had an adenocarcinoma of either the stomach ($n = 21$, 75%) or the gastroesophageal junction ($n = 7$, 25%) (Supplementary Table 2). Median follow up was 11 (IQR 5–14) months.

Patient selection

Detectable genomic alterations were found in the tumor of 19 of the 28 patients (68%). Clinical and pathological differences between the patients with and without detectable genomic alterations are displayed in Supplementary Table 2. In the 19 patients with detectable genomic alterations, cell-free tumor DNA analysis was performed on peritoneal fluid samples. In seven patients (with patient numbers 5, 8, 14, 15, 16, 17 and 19) multiregional samples were collected and analyzed, resulting in a total of 27 samples which were available for conventional cytological analysis and cell-free tumor DNA analysis (Fig. 1).

Detection of peritoneal dissemination by conventional cytology and cell-free DNA

The *positive group* consisted of 11 patients, who had PD at the moment of sampling. PD diagnosis was based on histological evidence of peritoneal metastases in 9 patients and malignant ascites in 2 patients. A total of 17 samples was collected including 4 samples of ascites and 13 peritoneal lavage samples. Conventional cytology tested tumor-positive in all *ascites* samples (4/4, 100%) and in 3 out of 13 (23%) *lavage* samples, accounting for 7 out of the 11 (64%) patients with PD. Tumor specific alterations of DNA were detected in all *ascites* samples (4/4, 100%) and in 8 of 13 (62%) *lavage* samples, corresponding with 10 of 11 (91%) patients with PD (Figs. 2 and 3).

The *negative group* consisted of 8 patients with a total of 12 peritoneal *lavage* samples, who had no PD during sampling. Median follow up of these patients was 11 months (IQR 5–14). At the time of sampling, conventional cytology reported all samples tumor-negative (12/12). Despite no evidence of peritoneal metastases during sampling, cell-free tumor DNA was detected in 2 out of 12 peritoneal *lavage* samples (Figs. 2 and 3). These samples were derived from two patients (patient 7 and 8), who developed histologically proven peritoneal metastases within 6 months after sampling. The remaining 6 patients in the negative group did not develop peritoneal metastases in the follow up period. No cell-free tumor DNA was detected in the peritoneal *lavage* sample of patient number 1, however the coverage was limited to 30 reads.

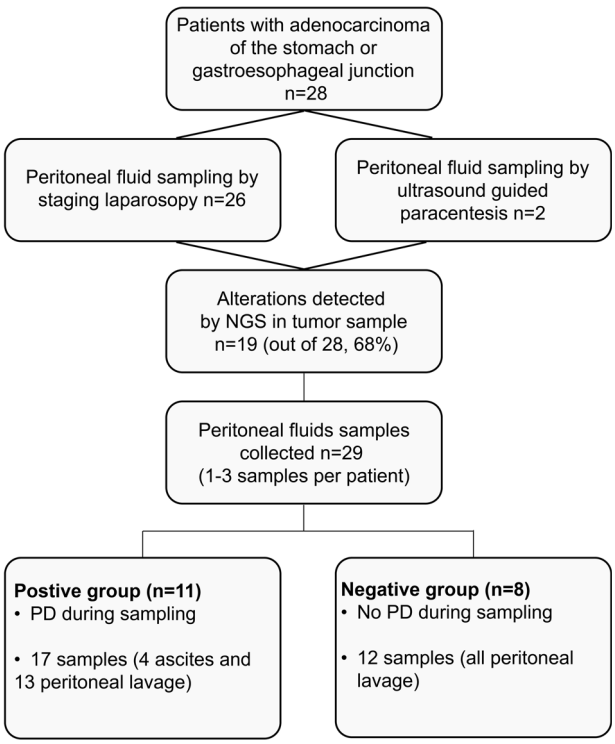

Fig. 1 Flowchart of patient selection and grouping of patients and samples. NGS next generation sequencing, PD peritoneal dissemination.

