RESEARCH ARTICLE

Assessing the Diagnostic Value of Serum Dickkopf-related Protein 1 Levels in Cancer Detection: a Case-control Study and Meta-analysis

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Abstract

> **Background:** This study aimed to summarize the potential diagnostic value of serum DKK1 levels in cancer detection. **Materials and Methods:** Serum DKK1 was measured using enzyme-linked immunosorbent assay in a case-control study. Then we performed a meta-analysis and the pooled sensitivity, specificity, diagnostic odds ratio, and summary receiver operating characteristic (sROC) curves were used to evaluate the overall test performance. **Results:** Serum DKK1 levels were found to be significantly upregulated in gastric cancer as compared to controls. ROC curve analysis revealed an AUC of 0.636, indicating the test has the potential to diagnose cancer with poor accuracy. The summary estimates of the pooled sensitivity, specificity and diagnostic odds ratio in meta-analysis were 0.55 with a 95% confidence interval (CI) (0.53-0.57), 0.86 (95%CI, 0.84-0.88) and 12.25 (95%CI, 5.31-28.28), respectively. The area under the sROC was 0.85. Subgroup analysis revealed that the diagnostic accuracy of serum DKK1 in lung cancer (sensitivity: 0.69 with 95%CI, 0.66-0.74; specificity: 0.95 with 95%CI, 0.92-0.97; diagnostic odds ratio: 44.93 with 95%CI, 26.19-77.08) was significantly higher than for any other cancer. **Conclusions:** Serum DKK1 might be useful as a noninvasive method for confirmation of cancer diagnosis, particularly in the case of lung cancer.

Keywords: Serum DKK1 - cancer - diagnosis

Introduction

Dickkopf-related protein 1 (DKK1) is a member of the Dickkopf gene family, which is comprised of five evolutionarily conserved members, DKK1-4, and a unique DKK3-related member, DKKL1 (Dickkopf-like protein 1, Soggy) (Katoh and Katoh, 2005). Dickkopf-1 is a 35kDa secreted protein involved in embryonic development; it plays a critical role in cell patterning, proliferation, and fate determination during embryogenesis as a potent inhibitor of the Wnt signaling pathway (Ogoshi et al., 2011).

Unlike in normal development, the role of DKK1 in cancer is not as well characterized. DKK1 is upregulated in certain tumor types, including breast cancer (Forget et al., 2007), hepatoblastomas, and Wilms' tumor (Wirths et al., 2003). Some studies showed that DKK1 levels have also been associated with cancer progression and poor prognosis. DKK1 is overexpressed in prostate, lung, esophageal, and hepatic cancers, and, in these diseases, it may serve as a serologic and prognostic biomarker (Yamabuki et al., 2007). DKK1 is frequently

overexpressed in ovarian carcinoma and involved in tumor invasion and progression (Wang and Zhang, 2011). Taken together, these studies support a role for DKK1 in tumor development and progression.

In a number of other tumor types, DKK1 is actually downregulated, suggesting that, in these cancers, it might act as a tumor suppressor. Downregulation or loss of DKK1 is observed in most melanoma cell lines (Kuphal et al., 2006; Yaccoby et al., 2007); it is also frequently depleted or lost in colon cancer (Gonzalez-Sancho et al., 2005) and breast cancer (Mikheev et al., 2008). Moreover, several reports have shown that DKK1 overexpression suppresses tumor cell growth (Cowling et al., 2007; DiMeo et al., 2009; Fillmore et al., 2009; Liu et al., 2009; Mitra et al., 2010; Hirata et al., 2011). Mikheev et al showed that DKK1 induces cell death in MDA-MB-435 melanoma cells (Mikheev et al., 2007). Lee et al showed that DKK1 inhibits cell growth and induces apoptosis in human mesothelioma cells (Lee et al., 2004). Dickkopf-1 has also been found to suppress the tumorigenicity of two human breast cancer cell lines that lack active Wnt signaling pathways (Mikheev et al., 2008). These reports

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support a role for DKK1 as a tumor suppressor. Clearly, its role as a tumor suppressor or promoter may be cancer type dependent.

