RESEARCH ARTICLE

Prognostic Role of Circulating Tumor Cells in Patients with Pancreatic Cancer: a Meta-analysis

Xue-Lei Ma[&], Yan-Yan Li[&], Jing Zhang[&], Jing-Wen Huang, Hong-Yuan Jia, Lei Liu*, Ping Li*

Abstract

 <u>Background</u>: Isolation and characterization of circulating tumor cells (CTCs) in patients suffering from a variety of different cancers have become hot biomarker topics. In this study, we evaluated the prognostic value of CTCs in pancreatic cancer. <u>Materials and Methods</u>: Initial literature was identified using Medline and EMBASE. The primary data were hazard ratios (HRs) with 95% confidence intervals (CIs) of survival outcomes, including overall survival (OS) and progression free survival/recurrence free survival (PFS/RFS). <u>Results</u>: A total of 9 eligible studies were included in this meta-analysis, published between 2002 and 2013. The estimated pooled HR and 95%CI for OS for all studies was 1.64 (95%CI 1.39-1.94, $p<0.00001$) and the pooled HR and 95%CI for RFS/DFS was 2.36 (95%CI 1.41-3.96, $p<0.00001$). The HRs and 95%CIs for OS and RFS/DFS in patients before treatment were 1.93 (95%CI 1.26-2.96, $p=0.003$) and 1.82 (95%CI 1.22-2.72, $p=0.003$), respectively. In patients receiving treatment, the HRs and 95%CI for OS and RFS/DFS were 1.37 (95%CI 1.00-1.86, $p=0.05$) and 1.89 (95%CI 1.01-3.51, $p=0.05$), respectively. Moreover, the pooled HR and 95%CI for OS in the post-treatment group was 2.20 (95%CI 0.80-6.02, $p=0.13$) and the pooled HR for RFS/DFS was 8.36 (95%CI 3.22-21.67, $p<0.0001$). <u>Conclusions</u>: The meta-analysis provided strong evidence supporting the proposition that CTCs detected in peripheral blood have a fine predictive role in pancreatic patients especially on the time point of post-treatment.

Keywords: CTCs - pancreatic - prognosis - meta-analysis

Introduction

Pancreatic cancer, with a patient survival rate among the worst of any solid cancer, is the fourth leading cause of cancer-related morbidity and mortality in Western countries (Tjensvoll et al., 2013). It leads to an estimated 227,000 deaths per year worldwide and the 5 year survival rate is only 4.6% due to its early metastasis (Zhou et al., 2011). Most of the pancreatic cancer patients are already at a late stage when diagnosed by imaging and it is hard to make a definite diagnosis with screening methods (Ghaneh et al., 2008). Pancreatic cancer can be screened using Sialic acid Lewis antigen CA 19-9 (CA19-9) and carcinoembryonic antigen (CEA), which are serum-based biomarkers mainly used as auxiliary indicator for early diagnosis, however, their utility are notoriously variable among patients (Kokhanenko et al., 2001). People have made attempt to find a new biomarker to detect the tumor and observe the progression.

Circulating tumor cells (CTCs) are tumor cells circulating through normal vessels and capillaries, and neovessles formed by tumor induced angiogenesis (Tjensvoll et al., 2013). When cancer cells metastasis or show invasive property, the first step is to invade vessels and disseminated into peripheral blood, therefore, the detection of pancreatic tumor cells in the peripheral circulation could be used to predict the prognosis of pancreatic cancer, due to their metastatic propensity (Zippelius and Pantel 2000). That is to say, CTCs can provide predictive and prognostic information in terms of disease relapse, and overall survival, as reported in breast cancer; (Cristofanilli et al., 2004; Dong et al., 2012; Tarhan et al., 2013), lung cancer (Hiltermann et al., 2012; Ma et al., 2012; Tarhan et al., 2013), colorectal cancer (Cohen et al., 2008; Uen et al., 2008) and prostate cancer (Goodman et al., 2011; Wang et al., 2011). At present, the common approaches to detect CTCs in patients with pancreatic cancer include: 1) immunological assays using antibodies directed against cell surface antigen; 2) PCR-based molecular assays for tumor-derived DNA or RNA extraction from CTCs; and; 3) technologies based on physical or biological properties of cancer cells (Cen et al., 2012).