Diagnostic accuracy

The diagnostic accuracy for detecting PD was calculated with the presence of PD during sampling as the golden standard. The two patients who were diagnosed with peritoneal metastases within 6 months after sampling were excluded from this analysis. Considering diagnostic accuracy at the sample level is determined per sample ($n = 25$), conventional cytology reached a sensitivity of 41% (7/17) and a specificity of 100% (8/8), whilst tumor-guided cell-free tumor DNA analysis achieved a sensitivity of 71% (12/17) and a specificity of 100% (8/8). For detection of PD per patient, sensitivity was 64% (7/11) following conventional cytology and 91% (10/11) following tumor-guided cell-free tumor DNA analysis (Fig. 4).

Genomic alterations

In the gastric tumor, point mutations were detected in 10 genes, of which TP53 was most frequently altered (in 17 patients, 89%). Additionally, mutations in PIK3CA, CDH1, CDKN2A, ALK, FBXW7, PTN11, MET and JAK3 genes were observed. Amplification of ERBB2 was present in 3 patients (16%). In addition, amplifications in KRAS, FGFR2, KIT, EGFR, PDGFRA and a deletion in TP53 were detected. All genomic alterations are displayed in Fig. 2 and Supplementary Table 3.

Cell-free DNA detection in peritoneal fluid samples. In the peritoneal fluid samples, a median cell-free DNA input of 1.2 ng (IQR 0.6–5.4) was used for NGS. The median input of cell-free DNA was significantly different between ascites and lavage samples, 29.5 ng (IQR 17.3–51.5) versus 1.0 ng (IQR 0.5–2.3), p-value = 0.026. Also, a significant difference in median input of cell-free DNA was found between the samples in the positive group (1.2 ng; IQR 0.7–5.4) and the negative group (0.3 ng; IQR 0.1–1.1), p-value = 0.008. For the samples in which cell-free tumor DNA was detected (n = 14), a median variant allele frequency (VAF) of 6.6 (IQR 2.0–14.3) was found (Supplementary Table 3). *Peritoneal lavage* samples ($n = 10$) had significantly lower VAFs than samples of *ascites* ($n = 4$), 2% (IQR 1.8–6.7) versus 13.5% (IQR 7.5–53.5) (p-value = 0.021), respectively. When grouped according to the extent of peritoneal disease, defined by the peritoneal cancer index (PCI), no significant difference in VAFs was found between the groups (p-value = 0.067). Although a trend towards higher VAFs with more peritoneal disease was observed (Supplementary Fig. 1). In total, 21 of the 36 (58%) detectable alterations were retrieved by cell-free tumor DNA analysis of the peritoneal fluids (Fig. 2). Additionally, in patient number 9, an extra KRAS