A huge benefit of a serum biomarker for cancer is that it allows comprehensive analysis of tumors without the need for biopsy, surgery, or other invasive procedures. Protein in serum or plasma could be novel potential biomarker for cancer diagnosis and prognosis(Baser et al., 2013; Ma et al., 2013). Along these lines, several groups have devoted time and energy to examining the utility of serum DKK1 as a cancer biomarker. Shen et al described a large-scale, multicenter validation study to assess the diagnostic accuracy of DKK1; they found that serum DKK1 could serve as a biomarker for the diagnosis of hepatocellular carcinoma (Shen et al., 2012). It may also be useful as a diagnostic marker in a number of cancers, including gastric, cervical, and endometrial cancers (Jiang et al., 2009; Lee et al., 2012). In contrast to these reports, Soydinc et al found that serum DKK1 did not display any diagnostic potential in colon or rectal cancers (Soydinc et al., 2011). An examination of multiple tumor types shows that DKK1 is significantly elevated in lung cancer (Sheng et al., 2009). Given the variability of DKK1 in cancer, we performed a case-control study and a meta-analysis to assess the diagnostic accuracy of serum DKK1 for cancer diagnosis.

Materials and Methods

Blood samples and isolation of serum

Blood samples from 98 gastric cancer (GC) patients (68 males and 30 females; mean age 54.1 years, range 32-74) and 80 healthy donors (47 males and 23 females; mean age 50.8 years, range 27-65) were collected from January 2011 to February 2012 in Zhejiang Provincial People's Hospital. Serum samples from gastric cancer patients were selected on the basis of the following criteria: (a) patients were newly diagnosed and (b) their tumors were pathologically diagnosed as gastric cancer. Serum was obtained at the time of diagnosis; it was centrifuged for 10 min at 1500×g in a swing bucket rotor at 4°C, and the supernatant was collected and stored at -80°C until use. Written informed consent was obtained from all participants involved, and the project was approved by the ethics committee of Zhejiang Provincial People's Hospital.

Determination of serum DKK1 levels by enzyme-linked immunosorbent assay (ELISA)

The serum concentration of DKK1 in gastric cancer patients and healthy controls was determined using the DKK1 ELISA Kit (Cusabio Biotech CO. Ltd., USA) according to the manufacturer's instructions. Each well of a 96-well plate, pre-coated with DKK1 antibody, was filled with 100 μl of standard (provided by manufacturer), sample (GC or healthy control), or sample diluent (blank) and incubated for 2 h at 37°C. Next, 100 μl of biotin-antibody working solution was added to each well and incubated for 1 h at 37°C. This was followed by addition of 100 μl horseradish peroxidase-avidin working solution. After incubating at 37°C for 1 h, the contents of each well were aspirated and replaced with 90 μl of the chromogenic

peroxidase substrate tetramethylbenzidene (TMB). The plate was protected from light and incubated for 30 min at 37°C. The reaction was terminated by adding 50 μl 'stop solution' (provided by manufacturer). The color intensity of each well was assessed using a microplate reader (Infinite M200, Tecan, Swtzerland) at an optical density of A450. The results are the means of at least three replicates.

Literature search

PubMed, Medline, and Google Scholar were used to search all relevant publications prior to 30th June 2013. Key index words included "serum DKK1", "serum Dickkopf-1", "cancer", "carcinoma", "tumor", and "neoplasm". All references of the included studies were also manually searched to identify any additional eligible studies.

The inclusion criteria for this meta-analysis were: 1) Papers must include a pathological diagnosis of cancer and measurements of serum DKK1 levels; 2) studies must include raw data, so that true-positives, false-positives, false-negatives, and true-negatives could be identified and calculated; 3) studies must include a reference standard for the diagnosis of cancer; 4) studies must include more than 20 patients. Exclusion criteria were: 1) studies with duplicate data reported in other studies; 2) studies not published in English.

Quality assessment

The methodological quality of included trials was assessed using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2 tool) by two independent reviewers; disagreements were resolved by consensus. Items included covered risk of bias and applicability concerns, including patient spectrum, reference standard, index test, and flow and timing. Items received a score of "1" if they were deemed "low risk"; aggregate scores were 7. Quality was reliable when the total score was ≥3 points.

