for advanced disease can palliate the disease and even prolong life[3,4]. Therefore, the accurate and appropriate diagnosis of the disease entity is required so that an individualized oncological approach can be used. The tumor-node-metastasis (TNM) staging system is the universally accepted method to describe the degree of cancer advancement[5,6]. As with other cancers, gastric cancer has disease-specific factors in its staging. One of them is the cytology of a peritoneal wash or ascites because peritoneal metastasis is the most frequent mode of distant metastasis and post-surgical recurrence. However, it is often difficult to diagnose peritoneal metastasis by conventional imaging modalities, such as computed tomography and positron emission tomography. The cytological detection of free cancer cells in the peritoneal cavity is a very important finding in gastric cancer. Positive cytology means that peritoneal metastasis exists anywhere in the peritoneal cavity even if it is invisible, so it implies a high probability of future manifestations of peritoneal metastasis[7-12]. Therefore, peritoneal lavage cytology findings as well as peritoneal metastasis are factors in gastric cancer staging in Japan as stage 4 disease[13]. The most recent TNM classification system includes intraperitoneal cancer cell detection as part of the staging process, denoting metastatic disease[5].

Peritoneal carcinomatosis is an incurable disease with poor prognosis. In cases of peritoneal carcinomatosis, although debate about surgical application still remains, palliative chemotherapy would be preferred[14-17]. From this point of view, peritoneal carcinomatosis needs to be precisely diagnosed before surgery or at the beginning of surgery for surgeons to determine the most appropriate therapeutic approach[18]. However, in reality, the uneven shape of the peritoneal cavity makes it impossible for the entire cavity to be thoroughly inspected and difficult for the surgeon to definitively judge whether the peritoneal cavity is completely free of metastatic foci. Consequently, peritoneal lavage cytology is needed for the indirect diagnosis or prediction of peritoneal metastasis, and it must be as accurate as possible. The accuracy in peritoneal lavage cytology depends greatly upon the experience of the cytopathologist; therefore, the diagnosis remains inevitably subjective. In addition, several studies indicate that the sensitivity and specificity of peritoneal lavage cytology is unsatisfactory and that there is still room for improvement[19]. Over the past decade, several new diagnostic approaches have been studied. As an alternative to conventional cytology by Papanicolaou staining, immunocytochemistry or PCR-based genetic detection of epithelial or malignant cells in the peritoneal fluid has emerged (Table 1). There are advantages and shortcomings of each approach[20]. In this review, we examine recent studies, summarize findings on the molecular biology-based diagnosis of peritoneal cancer cell existence, and discuss recent advances in the treatment of peritoneal carcinomatosis.

CONVENTIONAL CYTOLOGY

Since the method of lavage cytology was described by Moore *et al*[21] in 1961, several clinical studies have demonstrated the prognostic significance of intraperitoneal free cancer cells at the time of surgery[7,10,12,16,17,22-25]. The Japanese Classification of Gastric Carcinoma (2nd English edition) first included the result of peritoneal cytology as one of the staging parameters in 1999[26]; since then, the Japanese Gastric Cancer Association includes peritoneal cytology in their staging system[14]. Although the most recent TNM classification has included the detection of intraperitoneal free cancer cells as part of the staging process, denoting M1 disease[5], the application of peritoneal cytology in preoperative staging is still controversial. The European Society for Medical Oncology (ESMO) practice guidelines recommend laparoscopy, but regard cytology as optional, and the current National Comprehensive Cancer Network (NCCN) guidelines also do not include cytology in the treatment algorithm[27]. Nevertheless, peritoneal cytology has important clinical implications in the management of advanced gastric cancer[7,28].

In gastric cancer surgery, by either laparotomy or laparoscopic approach, about 100-200 mL of saline is usually instilled into the Douglas pouch (and occasionally into the left subphrenic space) and gently stirred. A washing sample is then aspirated and subjected to cytology. Traditionally, Papanicolaou or Giemsa stainings are employed, and specimens are diagnosed by experienced cytopathologists. The accuracy, sensitivity, and specificity of conventional cytology in predicting peritoneal recurrence was 73.0%-91.9%, 11.1%-80.0%, and 86.4%-100.0%, respectively[20]. Thus, sensitivity had a particularly wide range, which indicated the need for further advanced techniques.

CARCINOEMBRYONIC ANTIGEN IN PERITONEAL LAVAGE

Kanetaka *et al*[29] recently reported that the measurement of carcinoembryonic antigen (CEA) level in peritoneal lavage (pCEA) by an enzyme immunoassay can predict poor prognosis and may help to elucidate a cohort who need more intensive adjuvant chemotherapy to improve their prognosis. Since Asao *et al*[30] first reported that the CEA antigen level in peritoneal lavage could reflect the presence of peritoneal metastasis more accurately than conventional cytology in 1991, other investigators have demonstrated the clinical significance of pCEA levels[31-35]. Most of these reports showed a significant correlation between pCEA level and survival after surgery, implying that pCEA could be a potential predictor of poor prognosis. However, the pCEA level may reflect both the production of CEA in the peritoneal cavity and the serum CEA level and may not be specific as a marker for the existence of intraperitoneal free cancer cells or occult peritoneal metastasis.