With the aim of gaining a better insight into the prognostic value of CTCs in patients with pancreatic cancer, our comprehensive study was conducted by pooling published studies using standard meta-analysis techniques.

Cancer Center, State Key Laboratory of Biotherapy, West China Hospital, Sichuan University, Chengdu, China [&]Equal contributors
*For correspondence: lipinghx@gmail.com, Liuleihx@gmail.com

Materials and Methods

Search strategy

PubMed and EMBASE were searched on July 19th, 2013. We retrieved articles with combination of the following key words: circulating tumor cells, CTCs, RT-PCR, CK19 mRNA, CK20 mRNA, CEA mRNA, EpCAM and pancreatic cancer.

Study selection and inclusion/exclusion criteria

Titles and abstracts were reviewed for all searched papers, and full text was perused for potentially eligible studies according to our including criteria. To avoid duplicate data, the most integrated study with the longest follow-up time was included if several published studies with duplicate patients were performed in the same Research Center or if one study contained patients also described in another study. But if different patients were included in two studies of the same Research Center, we included both studies. Similarly, if there are multiple sets of data in one study, such as various sampling time (pre and post), we listed all data as separate ones.

In our meta-analysis, our inclusion criteria are as follows: 1) containing patient cases of pancreatic cancer, 2) measuring the presence of CTCs, 3) studies with data available regarding prognostic role of CTCs in pancreatic cancer patients with survival outcomes such as overall survival (OS) and progression free survival/recurrence free survival (PFS/RFS). Studies were excluded in our study: 1) with duplicate data, 2) lacking key information to calculate log hazard ratio (logHR) and SE (logHR) (SE).

Data extraction

Articles were reviewed independently by two investigators (Xuelei Ma and Jingwen Huang) for data extraction. Any discrepancy was further discussed to reach a consensus. Data was extracted from eligible studies by another two investigators (Yanyan Li and Jing Zhang) independently. The primary data was HR with 95% confidence interval (CI) of survival outcomes, including OS and PFS/RFS. Additional data obtained from the studies included publication year, patients' country, number of patients, histology type, average age of patients, sex ratio (Famale/Male), lymphnode invasion status (negative/positive), tumor differentiation (well/poor), TNM stage, follow-up time and detection method. The statistical data for calculating logHR and SE were also obtained following Parmar's method, such as HR (95%CI) or the Kaplan-Meier survival curves with *p* value.

Statistical methods

The logHR and SE were required in our analysis. Some of the original papers provided the logHR and SE directly, while other studies didn't. We utilized available data such as HR with 95%CI, or the *p*-value for log-rank test with Kaplan-Meier survival curve to calculate them instead. The methods were developed by Parmar (Parmar et al., 1998), Williamson (Williamson et al., 2002) and Tierney (Tierney et al., 2007). Those logHRs and SEs were calculated with the methods above.

As the outcome for analysis was survival in patients,

the significant outcome was defined as $p<0.05$. A combined HR>1 frequently indicated the poorer prognosis in CTCs positive cohort. $p<0.10$ or $I^2>50\%$ represents existed heterogeneity in combined HRs (Higgins et al., 2003). When homogeneity was fine ($p≥0.10$, $I^2≤50\%$), a fixed effects model was applied to secondary analysis, otherwise, a random effects model was performed. All the above calculations, and publication bias which was evaluated using the Begg's funnel plot, were performed by STATA 11.0 (STATA Corporation, College Station, TX).

Results

Eligible studies

The primary literature research yielded 466 articles. After screening their titles and abstracts, 420 studies were excluded because they were laboratory studies (180), review articles (48), articles on other cancers (21), duplicate studies (7), or didn't show the prognostic role of CTCs (164). The rest 46 full texts were reviewed repeatedly. Thirty-seven articles were further excluded because of the following reasons: no enough survival data. Finally, 9 studies (Uchikura et al., 2002; Mataki et al., 2004; Soeth et al., 2005; Kurihara et al., 2008; Sergeant et al., 2011; de Albuquerque et al., 2012; Khoja et al., 2012; Sergeant et al., 2012; Bidard et al., 2013) were included in this study, which were published between 2002 and 2013. (Figure 1)

The eligible studies encompassed 603 patients with the mean number of 67. The 9 studies were from 5 countries: France (Bidard et al., 2013), UK (Khoja et al., 2012), Germany (Soeth et al., 2005; de Albuquerque et al., 2012), Belgium (Sergeant et al., 2011; 2012) and Japan (Uchikura et al., 2002; Mataki et al., 2004; Kurihara et al., 2008). The histology types of the pancreatic cancer patients included pancreatic adenocarcinoma (N=113), biliary pancreatic cancer (N=120), pancreatic cancer (N=80) and pancreatic ductal adenocarcinoma (N=290). Also, the samples were collected at different times during the treatment: pre-, intra- and post-treatment. The main characteristics of the included studies were summarized in Table 1.