Statistical analysis

In this case-control study, statistical analyses were performed using SPSS version 13.0 software. Receiver operating characteristic (ROC) analysis was used to determine the diagnostic potential of serum DKK1. The cutoff level in ELISA was set by ROC to provide optimal diagnostic accuracy for DKK1.

Meta-analysis was performed using Meta-Disc 1.4 software. We used a bivariate regression approach to calculate the pooled sensitivity (SEN) and specificity (SPE), positive and negative likelihood ratios (PLR and NLR, respectively), and summary receiver operating characteristic (SROC) curves to summarize the study results. The area under the sROC curve was an alternative global measure of test performance. A pooled PLR value greater than 10 indicated that the positive result of the given test was a useful confirmation of cancer. In contrast, a pooled NLR value less than 0.1 indicated that the negative result is useful for the exclusion of the disease. The diagnostic odds ratio (DOR) describes the odds of positive test results in patients with cancer compared

with the odds of positive results in those without cancer. The inter-study heterogeneity was evaluated by the I^2 test for the pooled DOR. $I^2 \geq 50\%$ indicated substantial heterogeneity.

Results

Diagnostic accuracy of serum DKK1 in gastric cancer in a case-control study

DKK1 protein was detected in serum samples from all 98 gastric cancer patients and all 80 healthy individuals. Serum DKK1 levels in GC patients were significantly different from levels in healthy individuals (332.9pg/ml±291.4 *vs* 225.4 pg/ml±136.1; *p*=0.002, Mann-Whitney U test; Figure 1A). Using an ROC curve based on our data, we selected 186.8pg/ml as the optimal DKK1 serum level for diagnosing gastric cancer. At this value, high accuracy with sensitivity (71.9%) and specificity (48.7%) was achieved. Receiver operating characteristic (ROC) curve
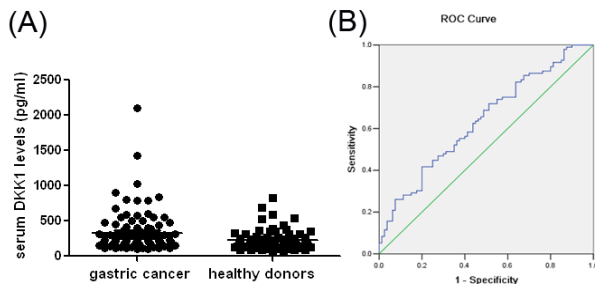

Figure 1. Serum DKK1 Levels in Gastric Cancer Patients and Healthy Donors. A) Serum DKK1 levels were significantly upregulated in gastric cancer compared to healthy controls (*p*<0.01); **B**) ROC curve analysis of diagnostic sensitivity and specificity of the serum DKK1 ELISA assay in gastric cancer. The area under the curve was 0.636
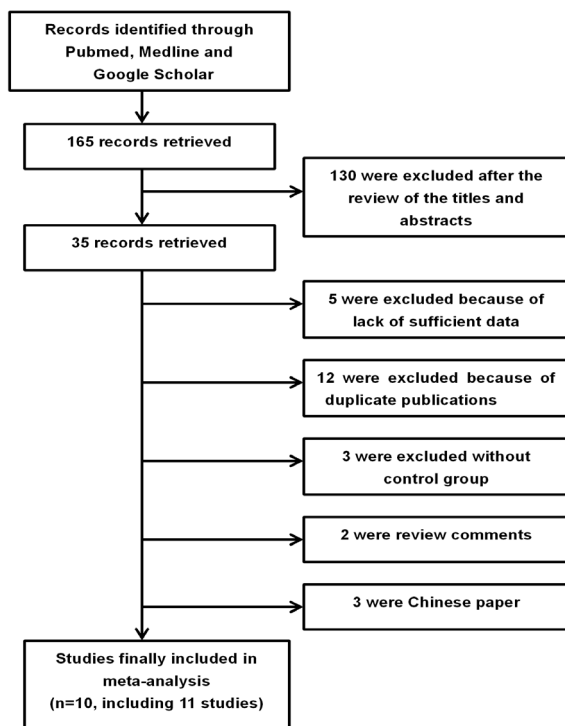

Figure 2. Flow Diagram of Identification of Relevant Studies

analysis revealed that the area under the curve (AUC) was 0.636, indicating that the test has the potential to classify cases as 'cancer' or 'normal' with poor some accuracy (Figure 1B).