Table 1 List of published studies regarding the molecular diagnosis of peritoneal fluid in gastric cancer

Ref.	Molecule	Technique	Number of patients	Results
Asao *et al*[30]	CEA	Enzyme immunoassay	120	Correlation with 2-yr survival rate
Irinoda *et al*[32]	CEA, sialyl-Tn antigen	Enzyme immunoassay	96	Correlation with peritoneal metastasis and prognosis
Abe *et al*[31]	CEA	Enzyme immunoassay	56	Correlation with peritoneal metastasis and overall survival
Cetin *et al*[34]	CEA	Enzyme immunoassay	70	Correlation with peritoneal metastasis and overall survival
Kanetaka *et al*[29]	CEA	Enzyme immunoassay	597	Correlation with overall survival and peritoneal recurrence free survival
Yamamoto *et al*[33]	CEA, CA125	Enzyme immunoassay	229	Correlation with overall survival and recurrent cites
Li *et al*[35]	CEA	Radioimmunoassay	64	Correlation with overall survival
Kodera *et al*[38]	CEA	RT-PCR	189	Correlation with overall survival and peritoneal recurrence-free survival
Wang *et al*[36]	CEA	RT-PCR	40	Correlation with peritoneal recurrence
Sugita *et al*[41]	CEA, CK20	RT-PCR	129	Correlation with overall survival and peritoneal recurrence-free survival
Dalal *et al*[37]	CEA, CK20, survivin, MUC2	RT-PCR	40	CEA had high sensitivity and specificity, while CK20, survivin, and MUC2 showed high false-positive rates
Takata *et al*[42]	CEA, CK20	RT-PCR	104	Predict peritoneal recurrence
Kodera *et al*[40]	CK20	RT-PCR	195	Not sufficiently sensitive as CEA
Yonemura *et al*[39]	MMP-7	RT-PCR	152	Improved the sensitivity for peritoneal dissemination in combination with cytology
Mori *et al*[43]	Multiple marker	Microarray	179	Correlation with disease-free survival and immuno-cytochemical cytology
Hiraki *et al*[52]	Aberrant gene methylation	Methylation-specific PCR	107	Correlation between positive methylation and peritoneal recurrence
Mori *et al*[56]	Telomerase activity	TRAP assay	46	Some concordance with cytology
Da *et al*[57]	Telomerase activity	TRAP assay	60	Correlation with high proliferating activity of gastric cancer
Wong *et al*[62]	Viral tropism	NDV-GFP imaging	30	Higher sensitivity and lower specificity than cytology
Kitayama *et al*[58]	EpCAM	Flow cytometry	195	Tumor cell/leukocyte ratio reflects peritoneal spread

CEA: Carcino-embryonic antigen; CA125: Cancer antigen 125; CK20: Cytokeratin 20; TRAP assay: Telomeric repeat amplification protocol assay; NDV-GFP: Newcastle disease virus-green fluorescent protein; MUC2: Mucin 2; RT-PCR: Reverse transcription polymerase chain reaction.

GENETIC DETECTION OF INTRAPERITONEAL GASTRIC CANCER CELLS

Molecular diagnosis with reverse transcriptase-polymerase chain reaction (RT-PCR) has been employed for the detection of minimal cancer cells due to its high sensitivity. Among the messenger RNA (mRNA) specific to cancer cells or epithelial cells, the most common target molecule is CEA mRNA. PCR evaluation of CEA mRNA in peritoneal fluid has increased sensitivity for the detection of peritoneal cancer cells as compared to cytology[36,37], and positive results have been associated with poor survival. Kodera *et al*[38] demonstrated that CEA PCR-positive patients had significantly worse overall survival and recurrence-free survival as compared to PCR-negative patients, independently of cytology. PCR appears to increase the accuracy of detection of occult disease.

In addition, molecular targets for PCR other than CEA have been investigated and include metalloprotease-7[39] and cytokeratin 20[40,41]. The expression level of a single gene was heterogeneous, so limited sensitivity hinders its use alone. To further improve the sensitivity and specificity of the mRNA detection approach, multiplex PCR may prove to be more clinically useful in capturing intraperitoneal free cancer cells[41-43].

Mori *et al*[44] tried to select marker candidates out of tens of thousands of genes with microarray analysis, and they identified the genes specific to cytology-positive samples. They further manufactured a microarray chip containing 10 marker genes as a "MiniChip" and demonstrated that the MiniChip assay has a sensitivity and specificity equal to or better than conventional cytology in detecting minimal free cancer cells in peritoneal fluid[43].

Recently, a new rapid genetic diagnostic technique to detect minute cancer cells has been developed and applied in the sentinel node navigation surgery as surgical decision making[45-48]. One-step nucleic acid amplification (OSNA) uses reverse transcription loop-mediated isothermal amplification (RT-LAMP) to detect mRNA expression of target sequences from crude samples without RNA purification[49]. The reaction can be completed in a single test tube and within 1 h. Kumagai *et al*[50] reported a multicenter study evaluating the clinical performance of the OSNA assay that detects cytokeratin 19 (CK19) mRNA in detecting lymph node (LN) metastases in gastric cancer patients, and this method showed high concordance rate to pathology. Although the OSNA assay is useful in the intraoperative rapid diagnosis of LN metastasis for gastric cancer, it remains unproven if this technique could be ap-

plied to detect intra-peritoneal free cancer cells. It needs to be determined how the different properties of cells in the peritoneal cavity interfere with the reaction and what the minimal number of cancer cells is for detection by this method.

DNA methylation is an important epigenetic change in cancer that leads to the recruitment of transcription repressors and chromatin changes, so methylation analysis has been used as a diagnostic modality for various cancers[51]. Hiraki *et al*[52,53] assessed whether gene methylation in peritoneal fluid from gastric cancer patients is clinically feasible for determining the peritoneal metastasis in gastric cancer. By using quantitative methylation-specific PCR to compare aberrant methylation status in gastric cancer, they isolated 6 genes (*BNIP3*, *CHFR*, *CYP1B1*, *MINT25*, *RASSF2* and *SFRP2*) as having cancer-specific DNA methylation, and they observed that there was a significant correlation between positive methylation in any of these 6 genes and peritoneal recurrence[52]. Thus, methylation analysis might improve the positive detection of gastric cancer cells in peritoneal lavage.