Correlation between CTCs and the survival outcome

Overall survival: HRs for OS were available in 2 articles, and the survival curves with *P* values were extracted in the remaining studies for calculation. The
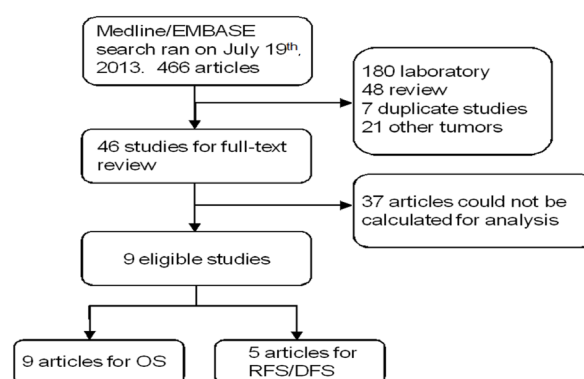

Figure 1. Selection of Studies

Table 1. Summary Table of the Meta-Analysis

Study	Country	N (F/M)	P (N)	Age	Histology type	Differentiation (well:poor)	TNM stage (T1/T2/T3/T4)	Lymph node invasion (-;+)	Sample collection	Follow up period	Detection methods
Bidard FC 2013	France	79 (45/34)	7	18-59:33 >60:46	pancreatic adenocarcinoma	30;13	-	45;34	pre and post	6 months	CellSearch
Yuko Mataki 2004	Japan	53 (20/23)	14	67±8.8	biliarypancreatic cancer	-	9/15/14/10	-	post	55.3±17.5 months	RT-PCR
Albuquerque A 2012	Germany	34 (14/20)	16	median 66.9	pancreatic adenocarcinoma	10;24	0/4/2/28	6;28	pre	Every 3 months	RT-PCR
Khoja L 2011	UK	54 (25/29)	21	33-85	pancreatic cancer	5;49	-	10;44	pre	2 years	CellSearch
Kurihara T 2007	Japan	26 (15/11)	11	69.5±13	pancreatic cancer	-	0/1/1/24	6;20	pre	233.3±170.9 days	CellSearch
Sergeant G2 2011	Belgium	40 (17/23)	-	63.3±10.3	PDAC	23;17	13/26/0/1	13;27	pre	0.7-41.3 months	RT-PCR
Sergeant G 2012	Belgium	78 (-)	-	50-74	PDAC	-	-	-	intra	2 years	CellSearch
Soeth E 2005	Germany	172 (89/83)	32	<50:25, 51-70:112 >70:35	PDAC	23;151	10/19/51/52	32;150	pre	70 months	RT-PCR
Uchikura K 2002	Japan	67 (-)	32	39-85	biliarypancreatic cancer	-	14/14/11/26	-	intra	>2 years	RT-PCR

*N: patients number; F/M:female/male; P: CTC positive patient number; PDAC:pancreatic ductal adenocarcinoma; -: not given; RT-PCR: real-time PCR

estimated pooled HR for OS was 1.64 (95%CI 1.39-1.94, *p*<0.00001), which indicated an increased risk of mortality in CTC positive patients. Only one study give the HR for RFS/DFS directly[24], and HRs and 95%CIs of the rest studies were calculated using the survival curves and the *p* values. The pooled HR and 95%CI for RFS/DFS was 2.36 (95%CI 1.41-3.96, *p*<0.00001), which suggested a significant increased risk of disease progression in patients with CTCs positivity (shown in Figure 2).

Subgroup analysis

Lymph node invasion (negative/positive) ≥25% or <25%: In lymph node invasion (negative/positive) ≥25% group, the HRs and 95%CI for OS and RFS/DFS were 2.80 (95%CI 1.84-4.27, *p*<0.00001) and 1.57 (95%CI 0.69-3.53, *p*=0.28) respectively (shown in Figure 3 E). While, in lymph node invasion (negative/positive) <25% group, the HR and 95%CI for OS (1.43, 95%CI 1.13-1.80, *p*=0.003) was much lower and the HR and 95%CI for RFS/DFS (1.91, 95%CI 1.21-3.03, *p*=0.006) was a little higher than the relative OS and RFS/DFS in lymph node invasion (negative/positive) ≥25% group.