Eligible articles for meta-analysis

The results of the literature research are presented in Figure 2. The initial search yielded a total of 165 potential relevant articles. 130 articles were excluded after the review of the titles and abstracts that is, these articles had no direct link to the main subject. Next, the 35 remaining full manuscripts were retrieved for detailed evaluation. Finally, 10 manuscripts in total (Yamabuki et al., 2007; Jiang et al., 2009; Sheng et al., 2009; Sato et al., 2010; Shen et al., 2010; Soydinc et al., 2011; Tung et al., 2011; Lee et al., 2012; Shen et al., 2012; Shi et al., 2013), which consisted of 11 studies, were included for evaluation according to the inclusion and exclusion criteria. The remaining 25 studies were excluded because of: a) lack of sufficient data (n=5); b) duplicate publications (n=12); c) lack of a control group (n=3); d) being a review article (n=2); or e) printed in Chinese (n=3).

The primary characteristics of the studies, including the characteristics of the present case-control study (12 studies in total), used in the meta-analysis are shown in Table 1. The 12 studies were conducted in different countries. Six studies were conducted in China, 2 studies in Japan, 1 in Taiwan, 1 in Hong Kong, 1 in Korea, and 1 study in Turkey. The publication years ranged from 2007 to 2013. Six studies used an ELISA kit to measure serum DKK1 levels; 3 studies used an ELISA pre-coated with their own antibody; and 3 studies performed ELISA with unclear or insufficient information. Additionally, 9 studies included healthy volunteers as controls; one study included both healthy volunteers and patients with benign disease as controls; one study used cancer-free volunteers as controls; and one chose benign patients as controls.

Quality assessment of the included studies

The quality and bias of the 12 studies were evaluated based on the evaluation criteria of the risk of bias and applicability concerns to evaluate the quality of the literature. The methodological quality assessment for the included studies is shown in Table 2 and in Figure 3. All
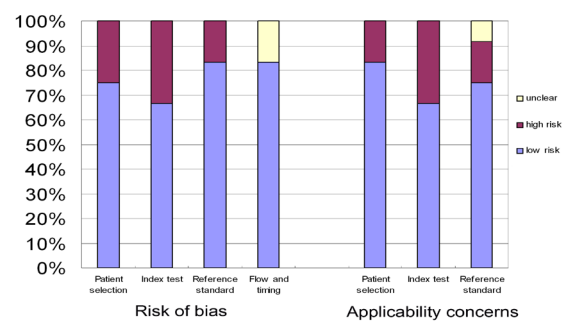

Figure 3. Overall Quality Assessment of Included Studies (QUADAS-2 tool): Proportion of Studies with Low, High, or Unclear Risk of Bias (left), Proportion of Studies with Low, High, or Unclear Concerns Regarding Applicability (right)

Table 1. Characteristics of Studies Included in the Meta-Analysis

First author	Year of Publication	Country of Origin	TP	FP	FN	TN	Tumor Type	Control	Detection Method
Lee	2012	korea	134	21	19	152	GCa	Healthy donors	ELISA kit
Shi	2012	China	28	0	9	50	ICCb	Healthy donors	ELISA kit
Shen	2012	China	293	20	131	193	HCCC	Healthy donors	ELISA kit
Soydinc	2011	Turkey	175	45	120	45	CRCd	Healthy donors	unclear
Sato	2010	Japan	469	10	437	197	PC+GC+HCC +BDC+BC+CCe	Healthy donors	ELISA**
Jiang	2009	China	32	7	8	23	CC	Healthy donors	unclear
Jiang	2009	China	19	9	9	20	ECf	Healthy donors	unclear
Sheng	2009	China	184	6	408	114	LC+GC+CRC +OC+CCg	Healthy and benign disease	ELISA
Yamabuki	2007	Japan	236	10	110	197	LC+SCC +SCLC+ESCCh	Healthy donors	ELISA
Shen	2010	Taiwan	39	15	36	60	UCi	Cancer-free volunteers	ELISA kit
Tung	2011	Hongkong	30	0	70	50	HCC	HBV carriers	ELISA kit
Jiang*	2013	China	68	41	28	39	GC	Healthy donors	ELISA kit

