Clinical Significance of Pim-1 in Human Cancers: A Meta-analysis of Association with Prognosis and Clinicopathological Characteristics

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Abstract

Background: Pim-1 is overexpressed in cancer tissues and plays a vital role in carcinogenesis. However, its clinical significance in cancers is not fully verified by meta-analysis, especially in relation to prognosis and clinicopathological features.

Methods: Four databases, PubMed, Embase, Cochrane Library, and Web of Science, were searched. Literature screening and data extraction according to the inclusion and exclusion criteria. The quality of the included literatures was evaluated using the Newcastle-Ottawa scale and the data analysis was performed using STATA and Review Manager software.

Results: 15 articles were finally included for meta-analysis, involving 1651 patients. Effect-size pooling analysis showed that high Pim-1 was related to poor overall survival (OS) (HR 1.68 [95% CI 1.17-2.40], *P* = .004) and disease-free survival (DFS) (HR 2.15 [95 %CI 1.15-4.01], *P* = .000). Subgroup analysis indicated that the detection techniques of Pim-1 were the main sources of heterogeneity, and 2 literatures using quantitative polymerase chain reaction (qPCR) for Pim-1mRNA had high homogeneity (I^2 = .0%, *P* = .321) in OS. Another 13 studies that applied immunohistochemistry (IHC) for Pim-1 protein had significant heterogeneity (I^2=82.2%, *P* = .000; I^2=92%, *P* = .000) in OS and DFS, respectively, and further analysis demonstrated that ethnicity, sample size, and histopathological origin were considered to be the main factors affecting their heterogeneity. In addition, high Pim-1 was associated with lymph node metastasis (OR 1.40 [95% CI 1.02-1.92], *P* = .04), distant metastasis (OR 2.69 [95%CI 1.67-4.35], *P* < .0001), and clinical stage III-IV (OR .7 [95% CI .50-.96, *P* = .03). Sensitivity analysis suggested that the pooled results of each effect-size were stable and reliable, and there was no significant publication bias (*P* = .138) in all included articles.

Conclusion: High Pim-1 can not only predict poor OS and DFS of cancer, but also help to infer the malignant clinical characteristics of tumor metastasis. Pim-1 may be a potential and promising biomarker for early diagnosis, prognostic analysis and targeted therapy of tumors.

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Introduction

Cancer has become a major disease that seriously threatens human life and health. In 2020, the age-standardized incidence rate by world standard population reached 201.0/100 000, while the age-standardized mortality rate by world standard population was 100.7/100 000.[1] Based on the development in mechanism research of cancer relying on multi-omics technologies such as genome, transcriptome, proteome, and metabolome, remarkable progress has also been made in the clinical efficacy. Moreover, with the advancement of detection technology, novel tumor markers such as oncogenes, proteins, non-coding RNAs, and circulating tumor DNA, have been discovered and used in clinical practice to provide more options and means for the precise diagnosis and treatment of malignant tumors.[2] Among them, a series of protein kinases have been recognized.[3] In particular, the functional biomarkers represented by serine/threonine kinases Pim kinase family in malignant tumors and their constructed signaling pathway network and clinical application prospects have received much attention.[4,5]

As an important Pim family member, Pim-1 was originally identified as a provirus insertion site for Moloney murine leukemia virus.[6] In addition to the induction of cytokines,[7] mitogens,[8] hypoxia,[9] hormones,[10] and infection factors,[11] the expression of Pim-1 is also regulated and activated through upstream signaling pathways, such as JAK-STAT,[12] PI3K-AKT-mTOR,[13] and NF-κB.[14] Studies have unveiled that Pim-1 is widely involved in the occurrence and development of multiple human cancers.[15] Mechanistically, Pim-1 can positively promote G1/S and G2/M at the cycle restriction point by activating cyclin CDC25 A and CDC25 C,[16,17] and inhibiting p21cip1/waf1 and p27Kip1 proteins.[18,19] Moreover, Pim-1 also take part in mediating the redistribution of mitosis and accelerating cell division progression.[20] In addition, Pim-1 kinase could suppress apoptosis via inactivating the pro-apoptotic protein BAD by direct phosphorylation, enhancing the activity of anti-apoptotic protein BCL-2, and indirectly inhibiting ASK1-mediated activation of the pro-apoptotic protein Caspase3 by phosphorylating the substrate ASK1.[21,22] Accumulating studies suggest that epithelial-mesenchymal transition (EMT) and stem cell characteristics in cancer cells are the key mechanisms of tumor invasion and metastasis.[23] Interestingly, Pim-1 kinase is considered to be the key factor in the mechanism of EMT induced by inflammatory factor IL6 in cancer cells, as evidenced by the decreased expression of Snail, N-cadherin, and Twist after Pim1 silencing. On the contrary, Pim-1 overexpression can down-regulate E-cadherin, up-regulate vimentin, and increase stem-like characteristics, ultimately.[24,25] In light of the vital roles of Pim1 in tumor progression, it is our believe that Pim-1 maybe a promising candidate biomarker for cancer with great clinical significance.