Tumor differentiation (well/poor) ≥50% or <50%

The HRs and 95%CIs for OS and RFS/DFS in tumor differentiation (well/poor) ≥50% patients were 2.31 (95%CI 1.27-4.21, *p*=0.006) and 2.10 (95%CI 1.21-3.65, *p*=0.009) respectively (shown in Figure 3 F). While, in tumor differentiation (well/poor) <50% patients, the HR for OS was 1.43 (95%CI 1.13-1.80, *p*=0.003) and the HR for RFS/DFS was 1.56 (95%CI 0.88-2.79, *p*=0.13).

Sampling time point: pre-, intra- and after-treatment

The HRs and 95%CIs for OS and RFS/DFS in patients before treatment were 1.93 (95%CI 1.26-2.96, *p*=0.003) and 1.82 (95%CI 1.22-2.72, *p*=0.003)
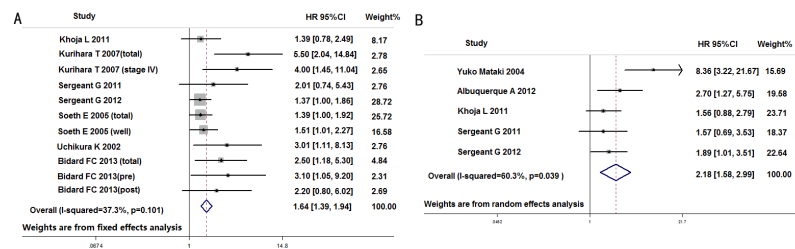

Figure 2. Estimated Hazard Ratios (HRs) Summary. A) Overall survival (OS) in all Patients; **B)** Disease-free survival/ recurrence free survival (DFS/RFS) in all patients
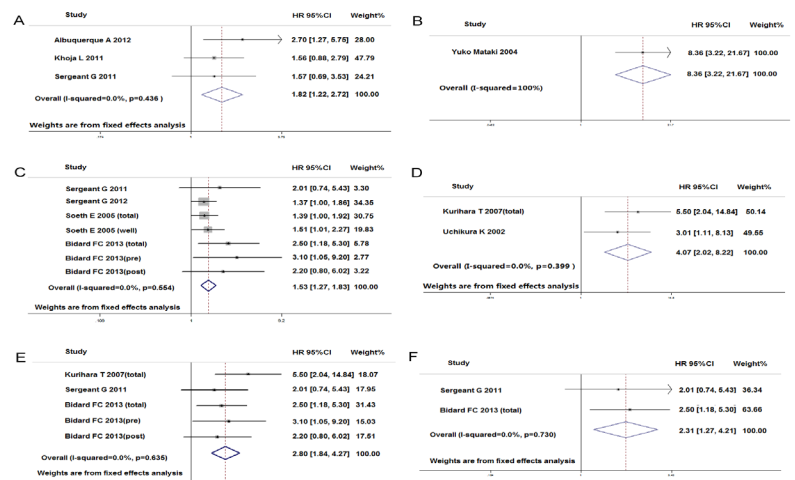

Figure 3. Estimated Hazard Ratios (HRs) Summary. A) Disease-free survival/ recurrence free survival (DFS/RFS) in patients before treatment; **B)** DFS/RFS in patients after treatment; **C)** overall survival (OS) in european patients; **D)** OS in non-european patient; **E)** OS in lymph node invasion (negative/positive) >25%patients; **F)** OS in tumor differentiation (well/poor)>50% patients

Table 2. Meta-Analyses of CTC Expression Hazard Ratios and Confidence Interval to Predict the Survival Outcome

