ANTICANCER RESEARCH *38*: 1255-1262 (2018)
doi:10.21873/anticanres.12347

Review

Gastric Cancer Cells in Peritoneal Lavage Fluid: A Systematic Review Comparing Cytological with Molecular Detection for Diagnosis of Peritoneal Metastases and Prediction of Peritoneal Recurrences

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Abstract. *Background/Aim: Detecting free tumor cells in the peritoneal lavage fluid of gastric cancer patients permits to assess a more accurate prognosis, predict peritoneal recurrence and select cases for a more aggressive treatment. Currently, cytology and molecular biology comprise the two most popular methods of detection that are under constant study by researchers. Materials and Methods: We burrowed into the available literature comparing cytological with molecular detection of free intraperitoneal gastric cancer cells. PubMed, Science Direct, Scopus and Google Scholar were the search engines investigated. Results: As of 2017, 51 dedicated studies have been published. Messenger RNA of carcinoembryonic antigen was the genetic target most frequently described. The genetic technique is usually superior to cytology in sensitivity (38-100% vs. 12.3-67% respectively), whereas cytological examination tends to show a slight pre-eminence in specificity (approximately 100%). Conclusion: So far, given the imperfection of each method, employment of both cytology and molecular examination seem to be mandatory.*

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Key Words: Gastric cancer, peritoneal lavage, peritoneal carcinomatosis, peritoneal recurrence, cytopathology, molecular detection, RT-PCR, review.

Currently, despite the amelioration and standardization of surgery techniques and multi-modal therapy, the prognosis of gastric cancer (GC), especially that of advanced GC (AGC) with serosal invasion (T3 or T4 cancers), remains very poor with a 5-year overall survival (OS) of less than 35% (1). Peritoneal dissemination is the most common route of metastasis followed by AGC and leads to peritoneal recurrence (PR) which is the most frequent cause of death (up to 60% of cases within 2 years) even if curative resection is performed (2). Hence, in the case of AGC, detecting intraperitoneal free cancer cells is of paramount importance because it is significantly related to the prediction of peritoneal metastasis (PM) and patients' prognosis (1, 2). Accordingly, since 1998 the Japanese Gastric Cancer Association (JGCA) recommends to perform peritoneal lavage cytology (PLC) to detect free floating malignant cells within the peritoneal cavity; in addition, in 2010, a positive PLC was classified as metastatic disease also in the 7th edition of the American Joint Committee on Cancer (AJCC) tumor node metastasis (TNM) staging system for GC (3). However, despite its high specificity, conventional PLC shows a questionable sensitivity (11.1 to 80%): in fact, cytology-negative cases often develop PR and meet with worse prognosis (1, 2). In an effort to enhance sensitivity, in the last decades researches have focused on the detection of several epithelial cell-related targets using molecular biology methods. Herein, we offer a meticulous review of the knowledge and progress so far achieved in terms of diagnostic and prognostic results through cytological and genetic examinations of peritoneal lavage (PL) in patients affected with AGC.

Materials and Methods

We systematically reviewed the world literature dealing with the detection of free intraperitoneal cancer cells and comparing cytopathological with molecular examination of peritoneal lavage fluid obtained from patients with GC and AGC. The investigation was carried out through four popular search engines (PubMed, Science Direct, Scopus and Google Scholar). GC, AGC, PC, PM, PLC, genetic detection, molecular diagnosis and Real time reverse chained transcriptase-polymerase chain reaction (RT-PCR) were the key words utilized for searching. Only works comparing the two aforementioned diagnostic techniques (both conventional PLC and molecular analysis) were included in the review.

Results

As of 2017, we found 51 studies dealing with PLC and molecular biological detection of free malignant cells in the peritoneum of GC patients (2, 4-54). Table I summarizes the principle features of the studies included in the review.

PLC. PL was collected by introducing, stirring and aspirating from the abdominal cavity an aliquot of saline solution ranging from 50 ml (9, 27) to 200 ml (24, 41). All the patients did not receive any neoadjuvant treatment and PLs were performed at the beginning of laparotomic gastrectomy (most works), laparoscopy surgery (conducted with curative or staging purpose) (35-40, 43), or paracentesis (only one case, 48). Conventional Papanicolaou staining was adopted in the vast majority of cases, followed by ordinary hematoxylin and eosin (H&E) coloration (9, 11) and Giemsa stain (17, 28). Immunocytochemistry (ICC) was described only occasionally (10, 31, 40, 53). Sensitivity (12.3% to 67%) and specificity (94% to 100%) of PLC were clearly expressed as a percentage ratio only in 12 and 8 studies respectively (Table I).