However, clinical significance of Pim-1 has not been widely recognized and is somewhat controversial in different tumors. Studies have shown that the expression of Pim-1 is negatively correlated with the prognosis of leukemia,[26] lymphoma,[27] head and neck tumors,[28-30] osteosarcoma,[31-33] gallbladder cancer,[34] colorectal cancer,[35,36] and non-small cell lung cancer (NSCLC).[37] Nevertheless, Reiser-Erkan et al[38] showed that Pim-1 kinase overexpression in pancreatic cancer had a good prognosis. Until now, there are no data evaluating the relationship between Pim-1 expression and prognostic value as well as clinical features through meta-analysis. Therefore, we conducted a meta-analysis to assess whether Pim-1 could be used as an emerging biomarker for human cancers in clinic.

Method

Literature Screening and Search Strategy

This meta-analysis was based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA),[39] a comprehensive search of four major electronic databases, PubMed (https://www.ncbi.nlm.nih.gov/pubmed), Cochrane library (https://www.cochranelibrary.com), Embase (https://www.embase.com), and Web of Science (https://www.webofknowledge.com), and the literature search was updated to December 31, 2020. The search strategy was as follows: (((((((((((((((((Neoplasm, Benign) OR (Benign Neoplasm)) OR (Neoplasms, Benign)) OR (Benign Neoplasms)) OR (Neoplasms, Malignant)) OR (Neoplasm, Malignant)) OR (Malignant Neoplasm)) OR (Malignant Neoplasms)) OR (Malignancies)) OR (Malignancy)) OR (Cancers)) OR (Cancer)) OR (Tumor)) OR (Tumors)) OR (Neoplasm)) OR (Neoplasia's)) OR (Neoplasia)) OR (Neoplasms)) AND ((((((pim-1 proto-oncogene protein) OR (pim-1 oncogene protein)) OR (pim-1 protein)) OR (proto-oncogene protein pim-1)) OR (Pim-1 protein)) OR (Pim-1)). Two authors participated in the whole process of the literature search and performed the first round of screening based on titles and abstracts to exclude studies on unrelated topics. The included articles were then screened by reading the full text, and studies that did not meet the inclusion criteria were excluded. This meta-analysis was registered with PROSPERO (https://www.researchregistry.com) before implementation.

Inclusion and Exclusion Criteria

The included studies should meet the following criteria: (1) Patients with pathologically confirmed malignant tumors and without receiving initial treatment before pathological sampling; (2) The long-term efficacy of tumors was compared by stratification of pim-1 expression levels in tumor tissues before treatment, and the detection techniques of pim-1 expression included qPCR and IHC; (3) The survival endpoint was OS and DFS; (4) The types of included studies included observational studies such as case-control or cohort and randomized controlled interventional studies; (5) All the

included studies were original English literatures that had already been published with full text. Exclusion criteria: (1) Literature review, case report, conference abstract, or animal experiments; (2) Without the data of efficacy between pim-1expression level and prognosis. (3) without clinicopathological characteristics data for analysis.

Data Extraction

Two authors performed data extraction from the included studies using a standardized data collection form, and disagreements between authors were resolved by consultation and, if necessary, with a third author. The extracted data include the publication year, study type, location, sample size, study subjects and other main clinicopathological characteristics as well as the number of samples for age, gender, depth of invasion (T stage), lymph node metastasis (N stage), distant metastasis (M stage), clinical stage at the high and low pim-1 expression levels in the tissues, as well as the primary outcomes (OS and DFS) between high and low pim-1 expression levels in the tissues, The HR, OR and 95% CI values clearly mentioned in the study were directly extracted, if there was no direct data, the survival curve was extracted using the software Engauge Digitizer 11.1, and then the HR and 95% CI values were calculated by the Excel table of Tierney,[40,41] and OR and 95% CI values were calculated by software Review Manager 5.4 to achieve indirect extraction.