TELOMERASE ACTIVITY IN THE PERITONEAL FLUID

Telomerase activity in cancer cells has been examined as a tag to detect cancer cells in the peritoneal cavity. Telomerase activity is one of the hallmarks of cancer and can be used to discriminate malignant cells from normal ones[54,55]. Mori *et al*[56] analyzed peritoneal lavage fluid employing a TRAP assay that reflects telomerase activity. To improve the efficacy of the assay, they enriched cancer cells with immunomagnetic beads coated with anti-Ber-EP4 antibody. Then, they successfully detected telomerase activity in the samples from gastric cancer patients with serosal or subserosal invasions, and they found some concordance with the results of cytology[56]. Da *et al*[57] have also investigated the telomerase activity in peritoneal lavage from gastric cancer patients without enrichment of cancer cells. Although the sample size was relatively small, their data demonstrated that all patients with peritoneal metastasis had detectable telomerase activity in peritoneal lavage fluid, and they found significant correlations between positive rate of telomerase activity and invasion depth, serosa-involved areas, and the presence and extent of peritoneal metastasis. While these methods were unique and appeared to be sensitive, they were not significantly superior to conventional cytology by itself. Nevertheless, telomerase activity analysis in peritoneal lavage fluid might be a helpful adjunct for the cytology in the diagnosis of occult peritoneal metastasis of gastric cancer.

FLOW CYTOMETRIC ANALYSIS OF FREE CANCER CELLS IN PERITONEAL LAVAGE FLUID

Kitayama *et al*[58] tried to quantify the free cancer cells recovered from ascites or peritoneal lavage fluid from gastric cancer patients by conventional flow cytometry. The peritoneal lavage fluid from gastric cancer patients contains erythrocytes, leukocytes, dissociated peritoneal mesothelium, and a small number of cancer cells. Therefore, molecular detection needs to distinguish cancer cells from normal cells co-existing in the peritoneal cavity. Kitayama *et al*[58] stained the cells with monoclonal antibodies to CD45 and CD326 (EpCAM), and CD326-positive and CD45-positive cells were classified as either cancer cell or leukocytes. Instead of using the total number of cancer cells, they calculated the cancer cell/leukocyte ratio and demonstrated that the ratio was significantly higher in the patients with peritoneal metastasis and positive cytology than in those without peritoneal spread. They further showed the ratio to reflect well the effect of intraperitoneal chemotherapy. They thus proposed that the flow cytometry-based measurement of the intraperitoneal CD326(+)/CD45(+) ratio could be a diagnostic marker that reflects the severity of peritoneal metastasis as well as the effectiveness of intraperitoneal chemotherapy.

Besides gastric cancer, ovarian cancer also often forms excess ascites due to peritoneal metastasis, which is routinely drained and discarded for symptomatic relief. Peterson *et al*[59] regard the ascites as a source of cancer cells for monitoring the treatment response of ovarian cancer. Miniaturizing and advancing flow cytometric technology, they developed and tested a new microfluidic chip to capture, enrich and analyze ascites tumor cells in ovarian cancer patients. This technology allows the detection of occult cancer cells and enables the molecular profiling of individual cells. The microfluidic chip might be applicable to the diagnostic and molecular analysis of peritoneal fluid from gastric cancer patients.

DIAGNOSTIC POTENTIAL OF THE VISUAL DETECTION OF CANCER CELLS IN PERITONEAL CYTOLOGY SAMPLES

As a unique approach, several groups examined virus-mediated fluorescent gene expression to visually detect rare cancer cells in the body fluid or the cytology samples against millions of normal cells[55,60,61]. Wong *et al*[62] evaluated a novel detection technique for intraperitoneal free cancer cells by using Newcastle disease virus-green fluorescent protein (NDV-GFP), which is genetically modified NDV that expresses the green fluorescent protein gene. Newcastle disease virus has been studied since the 1950s for its ability to infect and replicate specifically in tumors. NDV-GFP targets and infects specifically cancer cells, resulting in specific GFP expression. Wong *et al*[62] evaluated peritoneal lavage samples from 30 gastric cancer patients undergoing staging laparoscopy with NDV-GFP. They found that NDV-GFP-mediated detection offers a more sensitive method of identifying free peritoneal gastric cancer cells in peritoneal lavage fluid as compared to conventional Pap staining cytology to dem-

onstrate that NDV-GFP could be used diagnostically.

WHAT IS NEXT FOR THE IMPROVEMENT OF INTRAPERITONEAL DIAGNOSIS?

As described above, numerous efforts have been made to improve the detection of intraperitoneal free cancer cells. The purpose of most of these studies appeared to primarily be an improvement of the accuracy in cytology. The secondary purpose will be to make diagnosis more convenient and automatic than subjective conventional cytology. Once the accuracy and procedure is essentially improved over the conventional cytology, what should we do next? The identification of intraperitoneal free cancer cells confers poor prognosis. In patients with positive cytology without macroscopic peritoneal metastasis, the benefit of radical or aggressive surgery is still a matter of debate. While some of these patients are palliated, others may undergo more aggressive therapies. Along with the improved diagnostic modality, the treatment strategy would also have to be a coupled issue.

MULTIMODAL CLINICAL APPROACH FOR PERITONEAL SPREAD OF GASTRIC CANCER

Surgeons have witnessed some patients with peritoneal spread of gastric cancer who underwent radical surgery and experienced cures due to the recent improvements in multimodal treatment. A phase II study of whether gastrectomy with curative intent would be beneficial for patients with positive cytology but absence of macroscopic peritoneal seeding has been conducted[63,64]. The study showed that median overall survival time was 705 d, and the 5-year survival rate was 26% in the patients with positive cytology with no other non-curative factors, suggesting that surgery with curative intent could be indicated even for patients with positive cytology[63,64]. For gastric cancer patients with macroscopic peritoneal metastasis, Yamaguchi *et al*[65] evaluated intraperitoneal chemotherapy along with systemic chemotherapy as a phase II study. They reported a 1-year survival rate of 77.1%, which is surprisingly high. The same group also reported salvage gastrectomy after intravenous and intraperitoneal chemotherapy for the patients who had peritoneal metastasis but showed apparent shrinkage of their peritoneal nodules as well as negative cytology by the treatment[66]. Those patients who underwent salvage gastrectomy exhibited a 26.4-mo median survival period and 82% 1-year overall survival. Those results suggested that the more sensitive and specific peritoneal diagnosis with the molecular approach might allow gastric cancer patients to receive more suitable individualized multimodal therapies.