*The present study; ** ELISA precoated by their own antibody; aGC: gastric cancer; bICC: Intrahepatic cholangiocarcinoma; CHCC: Hepatocellular cancer; dCRC: Colorectal cancer; ePC: Pancreatic cance, BDC: bile duct cancer, BC: Breast cancer, CC: Cervical cancer; fEC: Endometrial cancer; gLC: lung cancer, OC: ovarian cancer; c SCC: squamous cell carcinoma, SCLC: small cell lung cancer, ESCC: esophageal squamous cell carcinoma; iUrothelial carcinoma; UC. TP: true positive; FP: false positive; FN: false negative; TN: true negative

Table 2. Measurement for Included Papers using Quality-Assessment Tool for Diagnostic Accuracy Studies (QUADAS-2 tool)

Studies	Risk of bias				Applicability concerns			
	Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard	
Lee et al 2012	High risk	Low risk	Low risk	Low risk	High risk	Low risk	Low risk	
Shi et al 2012	High risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	
Shen et al 2012	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	
Soydinc et al 2011	Low risk	High risk	Low risk	Unclear	Low risk	High risk	Low risk	
Sato et al 2010	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	
Jiang et al 2009	Low risk	High risk	Low risk	Low risk	Low risk	High risk	Low risk	
Jiang et al 2009	Low risk	High risk	Low risk	Low risk	Low risk	High risk	Low risk	
Sheng et al 2009	Low risk	Low risk	High risk	Low risk	Low risk	Low risk	High risk	
Yamabuki et al 2007	Low risk	Low risk	Low risk	Unclear	Low risk	Low risk	Low risk	
Shen et al 2010	High risk	Low risk	High risk	Low risk	High risk	Low risk	High risk	
Tung et al 2011	Low risk	High risk	Low risk	Low risk	Low risk	High risk	Unclear	
Jiang et al 2013*	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	

studies included in our meta-analysis met, on average, 5.3 of the 7 QUADAS criteria, which is indicative of high quality. Of the 12 eligible studies, 3 studies had QUADAS scores of 7, 2 studies had scores of 6, 4 studies had scores of 5, 2 studies had QUADAS scores of 4, and 1 had a QUADAS score of 3.

Threshold effect

For serum DKK1, computation of the Spearman correlation coefficient between the logit of sensitivity and the logit of 1-specificity was 0.284 (p=0.371), indicating no threshold effect existed in the present study. It also indicated that the positive correlation had no statistical significance

Data synthesis and meta-analysis

The ranges of the sensitivity and specificity were 30%-88% and 49%-100%, respectively (Table 3, Figure 4). The pooled sensitivity (SEN) of serum DKK1 was 0.55 with a 95% confidence interval (CI) (0.53-0.57), and the pooled specificity (SPE) was 0.86 (95%CI, 0.84-0.88). Additionally, the pooled positive likelihood ratio (PLR)