Molecular detection of intraperitoneal free cancer cells. As for the molecular method, the mature messenger ribonucleic acid (messenger RNA, mRNA) of carcinoembryonic antigen (CEA) has been the target most commonly studied (41 articles) (2, 4-43) followed by 11 studies dealing with cytokeratin 20 (CK-20) mRNA (18, 25, 29-32, 36-39, 42, 49), 10 of which examined CEA mRNA concomitantly (18, 25, 29-32, 36-39, 49). Besides these, 23 additional mRNAs of other molecules have been occasionally investigated (11, 20-22, 28, 31, 36, 39, 44-54). Concerning the type of molecular biological technique, qualitative RT-PCR has been the one most frequently adopted (27 studies), followed by quantitative RT-PCR (Q-RT-PCR) (24 studies). Recently, other ultra-rapid non-PCR tests, such as transcription-reverse transcription concerted reaction (TCR), and PCR-tests, such as reverse transcription loop-mediated isothermal amplification (RT-LAMP), have been successfully employed for genetic analysis (33, 34, 43, 54). Sensitivity and specificity of molecular examination were clearly expressed as a percentage ratio in 23 and 16 studies respectively (Table I). Sensitivity and specificity of CEA mRNA were respectively 38-100% and 7.3-100%, whereas for CK-20 mRNA they were 25-64% and 80.3 to 94% (Table I).

Discussion

Major routes of metastatic spread in GC are direct infiltration of contiguous structures, hematogenous metastasis to the liver, regional lymph node metastasis, intraperitoneal dissemination, mesogastric pathway and intragastric exfoliation (29, 55). Of these, peritoneal dissemination is reported to be the most frequent pattern of metastasis and recurrence (32-54%) in AGC (42). PM from GC results from a 2 step-process: the former is the exfoliation of free cancer cells from the serosal surface of the primary tumor into the peritoneal cavity, the latter is the attachment of cancer cells to a preferable intraperitoneal site (such as omentum, mesenterium and Douglas pouch) with subsequent growth and invasion of the abdominal cavity (2, 29, 42). Furthermore, PM is also recognized as the most important independent prognostic factor for GC PR (29, 30). In fact, due to the development of PM even after R0 tumor resections, prognosis of AGC patients remain poor: to date, except for some individual experiences, no systemic or intraperitoneal treatment proved to effect a complete cure of AGC related-PM (56, 57). For this motive, the cytological examination of peritoneal lavage fluid has been adopted in clinical practice since 1999 by JGCA to detect free tumor cells floating in the abdominal cavity and predict PR (3, 24, 41). However, despite an excellent specificity approximately of 100%, conventional PLC through Papanicolaou or other classical stainings lack sensitivity (11.1 to 80%) since PR is often observed in PLC-negative patients as well as in non-AGC cancers (that is not invading the serosal layer) (30, Table I). Such a disappointing constraint has been in part referred to the technical personal skills of the cytologist but, mostly, it has been reported that the manipulation of the tumor as well as surgical maneuvers (especially when the surgeons open the gastric wall or the lymphovascular vessels) can cause tumor spillage from gastric lumen to peritoneal cavity ensuing PM-PR (2, 58-64); in this sense, of interest, gastric lavage as well as PL might be helpful preventive methods to minimize the risk of spillage of GC cells and PR (2). To increase the sensitivity and reliability of cancer cell detection in PL, in the last two decades researchers employed ICC and molecular biological methods to detect epithelial cell-related targets (40). Compared to standard cytology, ICC with antibodies has been described improving the detection rate by 5% to 15%; nevertheless,

Table I. *Main features of the 51 studies dealing with cytological and molecular detection of free intraperitoneal tumor cells in gastric cancer patients*.