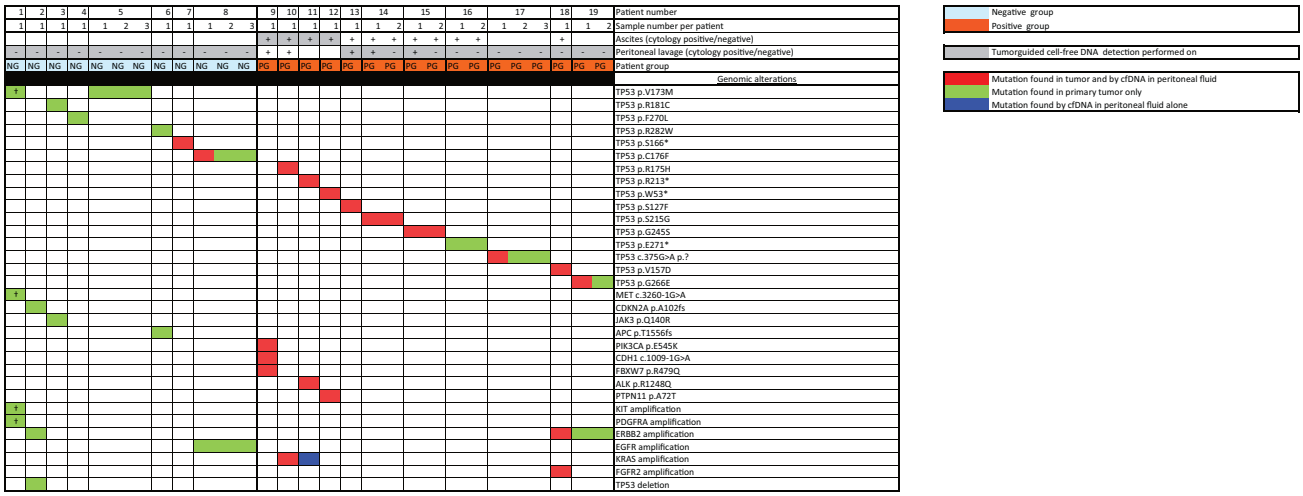

Fig. 2 Mutational landscape found in 29 peritoneal fluid samples of 19 patients with gastric cancer. NG negative group, PG positive group, *stop codon, †coverage in cell-free DNA was limited to 30 reads.

amplification was found by cell-free DNA detection only (not found in the primary tumor).

DISCUSSION

This is the first study to demonstrate the feasibility of tumor-guided cell-free DNA detection in peritoneal fluids (ascites and peritoneal lavage) of patients with gastric adenocarcinoma. Moreover, this method appeared more accurate than conventional cytology by identifying significantly more gastric cancer patients with peritoneal metastases. It is of note that it might even be possible to detect patients with occult peritoneal metastases that are too small to be detected using standard diagnostic tests.

Previous studies on cell-free tumor DNA detection in patients with gastric cancer have focused on plasma for disease monitoring and early detection [14]. To our knowledge, this is the first study that uses an amplicon-based NGS method for tissue. Instead of a dedicated and complex liquid biopsy based NGS method to detect cell-free tumor DNA in peritoneal fluids of patients with gastric cancer in a clinical diagnostic setting. The results are promising, as the diagnostic accuracy of tumor-guided cell-free DNA detection in peritoneal fluids by NGS in our small study had a sensitivity of 91% and a specificity 100%.

Approximately 20% of the gastric cancers are the genomic stable subtype, characterized by a lower mutational burden [16]. Therefore, single gene assays are unsuitable in the diagnostic trajectory of gastric cancer patients [17], unlike KRAS or/and BRAF digital droplet polymerase chain reaction used in peritoneal fluids of colon cancer patients [15]. Especially, diffuse type gastric cancer, which is more likely to metastasize to the abdominal cavity, is enriched (73%) in the genomic stable subtype [16]. Our robust and clinically validated multi-gene NGS panel detected mutations in the primary tumor of 19/28 (68%) study patients. Thus, about a third of gastric tumors had no or non-detectable mutations using our panel. Utilizing a panel designed for gastric cancer specific genetic alterations could partially enhance detection. Novel methodologies, like cell-free detection of DNA fragmentation profiles, may be valuable for patients without detectable mutations [18]. Genome wide cell-free DNA fragments have been detected in plasma in cancer patients. [18]. However, their feasibility in peritoneal fluids remains unproven.

One of the complexities with the presented technique is the relatively low amount of cell-free DNA in peritoneal lavage fluid leading to a lower coverage after sequencing. Additionally, the relatively low tumor fraction in peritoneal lavage leading to low allele frequencies (0.5% as lowest in this study). However, the combination of high-quality DNA in fresh material and the tumor-guided mutation detection allows for the presence of tumor DNA, although allelic dropout cannot be ruled out. Therefore, we cannot conclusively determine the absence of cell-free tumor DNA.

The clinical consequences of tumor-positive peritoneal cytology without macroscopic peritoneal metastases in patients with gastric cancer is debated. There are differing opinions whether tumor-positive cytology should be considered metastatic disease. Jamel et al. [4] reported on the negative prognostic impact of tumor-positive peritoneal cytology with a hazard ratio of 3.46 for overall survival compared to tumor-negative peritoneal cytology. The low sensitivity of conventional cytology (64%) observed in the current study is similar to previous findings [12], potentially underestimating the negative impact reported by Jamel et al.