was 4.99 (95%CI, 2.49-9.97), suggesting that patients with cancer have a nearly 5-fold higher chance of testing positive for serum DKK1 levels compared to patients without cancer. The negative likelihood ratio (NLR) was 0.45 (95%CI, 0.34-0.58), and the DOR was 12.25 (95%CI, 5.31-28.28). The SROC curve shows an overall summary of tests, which illustrates the relationship between SEN and SPE. As shown in Figure 5, the area under the SROC curve was 0.85 and the Q* was 0.78, indicating that serum DKK1 displayed reasonable accuracy in terms of differential diagnosis in cancer.
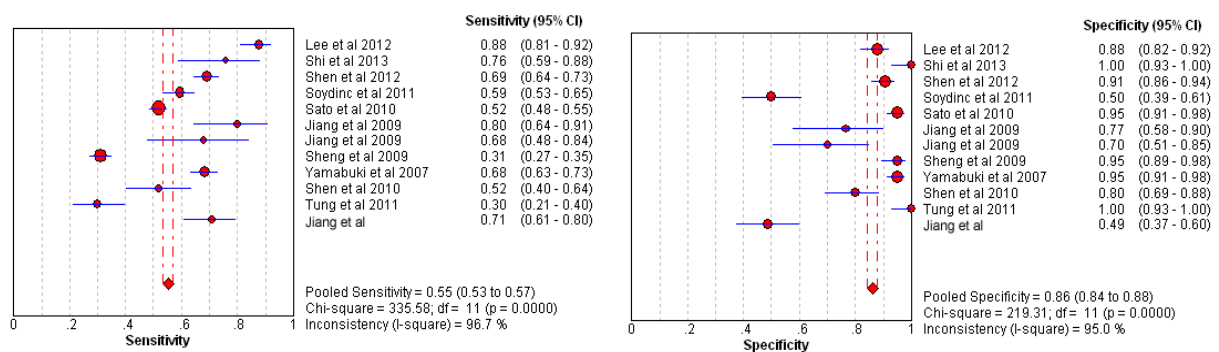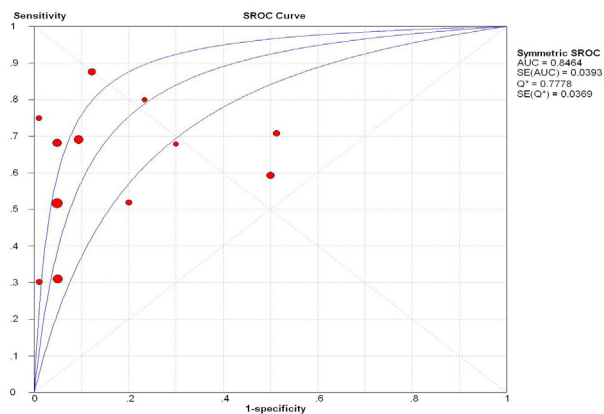
Heterogeneity assessment and meta-regression analysis

The I^2 test for the pooled DOR indicated the I^2 was 92.9% (p<0.001); it was 96.7% (p<0.001) and 95% (p<0.001) for SEN and SPE, respectively. This illustrates substantial heterogeneity among studies (Table 3). The meta-regression analysis was used to explore the overall heterogeneity and the possible sources of heterogeneity, including the type of negative control, country, assay method, and cutoff value. Our analysis showed that none of these factors were responsible for the observed

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Table 3. Pooled Results of the Meta-Analysis of the Diagnostic Accuracy of Serum DKK1 in Cancer

Studies	Sensitivity	Specificity	Positive LR	Negative LR	DOR
All studies	0.55 (0.53-0.57)	0.86 (0.84-0.88)	4.99 (2.49-9.97)	0.45 (0.34-0.58)	12.2 (5.31-28.3)
Lee et al 2012	0.88 (0.81-0.92)	0.88 (0.82-0.92)	7.22 (4.81-10.82)	0.14 (0.19-0.22)	51.0 (26.3-99.0)
Shi et al 2013	0.76 (0.59-0.88)	1.00 (0.83-1.00)	76.5 (4.82-1213.9)	0.25 (0.15-0.44)	303.0 (16.9-5401.3)
Shen et al 2012	0.69 (0.65-0.74)	0.91 (0.86-0.94)	7.36 (4.83-11.2)	0.34 (0.29-0.39)	21.6 (13.0-35.7)
Soydinc et al 2011	0.59 (0.54-0.65)	0.50 (0.39-0.61)	1.19 (0.95-1.49)	0.81 (0.64-1.04)	1.5 (0.91-2.34)
Sato et al 2010	0.52 (0.49-0.55)	0.95 (0.91-0.98)	10.7 (5.84-19.6)	0.51 (0.47-0.55)	21.1 (11.1-40.4)
Jiang et al 2009	0.80 (0.64-0.91)	0.77 (0.58-0.90)	3.43 (1.76-6.68)	0.26 (0.14-0.50)	13.1 (4.17-41.4)
Jiang et al 2009	0.68 (0.48-0.84)	0.70 (0.51-0.85)	2.26 (1.24-4.13)	0.46 (0.26-0.83)	4.93 (1.62-14.9)
Sheng et al 2009	0.31 (0.27-0.35)	0.95 (0.89-0.98)	6.22 (2.82-13.68)	0.73 (0.68-0.78)	8.57 (3.70-19.8)
Yamabuki et al 2007	0.68 (0.63-0.73)	0.95 (0.91-0.98)	14.1 (7.68-25.9)	0.33 (0.29-0.39)	42.3 (21.5-82.9)
Shen et al 2010	0.52 (0.40-0.64)	0.80 (0.69-0.88)	2.60 (1.57-4.29)	0.60 (0.46-0.78)	4.33 (2.09-8.95)
Tung et al 2011	0.30 (0.21-0.40)	1.00 (0.93-1.00)	30.8 (1.92-493.5)	0.71 (0.62-0.80)	43.7 (2.61-731.4)
Jiang et al 2013*	0.71 (0.62-0.79)	0.49 (0.37-0.62)	1.38 (1.08-1.77)	0.59 (0.41-0.88)	2.31 (1.24-4.29)
Heterogeneity p value (I^2)	<0.001 (96.7%)	<0.001 (95.0%)	<0.001 (95.8%)	<0.001 (96.5%)	<0.001 (92.9%)