Literature Quality Evaluation

Literature quality evaluation was performed independently by two other authors. Any disagreement was resolved by discussion until consensus was reached. If disagreement remained, the corresponding author was invited for discussion to obtain a final consensus. The quality of the included nonrandomized studies was assessed using the NOS.[42]

Statistical Analysis

Statistical analysis was performed using Stata 15.0 (Stata Corporation, College Station, TX) and Review Manager 5.4 (Cochrane Centre, Netherland) software, and the pooled effect size of the included studies was considered statistically significant at P-value <.05. The effect size of the included studies was judged homogeneous by the heterogeneity test, and the degree of heterogeneity was assessed by calculating the I^2 statistic and the chi-square test. When $I^2 \leq 50\%$ and $P > .1$ is considered that there is no significant heterogeneity, adopt the fixed effect model; when I2 > 50% and P ≤ .1 is considered that there is significant heterogeneity, adopt the random effect model to combine and analyze the effect size, and further conduct the sensitivity analysis of overall risk by deleting each study one by one, so as to evaluate the effect of single study.[43] The publication bias of the included studies (≥9) was evaluated by drawing funnel plots, and the pooled data were tested for asymmetry using the Begg test.

Results

Study Characteristics

Using the search strategy to search the four major electronic databases, 2538 relevant articles were initially retrieved, including PubMed 782, Embase 1236, Cochrane Library 5, and Web of Science 515. After screening the titles, abstracts and full texts successively, a total of 15 relevant studies that completely met the inclusion and exclusion criteria and could be used for data extraction were finally included in the meta-analysis. The literature screening process can be seen in the flow chart (Figure 1).

The 15 included articles were all observational studies, of which 11 studies were from China and the remaining 4 studies were from the United States, Germany, Finland, and Poland, respectively. There are only 2 studies to detect pretreatment Pim-1 mRNA by qPCR in cancerous tissues, and IHC was used to detect Pim-1 protein expression in the remaining 13. The cutoff value of Pim-1 mRNA expression level was defined by the $2^{-\triangle\triangle CT}$, while the cutoff value of Pim-1 protein expression level was defined by immunohistochemical staining results (IRS) based on the proportion of positive cells and the staining intensity of positive reactions. The total sample size is 1651 cases. Among the included studies, the minimum sample size is 43 cases and the maximum is 343. The study subjects involved 10 cancers: osteosarcoma, acute myelogenous leukemia, primary central nervous system lymphoma, bronchopulmonary neuroendocrine tumor, colorectal cancer, gallbladder cancer, salivary gland adenoid cystic carcinoma, pancreatic cancer, head and neck squamous-cell cancer, and NSCLC. Treatment was mentioned in 10 studies, of which 9 received the surgery or adjuvant chemoradiotherapy on this basis, and 1 received chemotherapy combined with hematopoietic stem cell transplantation. The follow-up time of the included studies ranged from 12 to 80 months (Table 1).

Quality Assessment

Among the included articles, the NOS scores of the 15 observational studies were above 5 points, and the quality of the included literatures was high overall (Table 2).

Meta-Analysis and Heterogeneity Test

Pim-1 expression and clinicopathological features. The age, gender, T stage, N stage, M stage, and clinical stage of patients with high and low expression of Pim-1 in each group were extracted from the literature, and the relationship between Pim-1 expression and clinicopathological characteristics was analyzed.
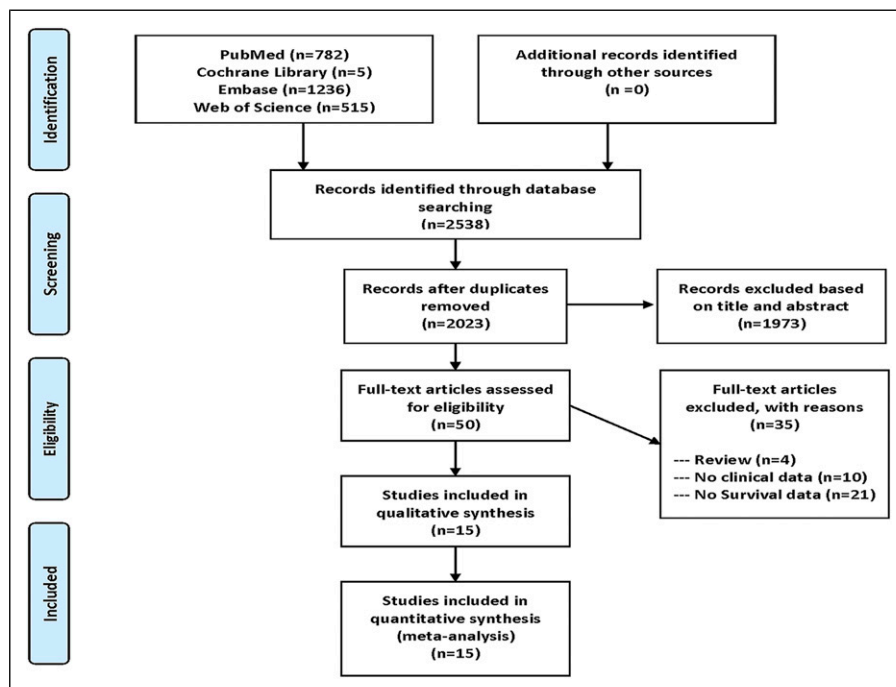
Figure 1. Flow diagram of study selection.