Year	Reference	Molecular marker	Molecular technique	Number of Gc patients	Main clinical observation	Sensitivity	Specificity
1997	4	CEA mRNA	RT-PCR	48	First study on RT-PCR for predicting GC PR	RT-PCR>PLC	PLC: 100% RT-PCR: 100%
1998	5	CEA mRNA	RT-PCR	148	Correlation of PLC+ and RT-PCR+ with OS ($p<0.002$)	n.a.	n.a.
1999	6	CEA mRNA	RT-PCR, Q-RT-PCR	199	Correlation of RT-PCR+ with poor OS ($p<0.001$)	n.a.	n.a.
2000	7	CEA mRNA	RT-PCR, Q-RT-PCR	109	Correlation between Q-RT-PCR+ and pT ($p<0.01$)	n.a.	n.a.
2000	8	CEA mRNA	RT-PCR	43	Correlation of RT-PCR+ with pT	PLC=33% RT-PCR>38%	n.a.
2001	9	CEA mRNA	RT-PCR	30	First study on omental milky spots as PR	n.a.	RT-PCR: open issue
2001	10	CEA mRNA	RT-PCR	17	Utility of both PLC and RT-PCR	PLC: 23% RT-PCR: 63%	n.a.
2001	11	CEA mRNA, telomerase	RT-PCR, telomerase assay	n.f.	Occurrence of PR in PLC- patients	n.f.	n.f.
2001	12	CEA mRNA, CK-19 mRNA,	RT-PCR	7	CEA>CK-19 as useful marker	n.a.	n.a.
2001	13	CEA mRNA	RT-PCR	230	PLC detects intraperitoneal cells and predicts PR more sensitively than RT-PCR	PLC: 46% RT-PCR: 31% Combined: 57%	PLC: 94% RT-PCR: 95%
2002	14	CEA mRNA	Q-RT-PCR	90	RT-PCR predicts PR more sensitively than PLC	PLC: 31% RT-PCR: 77%	PLC: 100% RT-PCR: 94%
2002	15	CEA mRNA	RT-PCR	86	RT-PCR as preferable method to detect cells/PR	PLC<RT-PCR	PLC<RT-PCR
2003	16	CEA mRNA	RT-PCR Q-RT-PCR	65	PLC combined with Q-RT-PLC as the most sensitive diagnostic test ($p<0.05$)	PLC: 51% RT-PCR: 49% QRT-PCR: 42% Combined: 70%	n.a.
2003	17	CEA mRNA	RT-PCR	136	Advisable adjuvant therapy for RT-PCR+ patients	n.a.	n.a.
2003	18	CEA mRNA, CK-20 mRNA	Q-RT-PCR	129	Multiplex Q-RT-PCR as more reliable than PLC	PLC<Q-RT-PCR	PLC<Q-RT-PCR
2004	19	CEA mRNA	Q-RT-PCR	195	CEA mRNA as independent risk factor for PR ($p=0.027$)	n.a.	n.a.
2004	20	CEA mRNA L3-PP mRNA	Q-RT-PCR	93	Clinical utility of L3-PP mRNA	PLC: n.f. L3-PP: 61% L3PP+CEA>L3PP	n.f.
2004	21	CEA mRNA, L3-PP mRNA	Q-RT-PCR	88	QRT-PCR combining L3-PP with CEA as the most sensitive test for detecting free intraperitoneal cells	PLC: 57% L3-PP: 57% CEA: 71% CEA+L3P: 85.7%	PLC: n.a. L3-PP: 93% CEA: 96% combined: n.a.
2004	22	CEA mRNA, DDC mRNA	Q-RT-PCR	114	Combination of CEA with DDC improves the accuracy of detecting free cancer cells	CEA: 73% DDC: 87% CEA+DDC: 93%	n.f.
2005	23	CEA mRNA	RT-PCR	40	RT-PCR correlates with pT-TNM-PR ($p<0.001$) and pN ($p=0.004$)	PLC<RT-PCR RT-PCR: 82%	n.a.
2005	24	CEA mRNA	Q-RT-PCR	80	CEA mRNA as independent prognostic factor ($p=0.0130$). No PLC analysis.	RT-PCR: 84.6%	RT-PCR: 87.7%
2005	25	CEA mRNA, CK-20 mRNA	Q-RT-PCR	230	High rates of false-positives with CK-20; high specificity but limited sensitivity of RT-PCR CK-20	PLC: n.a. CEA: n.a. CK-20: 64% CK20+CEA:73%	PLC: 95% CEA: 90% CK-20: 91% CK20+CEA:86%
2006	26	CEA mRNA	RT-PCR	284	RT-PCR+ as independent risk factor for cancer death	RT-PCR: 88.5%	RT-PCR: 81.6%