*The present study; LR: Likelihood Ratio; DOR: Diagnostic Odds Ratio

Figure 4. Forest Plot Showing Study-Specific and Mean Sensitivity and Specificity with Corresponding Heterogeneity Statistics

Figure 5. Summary ROC Curve (sROC) with Confidence and Prediction Regions around Mean Operating Sensitivity and Specificity Point

heterogeneity in serum DKK1 levels (data not shown).

Diagnostic accuracy of serum DKK1 in different cancer types (subgroup analysis)

Included in this study were 2 studies on gastric cancer, 2 hepatocellular carcinoma studies, 1 study on colorectal cancer, 1 intrahepatic cholangiocarcinoma study, 1 pancreatic cancer study, 1 study on bile duct cancer, and 1 study on esophageal squamous cell carcinoma. In total, there are 9 studies focused on serum DKK1 levels in digestive tract cancer. The pooled SEN of serum DKK1 was 0.54 (95%CI, 0.52-0.56), the pooled SPE of serum

DKK1 was 0.87 (95%CI, 0.85-0.89), and the DOR was 12.38 (95%CI, 4.23-36.04).

Additional, there was 1 study on cervical cancer, 1 on endometrial cancer, 1 on breast and cervical cancers, and 1 study on cancer of the ovaries and cervix. Overall, there are 4 studies that reported data on serum DKK1 levels in gynecological cancers. For these cases, the pooled SEN of serum DKK1 was 0.48 (95%CI, 0.44-0.52), the pooled SPE of serum DKK1 was 0.92 (95%CI, 0.89-0.94), and the DOR was 8.33 (95%CI, 2.18-31.78).

Two studies reported data on serum DKK1 levels in lung cancer. The pooled SEN of serum DKK1 was 0.69 (95%CI, 0.66-0.74), the pooled SPE of serum DKK1 was 0.95 (95%CI, 0.92-0.97), and the DOR was 44.93 (95%CI, 26.19-77.08). These data show that that the diagnostic accuracy of serum DKK1 in lung cancer is significantly higher than it is in any other cancer. The I^2 test for the pooled DOR indicated the I^2 was 0.00% (p=0.949). SEN was 0.0% (p=1.000) and SPE was 0.0% (p=0.946), showing no heterogeneity among the existing studies. The results also suggest that different cancer types may actually be the primary source of the heterogeneity.

Discussion

Serum DKK1 has emerged as a promising biomarker for cancer diagnosis. However, its diagnostic accuracy is quite variable from one report to the next. As a secreted protein, DKK1 is elevated in serum from patients with

diverse malignancies. In lung cancer, DKK1 was found to be a novel serologic and histochemical biomarker, as well as a therapeutic target (Yamabuki et al., 2007). Shen et al found DKK1 to be elevated in hepatocellular carcinoma, where it complemented measurement of AFP in the diagnosis of HCC (Shen et al., 2012). Kaba et al found that serum DKK-1 may represent a novel marker for bladder cancer determination and poor clinical outcome in Turkish patients(Kaba et al., 2014). On the contrary, in human colon tumors, DKK1 expression is actually decreased, and, in leukemia, DKK1 acts as a tumor suppressor; specifically, in leukemia, the DKK1 promoter is selectively hypermethylated, resulting in epigenetic silencing (Gonzalez-Sancho et al., 2005; Suzuki et al., 2007). In prostate cancer, DKK1 first increases and then decreases during progression from primary tumor to metastatic lesion (Hall et al., 2008). Other reports in several cancers, including colorectal, gastric, ovarian, and cervical cancers suggest that serum DKK1 levels may not actually have any diagnostic potential (Sheng et al., 2009; Soydinc et al., 2011). The reason for this discrepancy may be originated from racial difference and tissue specificity of different cancer. Thus, the exact value of serum DKK1 as a diagnostic tool requires additional investigation. In the present meta-analysis, we find that the range of sensitivity and specificity are 30%-88% and 49%-100%, respectively. The pooled SEN and SPE of serum DKK1 are 0.55 and 0.86, suggesting that the diagnostic accuracy of serum DKK1 is very limited and heterogeneous.