Age

3 articles related to age （≥60/＜60） were included. The heterogeneity test of fixed-effect model showed no significant heterogeneity (I^2 = 42%, P = .18) (Figure 2A). The pooled effect size showed that there was nonsignificant difference in the group of high Pim-1 expression around the age of 60 (OR 1.00 [95% CI .69-1.47], P = .99) (Figure 2A).

Gender

Eleven articles related to gender （male/female） were included, the results displayed no significant heterogeneity (I^2 = 22%, P = .24) (Figure 2B), and there was no significant correlation between high Pim-1 expression and gender (OR .80 [95% CI .63-1.02, P = .08) (Figure 2B).

T stage. Four literatures related to T stage (T1 + T2/T3 + T4) were included. The random-effects model heterogeneity test showed that there was significant heterogeneity (I^2 = 83%, P = .0006) (Figure 2C). The pooled effect size showed that there was no significant relation between degrees of infiltration and Pim-1 expression (OR .26 [95%CI .05-1.37], P = .11) (Figure 2C).

N Stage

Six literatures related to N stage (N0/N1-3) were included. A fixed-effects model was selected for the meta-analysis and the results suggested that no significant heterogeneity (I^2 = 30%, P

= .21) (Figure 3A). The pooled effect size showed that high expression of Pim-1 was related to lymph node metastasis (OR 1.40 [95% CI 1.02-1.92], P = .04) (Figure 3A).

M Stage

Seven literatures related to M stage （M0/M1） were included. The heterogeneity test of fixed-effect model demonstrated heterogeneity without significance (I^2 = 0%, P = .53) (Figure 3B). The pooled effect size revealed that high Pim-1 has correlation with distant metastasis (OR 2.69 [95% CI 1.67-4.35], P < .0001) (Figure 3B).

Clinical Stage

Five literatures related to clinical stage （I+II/III+IV） were included. A fixed-effects model was used for the meta-analysis. The results showed no significant heterogeneity (I^2=36%, P = .18) (Figure 3C). The pooled effect size indicated that high Pim-1 has relationship with advanced clinical stage (OR .7 [95% CI .50-.96], P = .03) (Figure 3C).

Pim-1 Expression and Prognosis

Fifteen articles with OS as the study endpoint and 5 articles with DFS as the study endpoint. Overall, the effect size HR and 95% CI of OS and DFS at high and low expression levels of Pim-1 in tumor tissues of all included literatures were tested for heterogeneity by random-effect model, respectively. The results demonstrated that there was significant heterogeneity

Table 1. Characteristics of All Included Studies.