For patients with peritoneal dissemination, systemic chemotherapy has long been the only therapeutic option, despite the limited effect due to the peritoneal plasma barrier [19]. Patient-tailored therapies have been proposed for patients with solitary tumor-positive peritoneal cytology. Firstly, intraperitoneal application of chemotherapy may target the peritoneal dissemination locally. Ongoing hyperthermic intraperitoneal chemotherapy (HIPEC) trails as the (prophylactic) GASTRICHIP [8] and (therapeutic) PERISCOPE II [9] investigate the role of HIPEC in these patients. The results are eagerly awaited. Cell-free tumor DNA analysis in peritoneal fluid may aid in more accurately identifying patients who could benefit from such extensive treatment strategy, and monitor the therapeutic efficacy [20]. Alternatively, conversion surgery is proposed for patients with only tumor-positive peritoneal cytology. Then, surgery with curative intent is performed for patients who converted to tumor-negative cytology at restaging after induction chemotherapy [10, 11]. Conversion surgery is a strategy which pre-selects chemo responsive tumors, although it relies heavily on the sensitivity of cytology techniques. The detection of tumor-guided cell-free DNA may to optimizing patient selection.

A large prospective study will be initiated to validate the accuracy of the test. Also, cut-off values of VAFs will be investigated with the potential to guide therapeutic decision making. And, the correlation between VAF and the extent of peritoneal disease will be subject of this larger study. Interestingly, in the current study, 3 patients had both negative and positive peritoneal samples, although they were sampled at the same point in time. In the larger upcoming study, we aim to investigate whether samples from a specific location (left subphrenic), right subphrenic Douglas pouch are more of the