	Survival Outcome	Data Sets (number)	Model	HR (95%CI)	Log-rank *p*	Heterogeneity (*p*, I²)
Total	OS	11	Fixed	1.64 (1.39, 1.94)	<0.00001	0.10, 37%
	RFS/DFS	5	Random	2.36 (1.41, 3.96)	0.001	0.04, 60%
Lymph node invasion	OS	5	Fixed	2.80 (1.84, 4.27)	<0.00001	0.63, 0%
(negative/positive) >25%	RFS/DFS	1	—	1.57 (0.69, 3.53)	0.28	—
Lymph node invasion	OS	3	Fixed	1.43 (1.13, 1.80)	0.003	0.94, 0%
(negative/positive) <25%	RFS/DFS	2	Fixed	1.91 (1.21, 3.03)	0.006	0.26, 21%
Pre-treatment	OS	5	Random	1.93 (1.26, 2.96)	0.003	0.08, 52%
	RFS/DFS	3	Fixed	1.82 (1.22, 2.72)	0.003	0.49, 0%
intra-treatment	OS	1	—	1.37 (1.00, 1.86)	0.05	—
	RFS/DFS	1	—	1.89 (1.01, 3.51)	0.05	—
after-treatment	OS	1	—	2.20 (0.80, 6.02)	0.13	—
	RFS/DFS	1	—	8.36 (3.22, 21.67)	<0.0001	—
Non-Europe	OS	2	Fixed	4.07 (2.02, 8.22)	<0.0001	0.40, 0%
	RFS/DFS	1	—	8.36 (3.22, 21.67)	<0.0001	—
Europe	OS	6	Fixed	1.53 (1.25, 1.83)	<0.0001	0.43, 0%
	RFS/DFS	4	Fixed	1.84 (1.31, 2.58)	0.0004	0.69, 0%
Tumor differentiation	OS	4	Fixed	2.31 (1.27, 4.21)	0.006	0.73, 0%
(well/poor)>50%	RFS/DFS	2	Fixed	2.10 (1.21, 3.65)	0.009	0.34, 0%
Tumor differentiation	OS	3	Fixed	1.43 (1.13, 1.80)	0.003	0.94, 0%
(well/poor)<50%	RFS/DFS	1	—	1.56 (0.88, 2.79)	0.13	—
stage (I-III/IV)>50%	OS	2	Random	2.54 (0.66, 9.73)	0.17	0.01, 85%
	RFS/DFS	1	—	2.70 (1.27, 5.75)	0.01	—
stage (I-III/IV)<50%	OS	1	—	2.01 (0.74, 5.43)	0.17	—
	RFS/DFS	1	—	8.36 (3.22, 21.67)	<0.0001	—
Only stage I-III	OS	1	—	5.50 (2.04, 14.84)	0.0007	—
Only stage IV	OS	1	—	3.01 (1.11, 8.13)	0.03	—

*OS, overall survival; RFS/DFS, recurrence free survival/disease free survival; HR, hazard ratio; CI, confidence interval

respectively (shown in Figure 3 A). In patients receiving treatment, the HRs and 95%CIs for OS and RFS/DFS were 1.37 (95%CI 1.00-1.86, *p*=0.05) and 1.89 (95%CI 1.01-3.51, *p*=0.05) respectively. Moreover, in the patients after treatment, the HR and 95%CI for OS was 2.20 (95%CI 0.80-6.02, *p*=0.13) and the HR and 95%CI for RFS/DFS was 8.36 (95%CI 3.22-21.67, *p*<0.0001) (shown in Figure 3B).

Europe and Non-Europe countries

Seven studies were from Europe and two were from Japan (Asia). The HRs and 95%CIs for OS and RFS/DFS in patients in Europe were 1.53 (95%CI 1.25-1.83, *p*<0.0001) and 1.84 (95%CI 1.31-2.58, *p*=0.004) respectively (shown in Figure 3 C). While, in the patients in Non-Europe (Japan), the HRs for OS and RFS/DFS were 4.07 and 8.36, respectively (shown in Figure 3 D). The results above and other results were shown in Table 2.

Publication bias

Begg's funnel plot and test was used to examine publication bias. Results of meta-analyses of CTC prediction value for OS and DFS/RFS were *p*=0.010 and *p*=0.142, respectively (Figure 4).

Discussion

As we know, it was the first time that a comprehensive and detailed meta-analysis revealed the prognostic role of CTCs for pancreatic cancer. The present meta-analysis is based on a relatively large pool of clinical studies and patients. Here, we identified 9 eligible studies which assessed the prognostic value of CTC detection by CellSearch system as well as the PCR-based molecular assays. This analysis provides coherent evidence that the expression of CTCs detected in the peripheral blood is of fine predictive role in patients with pancreatic cancer. The pooled results are fairly stable and not influenced by the CTC detection method, time point of blood withdrawal, source of patient and other characteristics.