Study ID	Country	Study design	Total	Gender (M/F)	Sample case				Detection method	Cancer type	HR (95% CI)	Follow-up (months)	Outcome
					High PIM-1		Low PIM-1						
					Definition	N	Definition	N					
Motylewska, E. 2020	POL	Retrospective	49	27/22	IRS ≥ 2	NA	IRS < 2	NA	IHC	Bronchopulmonary neuroendocrine neoplasm	4.63 (1.1-19.63)	NA	OS
Zhou, Y. 2018	CHN	Retrospective	57	30/27	NA	40	NA	17	IHC	Primary central nervous system lymphoma	2.102 (.777-5.691)	40 (max)	OS
Zhang, M. 2018	CHN	Retrospective	296	173/123	IRS ≥ 3	242	IRS < 3	54	IHC	Colorectal cancer	1.511 (.697-3.276)	80 (median)	DFS, OS
Xu, J. 2018	CHN	Retrospective	97	39/58	IRS≥5	82	IRS < 5	15	IHC	Salivary adenoid cystic carcinoma	NA	140 (max)	OS
Xue, C. 2018	CHN	Retrospective	66	19/47	IRS≥4	33	IRS < 4	33	IHC	Gallbladder cancer	1.289 (1.093-2.319)	NA	OS
Liu, Y. 2018	CHN	Retrospective	51	25/26	NA	29	NA	22	qPCR	Osteosarcoma	NA	NA	OS
Cheng, H. 2017	CHN	Retrospective	118	62/56	NA	80	NA	38	qPCR	Acute myeloid leukemia	NA	12 (median)	OS
Mou, S. 2016	CHN	Retrospective	43	16/27	IRS ≥ 3	33	IRS < 3	10	IHC	Osteosarcoma	NA	37 (median)	OS
Jiang, R. 2016	CHN	Retrospective	194	122/72	IRS > 4	84	IRS ≤ 4	111	IHC	Lung adenocarcinoma and squamous cell carcinoma	1.700 (1.139-2.536)	41 (median)	DFS, OS
Xu, J. 2016	CHN	Retrospective	87	56/31	IRS ≥ 6	63	IRS < 6	24	IHC	Pancreatic cancer	2.113 (1.046-4.266)	NA	OS
Liao, Y. 2016	USA	Retrospective	66	41/25	IRS≥5	31	IRS < 5	35	IHC	Osteosarcoma	3.51 (2.985-4.035)	250 (max)	DFS, OS
Zhu, X. 2014	CHN	Retrospective	54	23/31	IRS ≥ 5	45	IRS < 5	9	IHC	Salivary gland adenoid cystic carcinoma	NA	NA	OS
Peng, Y. H. 2013	CHN	Retrospective	343	196/147	IRS ≥ 2	283	IRS < 2	55	IHC	Colon cancer	NA	84 (max)	DFS, OS
Peltola, K. 2009	FIN	Retrospective	71	41/30	IRS ≥ 2	42	IRS < 2	29	IHC	Squamocellular carcinoma of head and neck	NA	24 (median)	OS
Reiser-erkan, C. 2008	DEU	Retrospective	59	31/28	IRS ≥ 2	38	IRS < 2	21	IHC	Pancreatic ductal adenocarcinoma	NA	20 (median)	OS

Abbreviations: ID, identity card; CHN, China; POL, Poland; FIN, Finland; DEU, Germany; USA, United States of America; M/F, Male/Female; NA, Not Available; IRS, immunohistochemical staining results; N, number; qPCR, quantitative polymerase chain reaction; IHC, immunohistochemistry; HR, hazard ratio; CI, confidence interval; Rep, reported; OS, overall survival; DFS, disease-free survival.

Table 2. The Risk of Bias and Quality of All Included Studies.