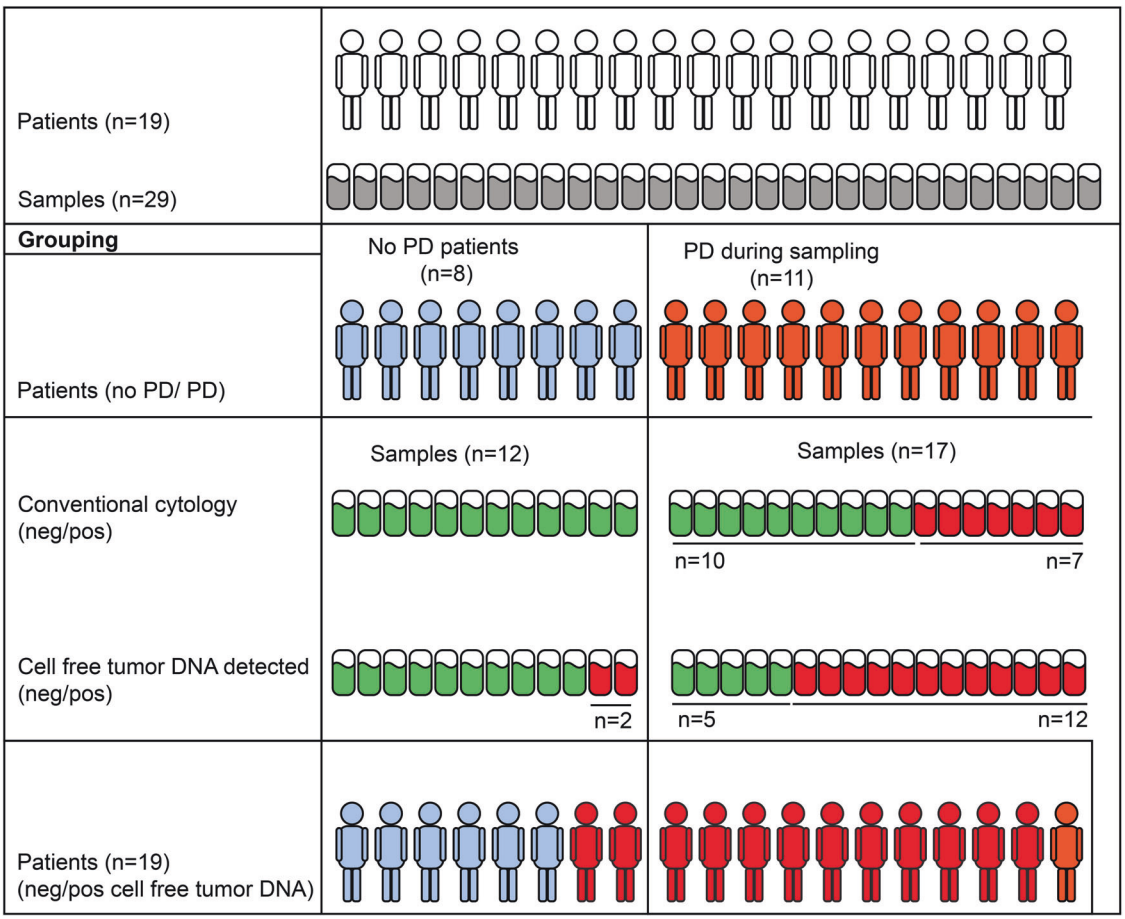

Fig. 3 Overview of study patients and samples. Blue no peritoneal dissemination (PD), Orange PD positive, Green tumor-negative, Red tumor-positive.

positive and if samples from different locations can be analyzed together.

In conclusion, this study has shown the feasibility of tumor-guided detection of cell-free DNA in peritoneal fluids of gastric cancer patients. Additionally, the diagnostic accuracy in detecting occult peritoneal dissemination by tumor-guided cell-free DNA was superior compared to conventional cytology. This technique could potentially detect occult peritoneal metastases, which might contribute to better patient selection for intensive treatment strategies.

METHODS

Patient and sample selection

Samples and data were prospectively collected between June 2021 and April 2023 and retrospectively analyzed. Patients with histologically proven adenocarcinoma of the stomach or gastroesophageal junction undergoing staging laparoscopy or ultrasound guided paracentesis of ascites were eligible. Patients were included if peritoneal fluids were available for analysis of cell-free tumor DNA. Clinical and pathological data were retrieved from the electronic patient files. The study was approved by the Institutional Review Board of the Netherlands Cancer Institute *IRBd21-146* and *IRB22-244*.

Tumor samples were derived from the primary tumor during gastroscopy or from macroscopic peritoneal metastases during staging laparoscopy. Peritoneal fluids were collected during staging laparoscopy or by ultrasound guided paracentesis. Staging laparoscopy was done according to the following local protocol: after inspection of the abdominal cavity the presence of macroscopic peritoneal disease was staged. Ascites, if present, was sampled. In case of low volume (< 20 cc) or in the absence of ascites, peritoneal lavage was performed by rinsing the left and right upper quadrant and Douglas pouch with 50 cc of saline 0.9% and collected afterwards.

Sample processing

For peritoneal fluids, routine cytological assessment consisted of centrifugation (10 min/3000 rpm) upon arrival at the pathology lab. The cell sediment was processed for cytospins (5 min/1250 rpm) and stained with Giemsa. For next-generation sequencing (NGS), supernatant was collected after centrifugation of the peritoneal fluid (10 min/3000 rpm) and biobanked at −80 °C. Cell-free DNA was isolated from the supernatant using the QIAamp® MinElute®ccfDNA kit (Qiagen, Hilden, Germany). For NGS, DNA was isolated from formalin-fixed paraffin-embedded (FFPE) tumor tissue sections using QIAamp® DNA FFPE Tissue kit (Qiagen, Hilden, Germany).