A NEW MOLECULAR-TARGETING THERAPY FOR INTRAPERITONEAL SPREAD OF GASTRIC CANCER

Along with the research for the improvement in detection of intraperitoneal cancer cells, molecular targeting therapies might be derived from the results of basic research. One of the molecular targets is epithelial cell adhesion molecule (EpCAM), a type I transmembrane glycoprotein functioning as a homotypic intercellular adhesion molecule[67]. High-level EpCAM expression was observed in 90.7% of gastric cancer[68]. Catumaxomab is an artificially engineered, tri-functional bispecific monoclonal antibody; Fab binding sites bind to EpCAM on cancer cells and CD3 on T cells, and the Fc region binds and activates accessory immune cells. The tri-cell complex of T-cells, tumor cells and accessory cells induces MHC-unrestricted but specific efficient tumor cell killing. The therapeutic benefit of Catumaxomab for patients with malignant ascites including gastric cancer patients has been reported in a pivotal clinical trial[69], which led to approval of Catumaxomab by the European Medicines Agency (EMA) in 2009. Intraperitoneal Catumaxomab treatment has been shown to trigger the activation of immune effector cells in the peritoneal cavity resulting in the depletion of EpCAM-positive tumor cells[70]. Thus, local strategies with molecular targeting agents might represent the appropriate option for treatment of the peritoneal spread of gastric cancer.

CONCLUSION

In the past decade, enormous strides have been made in the research for molecular detection of intraperitoneal free gastric cancer cells, and many new strategies have been clinically tested in gastric cancer patients. As with the conventional cytology, none of the candidate alternatives to conventional cytology are a perfect modality yet, whereas most of them would potentially be conducive to improve the conventional diagnosis and to predict prognosis. The uncertainty of a definition of positivity in these novel approaches and their clinical relevance remain potential limitations to the practical clinical use of these technologies. Too highly sensitive techniques such as PCR may result in the detection of clinically irrelevant metastatic disease, which could lead to either overtreatment with unnecessary chemotherapy, or worse, the withdrawal of potentially curative surgical treatment. Nevertheless, the development of more sensitive and rapid diagnostics in evaluating minimal peritoneal disease is needed for patients to be properly treated. Since peritoneal lavage cytology has recently been included in the staging criteria of gastric cancer, the cytology diagnosis has been focused on as having an important predictive

role in gastric cancer treatment, and the molecular diagnosis has undergone tremendous challenges. With the accumulated evidence, the molecular diagnosis of peritoneal cytology may be a reality in future gastric cancer practice.

REFERENCES

1 **Ferlay J**, Shin H, Bray F, Forman D, Mathers C, Parkin D. GLOBOCAN 2008 v2.0, Cancer incidence and mortality worldwide: IARC Cancer Base No.10. Available from: URL: http://globocan.iarc.fr

2 **Bray F**, Ren JS, Masuyer E, Ferlay J. Global estimates of cancer prevalence for 27 sites in the adult population in 2008. *Int J Cancer* 2013; **132**: 1133-1145 [PMID: 22752881 DOI: 10.1002/ijc.27711]

3 **Coburn N**, Seevaratnam R, Paszat L, Helyer L, Law C, Swallow C, Cardosa R, Mahar A, Lourenco LG, Dixon M, Bekaii-Saab T, Chau I, Church N, Coit D, Crane CH, Earle C, Mansfield P, Marcon N, Miner T, Noh SH, Porter G, Posner MC, Prachand V, Sano T, van de Velde C, Wong S, McLeod R. Optimal management of gastric cancer: results from an international RAND/UCLA expert panel. *Ann Surg* 2014; **259**: 102-108 [PMID: 23478525 DOI: 10.1097/SLA.0b013e318288dd2b]

4 **Brar SS**, Mahar AL, Helyer LK, Swallow C, Law C, Paszat L, Seevaratnam R, Cardoso R, McLeod R, Dixon M, Yohanathan L, Lourenco LG, Bocicariu A, Bekaii-Saab T, Chau I, Church N, Coit D, Crane CH, Earle C, Mansfield P, Marcon N, Miner T, Noh SH, Porter G, Posner MC, Prachand V, Sano T, van de Velde C, Wong S, Coburn NG. Processes of care in the multidisciplinary treatment of gastric cancer: results of a RAND/UCLA expert panel. *JAMA Surg* 2014; **149**: 18-25 [PMID: 24225775 DOI: 10.1001/jamasurg.2013.3959]

5 **Washington K**. 7th edition of the AJCC cancer staging manual: stomach. *Ann Surg Oncol* 2010; **17**: 3077-3079 [PMID: 20882416 DOI: 10.1245/s10434-010-1362-z]

6 **Sobin L GM**, Wittekind C. TNM Classification of Malignant Tumours, 7th ed. Oxford: Wiley-Blackwell, 2009

7 **Lee SD**, Ryu KW, Eom BW, Lee JH, Kook MC, Kim YW. Prognostic significance of peritoneal washing cytology in patients with gastric cancer. *Br J Surg* 2012; **99**: 397-403 [PMID: 22101572 DOI: 10.1002/bjs.7812]

8 **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. *Eur J Surg Oncol* 2010; **36**: 982-986 [PMID: 20591604 DOI: 10.1016/j.ejso.2010.06.007]

9 **Nakagohri T**, Yoneyama Y, Kinoshita T, Konishi M, Inoue K, Takahashi S. Prognostic significance of peritoneal washing cytology in patients with potentially resectable gastric cancer. *Hepatogastroenterology* 2008; **55**: 1913-1915 [PMID: 19102421]