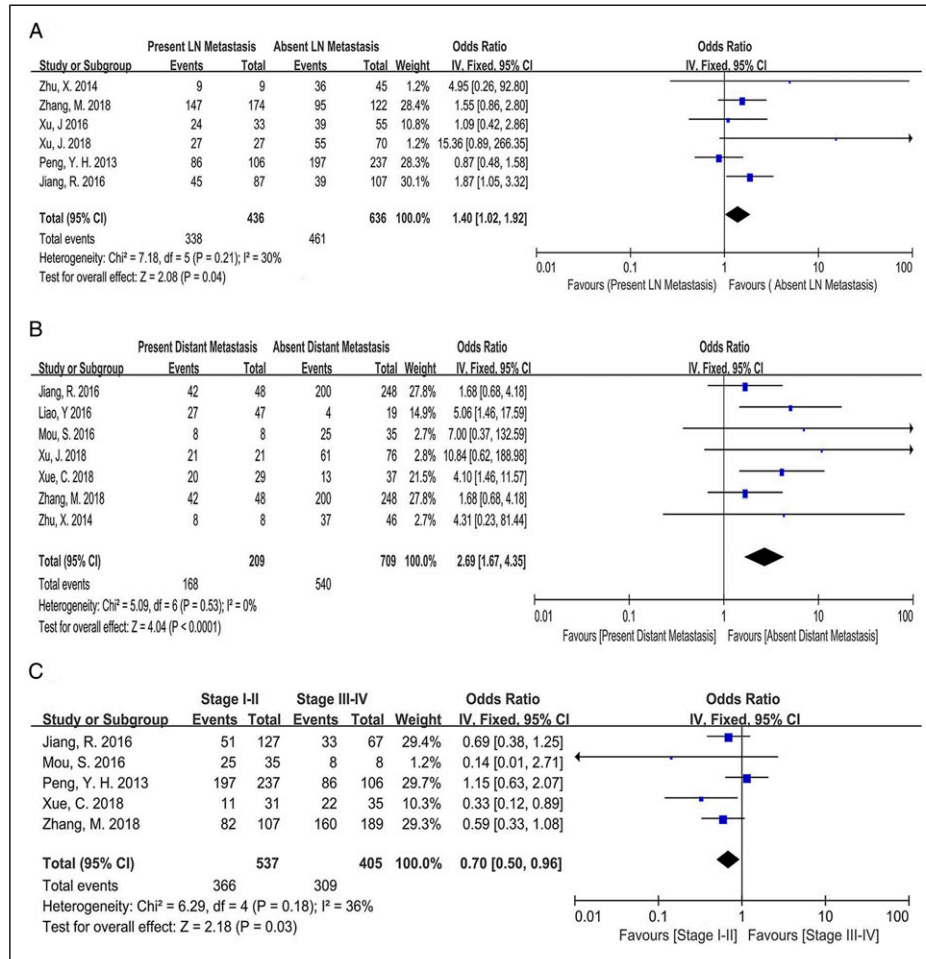| Study	Selection				Comparability		Outcome		Total
	Adequacy of case definition	Number of cases	Representativeness of the cases	Ascertainment of exposure	Ascertainment of detection method	Ascertainment of cut-off	Assessment of outcome	Adequate follow up	
Motylewska, E. 2020	—	—	0	—	—	—	—	—	7
Zhou, Y. 2018	—	—	0	—	—	0	—	—	6
Zhang, M. 2018	0	—	0	—	—	0	—	—	5
Xu, J. 2018	—	—	0	—	—	—	—	0	6
Xue, C. 2018	—	—	0	—	—	0	—	0	5
Liu, Y. 2018	—	—	0	—	—	—	—	—	7
Cheng, H. 2017	0	—	0	—	—	—	—	0	5
Mou, S. 2016	—	—	0	—	—	0	—	—	6
Jiang, R. 2016	—	—	0	—	—	—	—	—	7
Xu, J. 2016	—	—	0	—	—	—	—	—	5
Liao, Y. 2016	—	—	0	—	—	—	—	—	7
Zhu, X. 2014	0	—	0	—	—	—	—	0	5
Peng, Y. H. 2013	—	—	—	—	—	—	—	—	8
Peltola, K. 2009	—	—	0	—	—	—	—	—	7
Reiser-erkan, C. 2008	—	—	0	—	—	—	—	0	6

Figure 2. Forest plot for correlation between Pim-1 and age (2A), gender (2B), T stage (2C).

(I^2=80.1%, P=.000; I^2=92.3%, P=.000) (Tables 3 and 4). The pooled effect size results suggested that high Pim-1 was associated with unfavorable OS (HR 1.68 [95%CI 1.17-2.40], *P* = .004) and DFS (HR 2.15 [95% CI 1.15-4.01], *P* = .000) (Tables 3 and 4).

Subgroup Analysis

Detection technique. The analysis was performed according to different detection techniques, and heterogeneity test was applied by combining the outcome variables OS and DFS according to different expression products Pim-1 mRNA and Pim-1 protein detected, respectively.

Pim-1 mRNA and OS and DFS

In terms of OS, the heterogeneity test was performed on the included 2 articles using a fixed-effect model, and the results suggested that the heterogeneity disappeared (I^2 = .0%, *P* = .321) (Figure 4A), and high Pim-1 mRNA expression was associated with poor OS (HR 1.85 [95% CI 1.24-2.27], *P* = .023)

(Table 3). In terms of DFS, only one literature was included, so it was not analyzed.

Pim-1 Protein and OS and DFS

In terms of OS, the random-effects model was used to test the heterogeneity of the included 13 articles, and there was significant heterogeneity (I^2=82.2%, P=.000) (Figure 4A), but the effect size was combined to suggest that high expression of Pim-1 protein reflected unfavorable OS (HR 1.60 [95% CI 1.07-2.41], P=.003) (Table 3). In terms of DFS, the heterogeneity test for the included 4 articles also showed significant heterogeneity (I^2=92%, P=.000) (Figure 4A), but the combination of effect sizes still suggested that high Pim-1 protein revealed poor DFS (HR 2.37 [95%CI 1.19-4.73], P=.015) (Table 4).