Table I. *Continued*

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Year	Reference	Molecular marker	Molecular technique	Number of Gc patients	Main clinical observation	Sensitivity	Specificity
2006	27	CEA mRNA	Q-RT-PCR	65	Correlation of QRT-PCR+ with pT and stage	PLC: 12.3% QRT-PCR: 47.7%	n.a.
2006	28	CEA mRNA, IL-2 mRNA, IL-10 mRNA	Q-RT-PCR	110	CEA+ predicts poorer OS than CEA- (p=0.044)	n.a.	n.a.
2007	29	CEA mRNA, CK-20 mRNA	Q-RT-PCR	131	PLC+ and QRT-PCR+ have lower clinical significance in CRC than GC	n.a.	n.a.
2007	30	CEA mRNA, CK-20 mRNA	Q-RT-PCR	164	Combined QRT-PCR+ as independent risk factor for poor OS and DFS (p<0.001)	QRT-PCR>PLC	n.a.
2007	31	CEA, CK-20, FABP1, MUC2, TFF1, TFF2, MASPIN, GW112, PRSS4, TACSTD1	Multiplex RT-PCR	179	RT-PCR+ and ICC+ as portended poorer DFS than RT-PCR- and ICC-	RT-PCR>PLC	n.a.
2007	32	CEA mRNA, CK-20 mRNA	Q-RT-PCR	124	Worse OS for QRT-PCR+ than QRT-PCR- patients (p<0.001)	CEA: 72.7% CK-20: 54.6% combined: 81.1%	CEA: 82.7% CK-20: 80.3% combined: 79.7%
2007	33	CEA mRNA	TCR	n.f.	n.f.	n.f.	n.f.
2007	34	CEA mRNA	Q-RT-PCR, TCR	112	Correlation between TCR and QRT-PCR (p<0.0001)	PLC: 61.5% TCR: 84.6% QRT-PCR: 92.3%	PLC: 100% TCR: 100% QRT-PCR: 100%
2007	35	CEA mRNA	Q-RT-PCR	156	QRT-PCR+ patients had poorer survival (p=0.0003)	PLC: 61% QRT-PCR: 79%	n.a.
2008	36	CEA, CK-20, Survivin, MUC2	RT-PCR	34	CEA mRNA resulted to be the marker with best sensitivity and specificity. Limit: no marker mRNA quantificationby RT-PCR.	PLC: 67% CEA: 100% CK-20: 60% Survivin: 100% MUC2: 40%	PLC: 95% CEA: 91% CK-20: 94% Survivin: 74% MUC2: 100%
2011	2	CEA mRNA	Q-RT-PCR	38	Gastric and peritoneal irrigation prevents intraoperative cancer spillage	PLC<Q-RT-PCR	n.a.
2014	37	CEA mRNA, CK-20 mRNA	Q-RT-PCR	104	Worse DFS for PCR+ patients (p=0.007)	n.a.	n.a.
2014	38	CEA mRNA, CK-20 mRNA	RT-PCR	102	Ki-67 staining should be used to distinguish viable *vs*. inactive cancer cells	n.a.	n.a.
2014	39	CEA, CK-20, MMP-7, TGF-β1, CA125	Q-RT-PCR	116	PLC had the lowest sensitivity but the highest specificity (p<0.001). At multivariate analysis, CEA and MMP-7 mRNAs were found to be independent prognostic factors (p=0.028)	PLC: 42.2% CEA: 60% CK:20: 46.8% MMP-7: 53.3% CA125: 48.9% TGF-β1: 57.8% CEA+MMP7:71%	PLC: 95.8% CEA: 81.7% CK:20: 80.3% MMP-7: 90.1% CA125: 83.1% TGF-β1: 84.5% CEA+MMP7:74%
2015	40	CEA mRNA	Q-RT-PCR, OSNA	75	Concordance of 93.8% between PLC and OSNA	PLC: n.a. QRT-PCR: n.a. OSNA: 85%	PLC: n.a. QRT-PCR: n.a. OSNA: 97.7%
2016	41	CEA mRNA	RT-PCR	117	RT-PCR+ had shorter DFS (p=0.001)	PLC: n.a. CEA: 65%	PLC: n.a. CEA: 74.2%
2017	42	CEA mRNA CK-20 mRNA	RT-PCR	132	RT-PCR+ did not correlate with worse DFS (p=0.39)	PLC: n.a. CEA: n.a. CK-20: 25%	PLC: n.a. CEA: 7.3% CK-20: n.a.
2017	43	CEA mRNA	TCR	97	Association between reduced CEA mRNA after induction chemotherapy and longer survival (p<0.001)	n.a.	n.a.
1997	44	E-cadherin mRNA	RT-PCR	10	Possible clinical utility	RT-PCR>PLC	n.f.
1998	45	Trypsinogen-1 mRNA	RT-PCR	42	Possible clinical adoption	n.a.	n.a.
1999	46	E-cadherin mRNA	RT-PCR	52	Potentially valuable for diffuse GC	n.a.	low