10 **Euanorasetr C**, Lertsithichai P. Prognostic significance of peritoneal washing cytology in Thai patients with gastric adenocarcinoma undergoing curative D2 gastrectomy. *Gastric Cancer* 2007; **10**: 18-23 [PMID: 17334713 DOI: 10.1007/s10120-006-0402-7]

11 **Yajima K**, Kanda T, Ohashi M, Wakai T, Nakagawa S, Sasamoto R, Hatakeyama K. Clinical and diagnostic significance of preoperative computed tomography findings of ascites in patients with advanced gastric cancer. *Am J Surg* 2006; **192**: 185-190 [PMID: 16860627 DOI: 10.1016/j.amjsurg.2006.05.007]

12 **Kodera Y**, Yamamura Y, Shimizu Y, Torii A, Hirai T, Yasui K, Morimoto T, Kato T. Peritoneal washing cytology: prognostic value of positive findings in patients with gastric carcinoma undergoing a potentially curative resection. *J Surg Oncol* 1999; **72**: 60-64; discussion 64-65 [PMID: 10518099]

13 **Japanese Gastric Cancer Association**. Japanese classification of gastric carcinoma: 3rd English edition. *Gastric Cancer* 2011; **14**: 101-112 [PMID: 21573743 DOI: 10.1007/s10120-011-0041-5]

14 **Japanese Gastric Cancer Association**. Japanese gastric cancer treatment guidelines 2010 (ver. 3). *Gastric Cancer* 2011; **14**: 113-123 [PMID: 21573742 DOI: 10.1007/s10120-011-0042-4]

15 **Mezhir JJ**, Shah MA, Jacks LM, Brennan MF, Coit DG, Strong VE. Positive peritoneal cytology in patients with gastric cancer: natural history and outcome of 291 patients. *Indian J Surg Oncol* 2011; **2**: 16-23 [PMID: 22696066 DOI: 10.1007/s13193-011-0074-6]

16 **Fukagawa T**, Katai H, Saka M, Morita S, Sasajima Y, Taniguchi H, Sano T, Sasako M. Significance of lavage cytology in advanced gastric cancer patients. *World J Surg* 2010; **34**: 563-568 [PMID: 20054543 DOI: 10.1007/s00268-009-0355-1]

17 **Miyashiro I**, Takachi K, Doki Y, Ishikawa O, Ohigashi H, Murata K, Sasaki Y, Imaoka S, Nakaizumi A, Takenaka A, Furukawa H, Hiratsuka M. When is curative gastrectomy justified for gastric cancer with positive peritoneal lavage cytology but negative macroscopic peritoneal implant? *World J Surg* 2005; **29**: 1131-1134 [PMID: 16086213 DOI: 10.1007/s00268-005-7703-6]

18 **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**: 794-798 [PMID: 23532564 DOI: 10.1002/jso.23328]

19 **Fujiwara Y**, Doki Y, Taniguchi H, Sohma I, Takiguchi S, Miyata H, Yamasaki M, Monden M. Genetic detection of free cancer cells in the peritoneal cavity of the patient with gastric cancer: present status and future perspectives. *Gastric Cancer* 2007; **10**: 197-204 [PMID: 18095074 DOI: 10.1007/s10120-007-0436-5]

20 **Leake PA**, Cardoso R, Seevaratnam R, Lourenco L, Helyer L, Mahar A, Rowsell C, Coburn NG. A systematic review of the accuracy and utility of peritoneal cytology in patients with gastric cancer. *Gastric Cancer* 2012; **15** Suppl 1: S27-S37 [PMID: 21809111 DOI: 10.1007/s10120-011-0071-z]

21 **Moore GE**, Sako K, Kondo T, Badillo J, Burke E. Assessment of the exfoliation of tumor cells into the body cavities. *Surg Gynecol Obstet* 1961; **112**: 469-474 [PMID: 13772295]

22 **Suzuki T**, Ochiai T, Hayashi H, Nakajima K, Yasumoto A, Hishikawa E, Shimada H, Horiuchi F, Ohki S, Isono K. Importance of positive peritoneal lavage cytology findings in the stage grouping of gastric cancer. *Surg Today* 1999; **29**: 111-115 [PMID: 10030734]

23 **Makino T**, Fujiwara Y, Takiguchi S, Miyata H, Yamasaki M, Nakajima K, Nishida T, Mori M, Doki Y. The utility of preoperative peritoneal lavage examination in serosa-invading gastric cancer patients. *Surgery* 2010; **148**: 96-102 [PMID: 20096433 DOI: 10.1016/j.surg.2009.11.025]

24 **Bonenkamp JJ**, Songun I, Hermans J, van de Velde CJ. Prognostic value of positive cytology findings from abdominal washings in patients with gastric cancer. *Br J Surg* 1996; **83**: 672-674 [PMID: 8689216]

25 **Bando E**, Yonemura Y, Takeshita Y, Taniguchi K, Yasui T, Yoshimitsu Y, Fushida S, Fujimura T, Nishimura G, Miwa K. Intraoperative lavage for cytological examination in 1,297 patients with gastric carcinoma. *Am J Surg* 1999; **178**: 256-262 [PMID: 10527450]

26 **Japanese Gastric Cancer Association**. Japanese Classification of Gastric Carcinoma - 2nd English Edition - *Gastric Cancer* 1998; **1**: 10-24 [PMID: 11957040 DOI: 10.1007/s101209800016]

27 **Network NCC**. NCCN guideline: gastric cancer. Available from: URL: http://www.nccn.org

28 **Wu CC**, Chen JT, Chang MC, Ho WL, Chen CY, Yeh DC, Liu TJ, P'eng FK. Optimal surgical strategy for potentially curable serosa-involved gastric carcinoma with intraperitoneal free cancer cells. *J Am Coll Surg* 1997; **184**: 611-617 [PMID: 9179118]