Due to significant heterogeneity in 13 prognostic studies of all included IHC detection techniques, this group of included studies was further analyzed for sources of heterogeneity according to geographical race, sample size, histopathological origin.
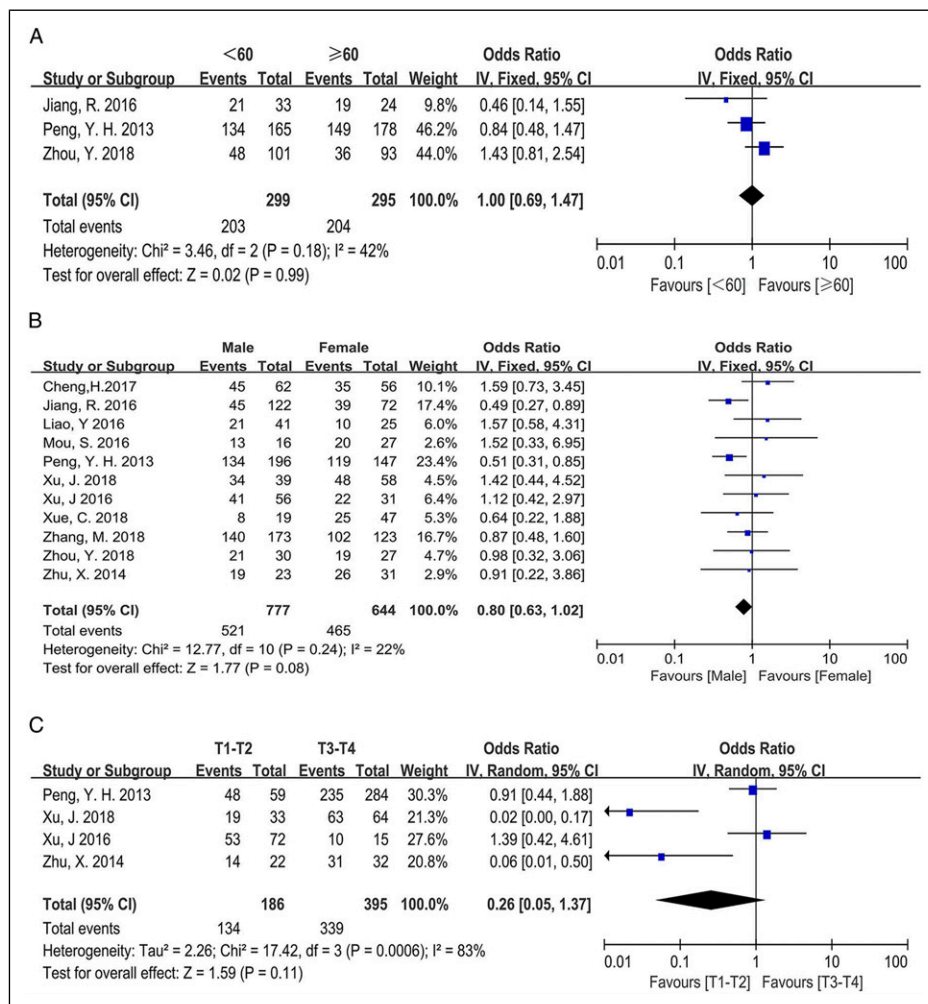
Figure 3. Forest plot for correlation between Pim-1 and N stage (3A), M stage (3B), clinical stage (3C).

Table 3. Summary of Meta-Analysis Results of High Pim-1 vs Low Pim-1 in OS.

Outcome	Subgroup		No. of Studies	Model	HR (95% CI)	p	Heterogeneity (I^2, p)
OS			15	Random effect model	1.68 (1.17-2.40)	.004	I^2 = 80.1%, P = .000
	Detection method						
	qPCR		2	Fixed effect model	1.85 (1.24-2.27)	.023	I^2 = .0%, P = .321
	IHC		13	Random effect model	1.60 (1.07-2.41)	.003	I^2 = 82.2%, P = .000
		Ethnicity					
		Asian	9	Fixed effect model	1.46 (1.19-1.79)	.000	I^2 = .0%, P = .666
		Caucasian	4	Random effect model	1.28 (.38-4.33)	.686	I^2 = 76.3%, P = .015
		Sample size					
		≥100	3	Fixed effect model	1.44 (1.09-1.91)	.010	I^2 = .0%, P = .448
		< 100	10	Random effect model	1.67 (.99-2.84))	.056	I^2 = 81.3%, P = .000
		Histological origin					
		Epithelial	9	Fixed effect model	1.35 (1.11-1.65)	.003	I^2 = 23.7%, P = .223
		Nonepithelial	4	Fixed effect model	3.48 (3.01-4.04)	.000	I^2 = .0%, P = .754

Abbreviations: NA, Not Available; qPCR, quantitative polymerase chain reaction; IHC, immunohistochemistry; HR, hazard ratio; CI, confidence interval; OS, overall survival.