Identification of genetic alterations

Genetic alterations were identified by standard of care molecular diagnostic assays at the Department of Pathology of our institute. This entailed isolated (cell-free) DNA prepping for NGS Ampliseq, with a maximum of 10 ng, according to manufacturer's instructions (Illumina Inc, San Diego, United States of America). A custom-made 52-gene amplicon-based cancer hotspot panel was used for identification of genetic alterations. The panel covered hotspot regions using 249 amplicons and including 98% of TP53 coding sequence, single nucleotide variations, small indels and copy number variations (Illumina) (Supplementary Table 1). A minimum of 100 reads was aimed to guarantee accuracy. Within the peritoneal fluids, a minimum VAF threshold of 0.5% was used to identify tumor-guided mutations.

Statistical analysis

Baseline characteristics are presented as medians with IQR for non-normally distributed variables or as percentages for categorical variables. Mann-Whitney *U* test was used to compare non-normally distributed variables between two groups, the chi square test to compare categorical

		Peritoneal metastases during sampling: positive	Peritoneal metastases during sampling: negative		
Conventional cytology	positive	7	0	7	PPV: 100%
	negative	4	6	10	NPV: 60%
		11	6	17	
		Sensitivity: 64%	Specificity: 100%		

		Peritoneal metastases during sampling: positive	Peritoneal metastases during sampling: negative		
Cell-free tumour DNA analysis	positive	10	0	10	PPV: 100%
	negative	1	6	7	NPV: 86%
		11	6	17	
		Sensitivity: 91%	Specificity: 100%		

Fig. 4 Diagnostic accuracy of conventional cytology versus detection of cell-free tumor DNA analysis calculated per patient in a study group of 17 patients with PD ($n = 11$) and without PD ($n = 6$) at the time of sampling. PPV positive predictive value, NPV negative predictive value.

variables between two groups and the Kruskal-Wallis test for three groups when dealing with non-normally distributed variables. Median follow up was calculated using Kaplan Meier. The diagnostic performance of conventional cytology and tumor-guided cell-free DNA analysis were evaluated in relation to the presence of PD. Diagnostic accuracy of both methods was quantified by calculating sensitivity and specificity, using the presence of PD during sampling as the golden standard. For this analysis, only the positive and negative group were used. Data were analyzed using SPSS version 29.0.0.0

DATA AVAILABILITY

The data generated in this study are available upon request from the corresponding author.