29 **Kanetaka K**, Ito S, Susumu S, Yoneda A, Fujita F, Takatsuki M, Kuroki T, Eguchi S. Clinical significance of carcinoembryonic antigen in peritoneal lavage from patients with gastric cancer. *Surgery* 2013; **154**: 563-572 [PMID: 23806263 DOI: 10.1016/j.surg.2013.03.005]

30 **Asao T**, Fukuda T, Yazawa S, Nagamachi Y. Carcinoembryonic antigen levels in peritoneal washings can predict peritoneal recurrence after curative resection of gastric cancer. *Cancer* 1991; **68**: 44-47 [PMID: 2049751]

31 **Abe N**, Watanabe T, Toda H, Machida H, Suzuki K, Masaki T, Mori T, Sugiyama M, Atomi Y, Nakaya Y. Prognostic significance of carcinoembryonic antigen levels in peritoneal washes in patients with gastric cancer. *Am J Surg* 2001; **181**: 356-361 [PMID: 11438272]

32 **Irinoda T**, Terashima M, Takagane A, Sasaki N, Abe K, Araya M, Nishizuka S, Yonezawa H, Nakaya T, Shimooki O, Oyama K, Ikeda K, Saito K. Carcinoembryonic antigen level in peritoneal washing is a prognostic factor in patients with gastric cancer. *Oncol Rep* 1998; **5**: 661-666 [PMID: 9538172]

33 **Yamamoto M**, Baba H, Toh Y, Okamura T, Maehara Y. Peritoneal lavage CEA/CA125 is a prognostic factor for gastric cancer patients. *J Cancer Res Clin Oncol* 2007; **133**: 471-476 [PMID: 17226046 DOI: 10.1007/s00432-006-0189-2]

34 **Cetin B**, Atalay C, Aslan S, Babacan B, Hatipoğlu C, Akinci M, Cetin A. Peritoneal carcinoembryonic antigen level for predicting locoregional and distant spread of gastric cancer. *Surg Today* 2005; **35**: 919-924 [PMID: 16249844 DOI: 10.1007/s00595-005-3057-9]

35 **Li JK**, Zheng M, Miao CW, Zhang JH, Ding GH, Wu WS. Peritoneal lavage cytology and carcinoembryonic antigen determination in predicting peritoneal metastasis and prognosis of gastric cancer. *World J Gastroenterol* 2005; **11**: 7374-7377 [PMID: 16437646]

36 **Wang JY**, Lin SR, Lu CY, Chen CC, Wu DC, Chai CY, Chen FM, Hsieh JS, Huang TJ. Gastric cancer cell detection in peritoneal lavage: RT-PCR for carcinoembryonic antigen transcripts versus the combined cytology with peritoneal carcinoembryonic antigen levels. *Cancer Lett* 2005; **223**: 129-135 [PMID: 15890245 DOI: 10.1016/j.canlet.2004.09.031]

37 **Dalal KM**, Woo Y, Kelly K, Galanis C, Gonen M, Fong Y, Coit DG. Detection of micrometastases in peritoneal washings of gastric cancer patients by the reverse transcriptase polymerase chain reaction. *Gastric Cancer* 2008; **11**: 206-213 [PMID: 19132482 DOI: 10.1007/s10120-008-0483-6]

38 **Kodera Y**, Nakanishi H, Ito S, Yamamura Y, Kanemitsu Y, Shimizu Y, Hirai T, Yasui K, Kato T, Tatematsu M. Quantitative detection of disseminated free cancer cells in peritoneal washes with real-time reverse transcriptase-polymerase chain reaction: a sensitive predictor of outcome for patients with gastric carcinoma. *Ann Surg* 2002; **235**: 499-506 [PMID: 11923605]

39 **Yonemura Y**, Fujimura T, Ninomiya I, Kim BS, Bandou E, Sawa T, Kinoshita K, Endo Y, Sugiyama K, Sasaki T. Prediction of peritoneal micrometastasis by peritoneal lavaged cytology and reverse transcriptase-polymerase chain reaction for matrix metalloproteinase-7 mRNA. *Clin Cancer Res* 2001; **7**: 1647-1653 [PMID: 11410502]

40 **Kodera Y**, Nakanishi H, Ito S, Yamamura Y, Fujiwara M, Koike M, Hibi K, Ito K, Tatematsu M, Nakao A. Prognostic significance of intraperitoneal cancer cells in gastric carcinoma: detection of cytokeratin 20 mRNA in peritoneal washes, in addition to detection of carcinoembryonic antigen. *Gastric Cancer* 2005; **8**: 142-148 [PMID: 16086116 DOI: 10.1007/s10120-005-0318-7]

41 **Sugita Y**, Fujiwara Y, Taniguchi H, Mori T, Ishii T, Niwa H, Okada Y, Takiguchi S, Yasuda T, Yano M, Monden M. Quantitative molecular diagnosis of peritoneal lavage fluid for prediction of peritoneal recurrence in gastric cancer. *Int J Oncol* 2003; **23**: 1419-1423 [PMID: 14532985]

42 **Takata A**, Kurokawa Y, Fujiwara Y, Nakamura Y, Takahashi T, Yamasaki M, Miyata H, Nakajima K, Takiguchi S, Mori M, Doki Y. Prognostic value of CEA and CK20 mRNA in the peritoneal lavage fluid of patients undergoing curative surgery for gastric cancer. *World J Surg* 2014; **38**: 1107-1111 [PMID: 24305936 DOI: 10.1007/s00268-013-2385-y]

43 **Mori K**, Suzuki T, Uozaki H, Nakanishi H, Ueda T, Matsuno Y, Kodera Y, Sakamoto H, Yamamoto N, Sasako M, Kaminishi M, Sasaki H. Detection of minimal gastric cancer cells in peritoneal washings by focused microarray analysis with multiple markers: clinical implications. *Ann Surg Oncol* 2007; **14**: 1694-1702 [PMID: 17294072 DOI: 10.1245/s10434-006-9321-4]