Additionally, we found significant heterogeneity between studies. As a result, we used DOR and AUC to evaluate the potential diagnostic value of serum DKK1. Although DOR is difficult to clinically interpret, it is useful in the assessment of the overall test accuracy in meta-analysis from a statistical point of view (Glas et al., 2003; Araujo et al., 2009). The DOR was 12.25, and the area under the SROC curve was 0.85, indicating that serum DKK1 had reasonable accuracy in terms of differential diagnosis between "normal" and "cancer". However, since there is considerable heterogeneity in the present analysis, the application of serum DKK1 in cancer diagnosis is limited. A primary goal of cancer screening is early detection; in many cases, this significantly improves patient outcome. Here, we show that serum DKK1 may be a convenient, noninvasive, low cost biomarker for cancer diagnosis. Clearly, further work must be done to validate these findings.

Different cut-off values were used for the included 12 studies. Thus, Spearman correlation coefficients were used to analyze the threshold effect. We found no statistically significant difference (R= 0.28, *p*=0.37), indicating that a threshold effect was not the source of the heterogeneity. Meta-regression analysis was then used to explore the possible sources of heterogeneity, including the type of negative control, assay method, country, and cutoff value. Results showed that none of the tested factors were responsible for serum DKK1 heterogeneity.

In our present case-control study of the Southeastern Han Chinese population, we found that serum DKK1 levels were significantly higher in gastric cancer patients than in healthy controls. However, the sensitivity and specificity were 71.9% and 48.7%, respectively. The area under the ROC curve was 0.636, indicating that the diagnostic accuracy of serum DKK1 in gastric cancer is insufficient for clinical application. In order to explore the sources of heterogeneity, sub-group analysis was also performed. Consistent with our present case-control study, the meta-analysis also revealed that the pooled SEN and SPE of serum DKK1 in digestive tract cancer was 0.54 and 0.87, respectively. Additionally, the DOR of the pooled studies was 12.38. For gynecological cancer, the pooled SEN and SPE of serum DKK1 was 0.48 and 0.92, and the DOR was 8.33. However, the pooled SEN and SPE of serum DKK1 in lung cancer was 0.69 and 0.95, and the DOR was 44.93. These results show that the diagnostic accuracy of serum DKK1 in lung cancer is significantly higher than it is in any other cancer. Thus, it may prove to be a useful screening tool specifically for lung cancer. The differential DOR values among different types of cancer suggest that the type of cancer itself may actually be the main source of heterogeneity. This idea is supported by data from the I^2 test.

It should be noted that there are several important limitations of the present study. First, we did not explore diagnostic accuracy for early stage (stage I-II) cancers; this is because of insufficient raw data in these cases. Furthermore, primary data to investigate the elevated or decreased serum DKK1 values as a marker for tumor type, histology, or stage was not available. Second, the healthy controls included in our study proved to be quite heterogeneous. For example, one study used healthy individuals as controls and another used those with benign disease. The proper control groups must be established so that the accuracy of serum DKK1 as a diagnostic tool may not be overestimated. Third, many of the studies suffered from incomplete or inaccurate reporting; in these cases, we cannot correctly identify potential sources of bias and variability.

In conclusion, the current evidence suggests that serum DKK1 has potential diagnostic value in cancer. However, its applicability is limited because of substantial heterogeneous. Of all cancer types, DKK1 may be most useful as a screening tool in lung cancer. We propose that serum DKK1 may be a useful tool for monitoring cancer in a convenient, noninvasive, and low cost way. However, further studies are needed to identify additional biomarkers and to identify specific patients that may most benefit from these tools.

Acknowledgements

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