REFERENCES

1. Al-Batran SE, Homann N, Pauligk C, Goetze TO, Meiler J, Kasper S, et al. Perioperative chemotherapy with fluorouracil plus leucovorin, oxaliplatin, and docetaxel versus fluorouracil or capecitabine plus cisplatin and epirubicin for locally advanced, resectable gastric or gastro-oesophageal junction adenocarcinoma (FLOT4): a randomised, phase 2/3 trial. Lancet. 2019;393:1948–57.
2. Feingold PL, Kwong ML, Davis JL, Rudloff U. Adjuvant intraperitoneal chemotherapy for the treatment of gastric cancer at risk for peritoneal carcinomatosis: a systematic review. J Surg Oncol. 2017;115:192–201.
3. Borgstein ABJ, van Berge Henegouwen MI, Lameris W, Eshuis WJ, Gisbertz SS, Dutch Upper GICA. Staging laparoscopy in gastric cancer surgery. A population-based cohort study in patients undergoing gastrectomy with curative intent. Eur J Surg Oncol. 2021;47:1441–8.
4. Jamel S, Markar SR, Malietzis G, Acharya A, Athanasiou T, Hanna GB. Prognostic significance of peritoneal lavage cytology in staging gastric cancer: systematic review and meta-analysis. Gastric Cancer. 2018;21:10–8.
5. Japanese Gastric Cancer A. Japanese Gastric Cancer Treatment Guidelines 2021 (6th edition). Gastric Cancer. 2023;26:1–25.
6. American Joint Committee on Cancer. AJCC Cancer Staging Manual 8th Edition 2017.
7. Lordick F, Carneiro F, Cascinu S, Fleitas T, Haustermans K, Piessen G, et al. Gastric cancer: ESMO clinical practice guideline for diagnosis, treatment and follow-up. Ann Oncol. 2022;33:1005–20.
8. Glehen O, Passot G, Villeneuve L, Vaudoyer D, Bin-Dorel S, Boschetti G, et al. GASTRICHIP: D2 resection and hyperthermic intraperitoneal chemotherapy in locally advanced gastric carcinoma: a randomized and multicenter phase III study. BMC Cancer. 2014;14:183.
9. Koemans WJ, van der Kaaij RT, Boot H, Buffart T, Veenhof A, Hartemink KJ, et al. Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy versus palliative systemic chemotherapy in stomach cancer patients with peritoneal dissemination, the study protocol of a multicentre randomised controlled trial (PERISCOPE II). BMC Cancer. 2019;19:420.
10. Valletti M, Eshmuminov D, Gnecco N, Gutschow CA, Schneider PM, Lehmann K. Gastric cancer with positive peritoneal cytology: survival benefit after induction chemotherapy and conversion to negative peritoneal cytology. World J Surg Oncol. 2021;19:245.
11. Yoshida K, Yasufuku I, Terashima M, Young Rha S, Moon Bae J, Li G, et al. International retrospective cohort study of conversion therapy for stage IV gastric cancer 1 (CONVO-GC-1). Ann Gastroenterol Surg. 2022;6:227–40.
12. Mezhir JJ, Posner MC, Roggin KK. Prospective clinical trial of diagnostic peritoneal lavage to detect positive peritoneal cytology in patients with gastric cancer. J Surg Oncol. 2013;107:794–8.
13. Corcoran RB, Chabner BA. Application of cell-free DNA analysis to cancer treatment. N Engl J Med. 2018;379:1754–65.
14. Maron SB, Chase LM, Lomnicki S, Kochanny S, Moore KL, Joshi SS, et al. Circulating tumor DNA sequencing analysis of gastroesophageal adenocarcinoma. Clin Cancer Res. 2019;25:7098–112.
15. Van't Erve I, Rovers KP, Constantinides A, Bolhuis K, Wassenaar EC, Lurvink RJ, et al. Detection of tumor-derived cell-free DNA from colorectal cancer peritoneal metastases in plasma and peritoneal fluid. J Pathol Clin Res. 2021;7:203–8.
16. Cancer Genome Atlas Research N. Comprehensive molecular characterization of gastric adenocarcinoma. Nature. 2014;513:202–9.
17. Zhao D, Yue P, Wang T, Wang P, Song Q, Wang J, et al. Personalized analysis of minimal residual cancer cells in peritoneal lavage fluid predicts peritoneal dissemination of gastric cancer. J Hematol Oncol. 2021;14:164.
18. Cristiano S, Leal A, Phallen J, Fiksel J, Adleff V, Bruhm DC, et al. Genome-wide cell-free DNA fragmentation in patients with cancer. Nature. 2019;570:385–9.
19. Jacquet P, Sugarbaker PH. Peritoneal-plasma barrier. Cancer Treat Res. 1996;82:53–63.
20. Pu X, Li Z, Wang X, Jiang H. Ascites and serial plasma circulating tumor DNA for predicting the effectiveness of hyperthermic intraperitoneal chemotherapy in patients with peritoneal carcinomatosis. Front Oncol. 2022;12:791418.

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AUTHOR CONTRIBUTIONS

Conceptualization: KvdS, JWvS, MCB, LLK; Methodology: KvdS, JWvS, MCB, LLK; Formal Analysis: KvdS, EV, MCB, LLK; Resources: LLK; Data Curation: KvdS, JWvS, NH, KJH, AAFAV, EV, MCB, LLK; Writing-original draft: KvdS, JWvS, MV, MCB, LK; Writing-Review, editing: KvdS, JWvS, MAV, JMvD, NH, KJH, AAFAV, EV, JvdB, PS, MN, TvW, MCB, LLK; Visualization; KvdS; Supervision: JWvS, MAV, MCB, LLK; Funding: KvdS, JWvS, LLK.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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