44 **Mori K**, Aoyagi K, Ueda T, Danjoh I, Tsubosa Y, Yanagihara K, Matsuno Y, Sasako M, Sakamoto H, Mafune Ki, Kaminishi M, Yoshida T, Terada M, Sasaki H. Highly specific marker genes for detecting minimal gastric cancer cells in cytology negative peritoneal washings. *Biochem Biophys Res Commun* 2004; **313**: 931-937 [PMID: 14706632]

45 **Castellano I**, Macrì L, Deambrogio C, Balmativola D, Bussone R, Ala A, Coluccia C, Sapino A. Reliability of whole sentinel lymph node analysis by one-step nucleic acid amplification for intraoperative diagnosis of breast cancer metastases. *Ann Surg* 2012; **255**: 334-342 [PMID: 21975319 DOI: 10.1097/SLA.0b013e31823000ed]

46 **Matsuzuka T**, Takahashi K, Kawakita D, Kohno N, Nagafuji H, Yamauchi K, Suzuki M, Miura T, Furuya N, Yatabe Y, Matsuo K, Omori K, Hasegawa Y. Intraoperative molecular assessment for lymph node metastasis in head and neck squamous cell carcinoma using one-step nucleic acid amplification (OSNA) assay. *Ann Surg Oncol* 2012; **19**: 3865-3870 [PMID: 22618721 DOI: 10.1245/s10434-012-2409-0]

47 **Tamaki Y**, Sato N, Homma K, Takabatake D, Nishimura R, Tsujimoto M, Yoshidome K, Tsuda H, Kinoshita T, Kato H, Taniyama K, Kamio T, Nakamura S, Akiyama F, Noguchi S. Routine clinical use of the one-step nucleic acid amplification assay for detection of sentinel lymph node metastases in breast cancer patients: results of a multicenter study in Japan. *Cancer* 2012; **118**: 3477-3483 [PMID: 22252672 DOI: 10.1002/cncr.26683]

48 **Yaguchi Y**, Sugasawa H, Tsujimoto H, Takata H, Nakabayashi K, Ichikura T, Ono S, Hiraki S, Sakamoto N, Horio T, Kumano I, Otomo Y, Mochizuki H, Yamamoto J, Hase K. One-step nucleic acid amplification (OSNA) for the application of sentinel node concept in gastric cancer. *Ann Surg Oncol* 2011; **18**: 2289-2296 [PMID: 21301968 DOI: 10.1245/s10434-011-1591-9]

49 **Muto Y**, Matubara H, Tanizawa T, Nabeya Y, Kawahira H, Akai T, Hoshino I, Hayashi H. Rapid diagnosis of micrometastasis of gastric cancer using reverse transcription loop-mediated isothermal amplification. *Oncol Rep* 2011; **26**: 789-794 [PMID: 21769432 DOI: 10.3892/or.2011.1389]

50 **Kumagai K**, Yamamoto N, Miyashiro I, Tomita Y, Katai H, Kushima R, Tsuda H, Kitagawa Y, Takeuchi H, Mukai M, Mano M, Mochizuki H, Kato Y, Matsuura N, Sano T. Multicenter study evaluating the clinical performance of the OSNA assay for the molecular detection of lymph node metastases in gastric cancer patients. *Gastric Cancer* 2014; **17**: 273-280 [PMID: 23743877 DOI: 10.1007/s10120-013-0271-9]

51 **Nagasaka T**, Tanaka N, Cullings HM, Sun DS, Sasamoto H, Uchida T, Koi M, Nishida N, Naomoto Y, Boland CR, Matsubara N, Goel A. Analysis of fecal DNA methylation to detect gastrointestinal neoplasia. *J Natl Cancer Inst* 2009; **101**: 1244-1258 [PMID: 19700653 DOI: 10.1093/jnci/djp265]

52 **Hiraki M**, Kitajima Y, Koga Y, Tanaka T, Nakamura J, Hashiguchi K, Noshiro H, Miyazaki K. Aberrant gene methylation is a biomarker for the detection of cancer cells in peritoneal wash samples from advanced gastric cancer patients. *Ann Surg Oncol* 2011; **18**: 3013-3019 [PMID: 21409489 DOI: 10.1245/s10434-011-1636-0]

53 **Hiraki M**, Kitajima Y, Sato S, Nakamura J, Hashiguchi K, Noshiro H, Miyazaki K. Aberrant gene methylation in the peritoneal fluid is a risk factor predicting peritoneal recurrence in gastric cancer. *World J Gastroenterol* 2010; **16**: 330-338 [PMID: 20082478]

54 **Kishimoto H**, Kojima T, Watanabe Y, Kagawa S, Fujiwara T, Uno F, Teraishi F, Kyo S, Mizuguchi H, Hashimoto Y, Urata Y, Tanaka N, Fujiwara T. In vivo imaging of lymph node

metastasis with telomerase-specific replication-selective adenovirus. *Nat Med* 2006; **12**: 1213-1219 [PMID: 17013385 DOI: 10.1038/nm1404]

55 **Kojima T**, Hashimoto Y, Watanabe Y, Kagawa S, Uno F, Kuroda S, Tazawa H, Kyo S, Mizuguchi H, Urata Y, Tanaka N, Fujiwara T. A simple biological imaging system for detecting viable human circulating tumor cells. *J Clin Invest* 2009; **119**: 3172-3181 [PMID: 19729837 DOI: 10.1172/JCI38609]

56 **Mori N**, Oka M, Hazama S, Iizuka N, Yamamoto K, Yoshino S, Tangoku A, Noma T, Hirose K. Detection of telomerase activity in peritoneal lavage fluid from patients with gastric cancer using immunomagnetic beads. *Br J Cancer* 2000; **83**: 1026-1032 [PMID: 10993650 DOI: 10.1054/bjoc.2000.1408]

57 **Da MX**, Wu XT, Guo TK, Zhao ZG, Luo T, Qian K, Zhang MM, Wang J. Clinical significance of telomerase activity in peritoneal lavage fluid from patients with gastric cancer and its relationship with cellular proliferation. *World J Gastroenterol* 2007; **13**: 3122-3127 [PMID: 17589931]