The results of our collective evaluation of the literature on pancreatic cancer suggested that CTCs in the peripheral blood have a predictive value. As a thumb, RR>2 was considered as prognostic biomarker in practical. The combined HR and 95%CI for RFS/PFS meet this standard, while the combined HR and 95%CI for OS suggested a predictive marker but not in practical. These results suggested that CTCs detected in peripheral blood was a promising biomarker to speculate survival outcome, evaluate tumor progression and perform as a drug target.

In addition, the adverse prognostic effect of CTCs detection in the peripheral blood was confirmed throughout all performed subgroup analyses. When we divided the results by different time point of blood samples withdrawal including pre-treatment, intra-treatment and post-treatment, the subgroup analysis suggested that CTCs detected in different sampling time have prognostic value. Compared the pooled HR of different sampling time, the pooled HR for RFS/PFS in post-treatment group was
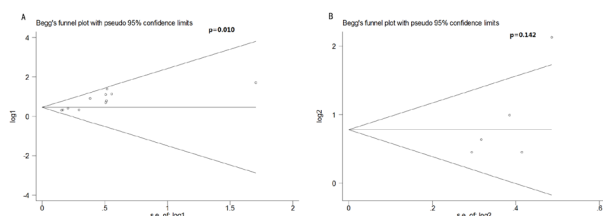
Figure 4. Begg's funnel plots of publication bias summary. A) Overall survival (OS); **B)** Disease-free survival/relapse free survival (DFS/RFS) in all patients

overlaid 2, while the other two time point groups were not. These results showed that post-treatment group showed a better predictive role than other time point groups. The main reason might be that post-treatment time incorporated the pretreatment status of CTCs and intra-treatment tumor cells released by surgery, which meant that CTCs detected at post-treatment time were the most relevant to affect tumor prognosis and patient survival time.

If we divided the results by tumor differentiation, we could easily find that the patients group with more well differentiation patients (well differentiation /poor differentiation>50%) have a higher predictive value than other groups. And if we divided the results by lymph node invasion, we could find that the patients with more lymph node invasion (negative/positive lymph node invasion>25%) also have a higher predictive value. We hypothesized that pancreatic cancer cells with poor differentiation or lymph node invasion are easy to metastasis, so CTCs could widely be detected positive in the poor differentiation and lymph node invasion group. Researchers could not predict the survival time in above state. When we divided the studies by the nation of pooled patients, HR for OS and RFS/PFS have a fine result above 2 in non-European group patients, while the HR OS and RFS/PFS was less than 2 in European patients. It suggests that CTCs have a significant strong predictive value in non-European patients rather than European group.

Though, no heterogeneity has been found in our meta-analysis, differences in the detection methods, sampling time, as well as in demographic or clinic pathologic data of included patients ought to be considered as potential sources of heterogeneity. We divided these studies into several subgroups according to above clinical characteristics. When we grouped the analysis into tumor differentiation (well/poor) >50% and <50% groups, the heterogeneity for OS could shrink as 0% and 0%, which means no heterogeneity. When we grouped the analysis into lymph node invasion (negative/positive) >25% and <25% groups, the heterogeneity for OS could also shrink as 0% and 0%. When we grouped the analysis into Europe and Non-Europe countries groups, the heterogeneity for OS could also shrink as 0% and 0%. This meant that the heterogeneity could be eliminated by the subgroup analysis of tumor differentiation, patient's nation and lymph node status. It indicated that these factors might be the reason of heterogeneity, and we used the subgroup analysis in fix model.

Although, the pool analysis of these studies could support a fine practical role for CTCs analysis in pancreatic cancer, challenges exist in this field because many clinical and technical characteristics could affect the newly detecting method. Firstly, there is growing evidence that the CTCs population is heterogeneous, and co-expression of candidate gene markers and CTCs could be useful for disease monitoring, prediction of survival, and response to therapy. Secondly, some CTCs may have weak or no cytokeratin expression because cell differentiation inducing loss of these antigens can be present during EMT (Hofman et al., 2011). Thirdly, activated leukocytes number is higher in patients with cancer than in controls, and may express markers used to detect CTCs. Last but not the least, we combined the different time point of blood withdraw, as we know, the postoperative sampling time might reflect the most relevant CTCs status, rather than preoperative and intro-treatment CTCs level. (Muller & Schlimok 2000)All these limitation indicated that the detection of CTCs have some false negative and positive result technically. (Kowalewska et al., 2006)