Table I. *Continued*

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Year	Reference	Molecular marker	Molecular technique	Number of Gc patients	Main clinical observation	Sensitivity	Specificity
2000	47	telomerase	TRAP assay	12	possible clinical use	n.a.	n.a.
2001	48	MMP-7 mCRNA	RT-PCR	152	Both PLC and MMP-7 mRNA are independent predictors of PR (p<0.001)	PLC: 46% RT-PCR: 33% combined: 62%	PLC: 95% RT-PCR: 88% combined: n.a.
2004	49	CK-20, FABP1, MUC2, TFF1, TFF2	RT-PCR	99	Advocating RT-PCR *vs*. Q-RT-PCR	n.a.	multi-RT-PCR: 91-100%
2004	50	Survivin mRNA	RT-PCR	48	Promising help to future therapeutic strategy	RT-PCR+PLC: 66.7% (>PLC)	n.a.
2004	51	TGFβ, IL-2, IL-6	Q-RT-PCR	78	No significant difference in expression between GC and control patients	n.a.	n.a.
2005	52	Heparanase mRNA	RT-PCR	48	Possible marker for detecting PM	RT-PCR>PLC	n.a.
2014	53	IL-17 mRNA	Q-RT-PCR	114	Low expression as independent predictive factor for prognosis (p=0.0098)	n.a.	n.a.
2014	54	CK-19 MRNA	RT-LAMP	52	RT-LAMP+ significant for PR (p<0.005)	RT-LAMP>PLC	n.a.

Q-RT-PCR: Real-time quantitative PCR; CEA: carcinoembryonic antigen; PR: peritoneal recurrence; PLC: peritoneal lavage cytology; CK: cytokeratin; L3-PP: L-3 phosphoserine phosphatase; DDC: dopa decarboxylate; IL: interleukin; OS: overall survival; DFS: disease free survival; TCR: transcription-reverse transcription concerted reaction; MMP: matrix metalloproteinase; OSNA: one-step nucleic acid amplification; TRAP: telomeric repeat amplification protocol; RT-LAMP: reverse transcription-loop mediated isothermal amplification; CRC: colorectal cancer; ICC: immunocytochemical cytology; n.a.: not assessed; n.f.: not findable.

PM can still occur in PLC-ICC negative cases (36, 40). Molecular detection with qualitative or quantitative RT-PCR have identified potent molecular markers of various target genes such as transcripts of CEA, CK-20, MMP-7, heparanase and many other molecules (Table I). In most cases, if not all, molecular results (especially those on CEA mRNA) have proved to be superior to cytological ones in sensitivity and prognostic prediction for survival (10, 14, 27, 34-36, 39); in particular, GC patients expressing molecular positive tests, have been identified to benefit from more aggressive adjuvant treatment including intraperitoneal chemotherapy with paclitaxel (43). Concerning specificity, on the other hand, both methods have often achieved 100% (4, 34) and PLC not infrequently was superior (14, 25, 39, 48). Currently however, yet promising, the detection molecular methods are not applied for routine use everywhere: sometimes, in fact, they provided controversial results (such as false-positive cases with mRNAs released by lymphocytes and sane mesothelial cells and other false-positives results due to isolated tumor cells -that is clinically insignificant cells without metastatic potential- and not to micrometastases -the utter active metastatic cells-) and, most of all, they are time-consuming, labor-intensive and expensive (1, 32, 24, 40). With this respect, testing novel ultra-rapid molecular methods (such as OSNA, TCR and LAMP) (33, 34, 40, 43, 54) with innovative targets isolated not only from PLs but also from blood or tissue specimens of AGC patients (65, 66) could surmount some temporal and costly limitations of the current genetic techniques. Furthermore, they could provide an opportunity to perform reliable tailor-made surgery for GC as a common procedure in general hospitals (54).

Conclusion

Detecting free tumor cells in peritoneal lavage fluid of AGC patients is of paramount importance in order to predict PR, assess a more accurate prognosis and select cases for more intensive treatment. So far, given the imperfection of each method, employment of both cytology and molecular method seem to be mandatory for achieving this aim.

Conflicts of Interest

The Authors declare no conflicts of interest.

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Received December 15, 2017
Revised January 27, 2018
Accepted January 29, 2018