58 **Kitayama J**, Emoto S, Yamaguchi H, Ishigami H, Kamei T, Yamashita H, Seto Y, Matsuzaki K, Watanabe T. Flow cytometric quantification of intraperitoneal free tumor cells in patients with peritoneal metastasis. *Cytometry B Clin Cytom* 2014; **86**: 56-62 [PMID: 24115348 DOI: 10.1002/cyto.b.21126]

59 **Peterson VM**, Castro CM, Chung J, Miller NC, Ullal AV, Castano MD, Penson RT, Lee H, Birrer MJ, Weissleder R. Ascites analysis by a microfluidic chip allows tumor-cell profiling. *Proc Natl Acad Sci USA* 2013; **110**: E4978-E4986 [PMID: 24297935 DOI: 10.1073/pnas.1315370110]

60 **Adusumilli PS**, Gholami S, Chun YS, Mullerad M, Chan MK, Yu Z, Ben-Porat L, Rusch VW, Fong Y. Fluorescence-assisted cytological testing (FACT): Ex Vivo viral method for enhancing detection of rare cancer cells in body fluids. *Mol Med* 2011; **17**: 628-634 [PMID: 21487639 DOI: 10.2119/molmed.2011.00078]

61 **Maida Y**, Kyo S, Sakaguchi J, Mizumoto Y, Hashimoto M, Mori N, Ikoma T, Nakamura M, Takakura M, Urata Y, Fujiwara T, Inoue M. Diagnostic potential and limitation of imaging cancer cells in cytological samples using telomerase-specific replicative adenovirus. *Int J Oncol* 2009; **34**: 1549-1556 [PMID: 19424572]

62 **Wong J**, Schulman A, Kelly K, Zamarin D, Palese P, Fong Y. Detection of free peritoneal cancer cells in gastric cancer using cancer-specific Newcastle disease virus. *J Gastrointest Surg* 2010; **14**: 7-14 [PMID: 19902312 DOI: 10.1007/s11605-009-1071-8]

63 **Kodera Y**, Ito S, Mochizuki Y, Ohashi N, Tanaka C, Kobayashi D, Kojima H, Matsui T, Kondo K, Fujiwara M. Long-term follow up of patients who were positive for peritoneal lavage cytology: final report from the CCOG0301 study. *Gastric Cancer* 2012; **15**: 335-337 [PMID: 22527184 DOI: 10.1007/s10120-012-0156-3]

64 **Kodera Y**, Ito S, Mochizuki Y, Kondo K, Koshikawa K, Suzuki N, Kojima H, Kojima T, Matsui T, Takase T, Tsuboi K, Fujiwara M, Nakao A. A phase II study of radical surgery followed by postoperative chemotherapy with S-1 for gastric carcinoma with free cancer cells in the peritoneal cavity (CCOG0301 study). *Eur J Surg Oncol* 2009; **35**: 1158-1163 [PMID: 19328643 DOI: 10.1016/j.ejso.2009.03.003]

65 **Yamaguchi H**, Kitayama J, Ishigami H, Emoto S, Yamashita H, Watanabe T. A phase 2 trial of intravenous and intraperitoneal paclitaxel combined with S-1 for treatment of gastric cancer with macroscopic peritoneal metastasis. *Cancer* 2013; **119**: 3354-3358 [PMID: 23798046 DOI: 10.1002/cncr.28204]

66 **Kitayama J**, Ishigami H, Yamaguchi H, Yamashita H, Emoto S, Kaisaki S, Watanabe T. Salvage gastrectomy after intravenous and intraperitoneal paclitaxel (PTX) administration with oral S-1 for peritoneal dissemination of advanced gastric cancer with malignant ascites. *Ann Surg Oncol* 2014; **21**: 539-546 [PMID: 23975319 DOI: 10.1245/s10434-013-3208-y]

67 **Litvinov SV**, Velders MP, Bakker HA, Fleuren GJ, Warnaar SO. Ep-CAM: a human epithelial antigen is a homophilic cell-cell adhesion molecule. *J Cell Biol* 1994; **125**: 437-446 [PMID: 8163559]

68 **Went P**, Vasei M, Bubendorf L, Terracciano L, Tornillo L, Riede U, Kononen J, Simon R, Sauter G, Baeuerle PA. Frequent high-level expression of the immunotherapeutic target Ep-CAM in colon, stomach, prostate and lung cancers. *Br J Cancer* 2006; **94**: 128-135 [PMID: 16404366 DOI: 10.1038/sj.bjc.6602924]

69 **Heiss MM**, Murawa P, Koralewski P, Kutarska E, Kolesnik OO, Ivanchenko VV, Dudnichenko AS, Aleknaviciene B, Razbadauskas A, Gore M, Ganea-Motan E, Ciuleanu T, Wimberger P, Schmittel A, Schmalfeldt B, Burges A, Bokemeyer C, Lindhofer H, Lahr A, Parsons SL. The trifunctional antibody catumaxomab for the treatment of malignant ascites due to epithelial cancer: Results of a prospective randomized phase II/III trial. *Int J Cancer* 2010; **127**: 2209-2221 [PMID: 20473913 DOI: 10.1002/ijc.25423]

70 **Jäger M**, Schoberth A, Ruf P, Hess J, Hennig M, Schmalfeldt B, Wimberger P, Ströhlein M, Theissen B, Heiss MM, Lindhofer H. Immunomonitoring results of a phase II/III study of malignant ascites patients treated with the trifunctional antibody catumaxomab (anti-EpCAM x anti-CD3). *Cancer Res* 2012; **72**: 24-32 [PMID: 22044753 DOI: 10.1158/0008-5472.CAN-11-2235]

P- Reviewer: Bentrem D, Majumdar APN
S- Editor: Ding Y **L- Editor**: A **E- Editor**: Liu XM