Publication bias is a major problem in assessing the validity of clinical research studies. The publication bias for OS group analysis has been found by the Begg's test and funnel plot (Begg and Mazumdar 1994). Begg's test and funnel plot suggested that there was significant publication bias for OS group analysis (p=0.01) and no bias for PFS/RFS group analysis (p=0.142). We attempted to eliminated the publication bias by excluding the article caused the major bias, however, we did not find an article according the criteria. We hypothesized that the publication bias might be inherent in the analysis. One reason aroused the publication bias might be a small number of primary studies, and another reason was different detecting methods, sampling time, tumor differentiation, tumor stages and many other characteristics. In turn, we did not find evidence that publication bias may be significantly influencing our results. It should also be noted that our meta-analysis could not completely exclude biases. For example, positive results tend to be accepted by journals, while negative results are often rejected or even not submitted, which probably introduced bias.

Certain limitation still existed in statics method. Firstly, the multivariate analysis of logHR and SE could only be extracted using direct method, while the logHR and SE extracted from the survival curve and p-value are univariate analysis, these univariate analysis and multivariate analysis were combined together to ensure data integrity. Secondly, another source of biases was related to the eligible data extraction based on the Kaplan-Meier survival curve. LogHR and SE could not be reached reasonably well in a few cases.

In conclusion, the meta-analysis show available evidence supporting the proposition that CTCs detected in peripheral blood have a fine predictive role in pancreatic patients especially on the time point of post-treatment. It may be speculated that CTCs could perform as a fine detecting method in pancreatic cancer.

Acknowledgements

References

Begg CB, Mazumdar M (1994). Operating characteristics of a rank correlation test for publication bias. *Biometrics*, **50**, 1088-101.

Bidard FC, Huguet F, Louvet C, et al (2013). Circulating tumor cells in locally advanced pancreatic adenocarcinoma: the ancillary CirCe 07 study to the LAP 07 trial. *Ann Oncol*, **24**, 2057-61.

Cen P, Ni X, Yang J, et al (2012). Circulating tumor cells in the diagnosis and management of pancreatic cancer. *Biochim Biophys Acta*, **1826**, 350-6.

Cohen SJ, Punt CJ, Iannotti N, et al (2008). Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer. *J Clin Oncol*, **26**, 3213-21.

Cristofanilli M, Budd GT, Ellis MJ, et al (2004). Circulating tumor cells, disease progression, and survival in metastatic breast cancer. *N Engl J Med*, **351**, 781-91.

de Albuquerque A, Kubisch I, Breier G, et al (2012). Multimarker gene analysis of circulating tumor cells in pancreatic cancer patients: a feasibility study, *Oncology*, **82**, 3-10.

Dong X, Alpaugh KR, Cristofanilli M (2012). Circulating tumor cells (CTCs) in breast cancer: a diagnostic tool for prognosis and molecular analysis. *Chin J Cancer Res*, **24**, 388-98.

Ghaneh P, Costello E, Neoptolemos JP (2008). Biology and management of pancreatic cancer. *Postgrad Med J*, **84**, 478-97.

Goodman OB, Jr Symanowski JT, Loudyi A, et al (2011). Circulating tumor cells as a predictive biomarker in patients with hormone-sensitive prostate cancer. *Clin Genitourin Cancer*, **9**, 31-8.

Higgins JP, Thompson SG, Deeks JJ, et al (2003). Measuring inconsistency in meta-analyses. *BMJ*, **327**, 557-60.

Hiltermann TJ, Pore MM, van den Berg, et al (2012). Circulating tumor cells in small-cell lung cancer: a predictive and prognostic factor. *Ann Oncol*, **23**, 2937-42.

Hofman V, Ilie MI, Long E, et al (2011). Detection of circulating tumor cells as a prognostic factor in patients undergoing radical surgery for non-small-cell lung carcinoma: comparison of the efficacy of the CellSearch Assay and the isolation by size of epithelial tumor cell method. *Int J Cancer*, **129**, 1651-60.

Khoja L, Backen A, Sloane R, et al (2012). A pilot study to explore circulating tumour cells in pancreatic cancer as a novel biomarker. *Br J Cancer*, **106**, 508-16.

Kokhanenko, NIu, Ignashov AM, et al (2001). Role of the tumor markers CA 19-9 and carcinoembryonic antigen (CEA) in diagnosis, treatment and prognosis of pancreatic cancer. *Vopr Onkol*, **47**, 294-7.

Kowalewska M, Chechlinska M, Markowicz S, et al (2006). The relevance of RT-PCR detection of disseminated tumour cells is hampered by the expression of markers regarded as tumour-specific in activated lymphocytes. *Eur J Cancer*, **42**, 2671-4.

Kurihara T, Itoi T, Sofuni A, et al (2008). Detection of circulating tumor cells in patients with pancreatic cancer: a preliminary result. *J Hepatobiliary Pancreat Surg*, **15**, 189-95.

Ma XL, Xiao ZL, Liu L, et al (2012). Meta-analysis of circulating tumor cells as a prognostic marker in lung cancer. *Asian Pac J Cancer Prev*, **13**, 1137-44.

Mataki Y, Takao S, Maemura K, et al (2004). Carcinoembryonic antigen messenger RNA expression using nested reverse transcription-PCR in the peripheral blood during follow-up period of patients who underwent curative surgery for biliary-pancreatic cancer: longitudinal analyses. *Clin Cancer Res*, **10**, 3807-14.

Muller P, Schlimok G (2000). Bone marrow "micrometastases" of epithelial tumors: detection and clinical relevance. *J Cancer Res Clin Oncol*, **126**, 607-18.

Parmar MK, Torri V, Stewart L (1998). Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. *Stat Med*, **17**, 2815-34.

Sergeant G, Roskams T, van Pelt J, et al (2011). Perioperative cancer cell dissemination detected with a real-time RT-PCR assay for EpCAM is not associated with worse prognosis in pancreatic ductal adenocarcinoma. *BMC Cancer*, **11**, 47.

Sergeant G, van Eijsden R, Roskams T, et al (2012). Pancreatic cancer circulating tumour cells express a cell motility gene signature that predicts survival after surgery. *BMC Cancer*, **12**, 527.

Soeth E, Grigoleit U, Moellmann B, et al (2005). Detection of tumor cell dissemination in pancreatic ductal carcinoma patients by CK 20 RT-PCR indicates poor survival. *J Cancer Res Clin Oncol*, **131**, 669-76.

Tarhan MO, Gonel A, Kucukzeybek Y, et al (2013). Prognostic significance of circulating tumor cells and serum CA15-3 levels in metastatic breast cancer, single center experience, preliminary results. *Asian Pac J Cancer Prev*, **14**, 1725-9.

Tierney JF, Stewart LA, Ghersi D, et al (2007). Practical methods for incorporating summary time-to-event data into meta-analysis. *Trials*, **8**, 16.

Tjensvoll K, Nordgard O, Smaaland R (2013). Circulating tumor cells in pancreatic cancer patients: Methods of detection and clinical implications, *Int J Cancer*, **8**, 16.

Uchikura K, Takao S, Nakajo A, et al (2002). Intraoperative molecular detection of circulating tumor cells by reverse transcription-polymerase chain reaction in patients with biliary-pancreatic cancer is associated with hematogenous metastasis. *Ann Surg Oncol*, **9**, 364-70.

Uen YH, Lu CY, Tsai HL, et al (2008). Persistent presence of postoperative circulating tumor cells is a poor prognostic factor for patients with stage I-III colorectal cancer after curative resection. *Ann Surg Oncol*, **15**, 2120-8.

Wang FB, Yang XQ, Yang S, et al (2011). A higher number of circulating tumor cells (CTC) in peripheral blood indicates poor prognosis in prostate cancer patients--a meta-analysis. *Asian Pac J Cancer Prev*, **12**, 2629-35.

Williamson PR, Smith CT, Hutton JL, et al (2002). Aggregate data meta-analysis with time-to-event outcomes, *Stat Med*, **21**, 3337-51.

Zhou J, Hu L, Yu Z, et al (2011). Marker expression in circulating cancer cells of pancreatic cancer patients. *J Surg Res*, **171**, 631-6.

Zippelius A, Pantel K (2000). RT-PCR-based detection of occult disseminated tumor cells in peripheral blood and bone marrow of patients with solid tumors. An overview. *Ann N Y Acad Sci*, **906**, 110-23.