Table 4. Summary of Meta-Analysis Results of High Pim-1 vs Low Pim-1 in DFS.

Outcome	Subgroup	No. of Studies	Model	HR (95% CI)	p	Heterogeneity (I^2, p)
DFS		5	Random effect model	2.15 (1.15-4.01)	.000	I^2 = 92.3%, P = .000
	Detection method					
qPCR		1	NA	NA	NA	NA
IHC		4	Random effect model	2.37 (1.19-4.73)	.015	I^2 = 92.0%, P = .000
	Ethnicity					
	Asian	3	Fixed effect model	1.65 (1.23-2.21)	.001	I^2 = 31.9%, P = .230
	Caucasian	1	NA	NA	NA	NA
	Sample size					
	≥100	3	Fixed effect model	1.65 (1.23-2.21)	.001	I^2 = 31.9%, P = .230
	< 100	1	NA	NA	NA	NA
	Histological origin					
	Epithelial	3	Fixed effect model	1.65 (1.23-2.21)	.001	I^2 = 31.9%, P = .230
	Nonepithelial	1	NA	NA	NA	NA

Abbreviations: NA, Not Available; qPCR, quantitative polymerase chain reaction; IHC, immunohistochemistry; HR, hazard ratio; CI, confidence interval; DFS, disease-free survival.

Ethnicity

In terms of OS, the heterogeneity test of the included studies (9 for Asian, 4 for Caucasian) showed that the heterogeneity of Asian group disappeared (I^2 = 0%, P = .666) (Figure 4B), and the effect size combination results showed that the high expression of Pim-1protein still reflected the poor OS (HR 1.46 [95%CI 1.19-1.79], P=.000) (Table 3).

In terms of DFS, the heterogeneity test of Asian population in the included studies (3 for Asian, 1 for Caucasian) similarly showed high homogeneity (I^2 = 31.9%, P=.230) (Figure 5B), and high Pim-1 protein expression was associated with poor DFS (HR 1.65 [95% CI 1.23-2.21], P=.001) (Table 4).

Sample Size

In terms of OS, the heterogeneity test of the included studies (3 items for samples ≥100 cases, 10 items for samples <100 cases) showed high homogeneity (I^2 = 0%, P = .448) (Figure 4C) in the group with study samples ≥100 cases, and the effect size combination results suggested that high expression of Pim-1 protein was related to poor OS (HR 1.44 [95% CI 1.09-1.91], P=.010) (Table 3).

In terms of DFS, the heterogeneity test of the included studies (3 items for samples ≥100 cases, 1 item for samples <100 cases) also showed high homogeneity (I^2=31.9%, P=.230) (Figure 5C) in the group with ≥100 study samples, and the combination of effect sizes suggested that high Pim-1 was associated with poor DFS (HR 1.65 [95%CI 1.23-2.21], P=.001) (Table 4).

Histopathological Origin

In terms of OS, the heterogeneity test of included studies (9 epithelial homology, 4 non-epithelial homology) showed that there was high homogeneity in the two subgroups (I^2=23.7%, P=.223; I^2=0%, P=.754) (Figure 4D), respectively. And the combination of effect sizes showed that the high Pim-1 protein in cancer tissues from different histopathological origin was related to poor OS (HR 1.35 [95%CI 1.11-1.65], P=.003; HR 3.48 [95%CI 3.01-4.04], P=.000) (Table 3).

In terms of DFS, the heterogeneity test of the included studies (3 epithelial homology, 1 non-epithelial homology) also displayed no significant heterogeneity of epithelial tumors (I^2 = 31.9%, P = .230) (Figure 5D), and the pooled results showed that Pim-1 protein expression was negatively correlated with DFS (HR 1.65 [95% CI 1.23-2.21], P=.001) (Table 4).

Sensitivity Analyses

Sensitivity analysis was carried out by including all studies with OS and DFS as study endpoints, respectively. After literatures Liao, Y. 2016 were deleted, the heterogeneity of the OS and DFS disappeared (I^2 = 24.6%, P = .188; I^2=3.9%, P = .373) (Figure S1A and S1B), the combined effect sizes were not affected (HR 1.50 [95% CI 1.20-1.88], P = .000; HR 1.60 [95% CI 1.24-2.06], P = .000), and the study results were robust and reliable (Figures 6A and 6B).

Sensitivity analysis was also performed by including all studies related to clinical characteristics in each group. After excluding the studies included in any clinical characteristic group one by one, the corresponding combined
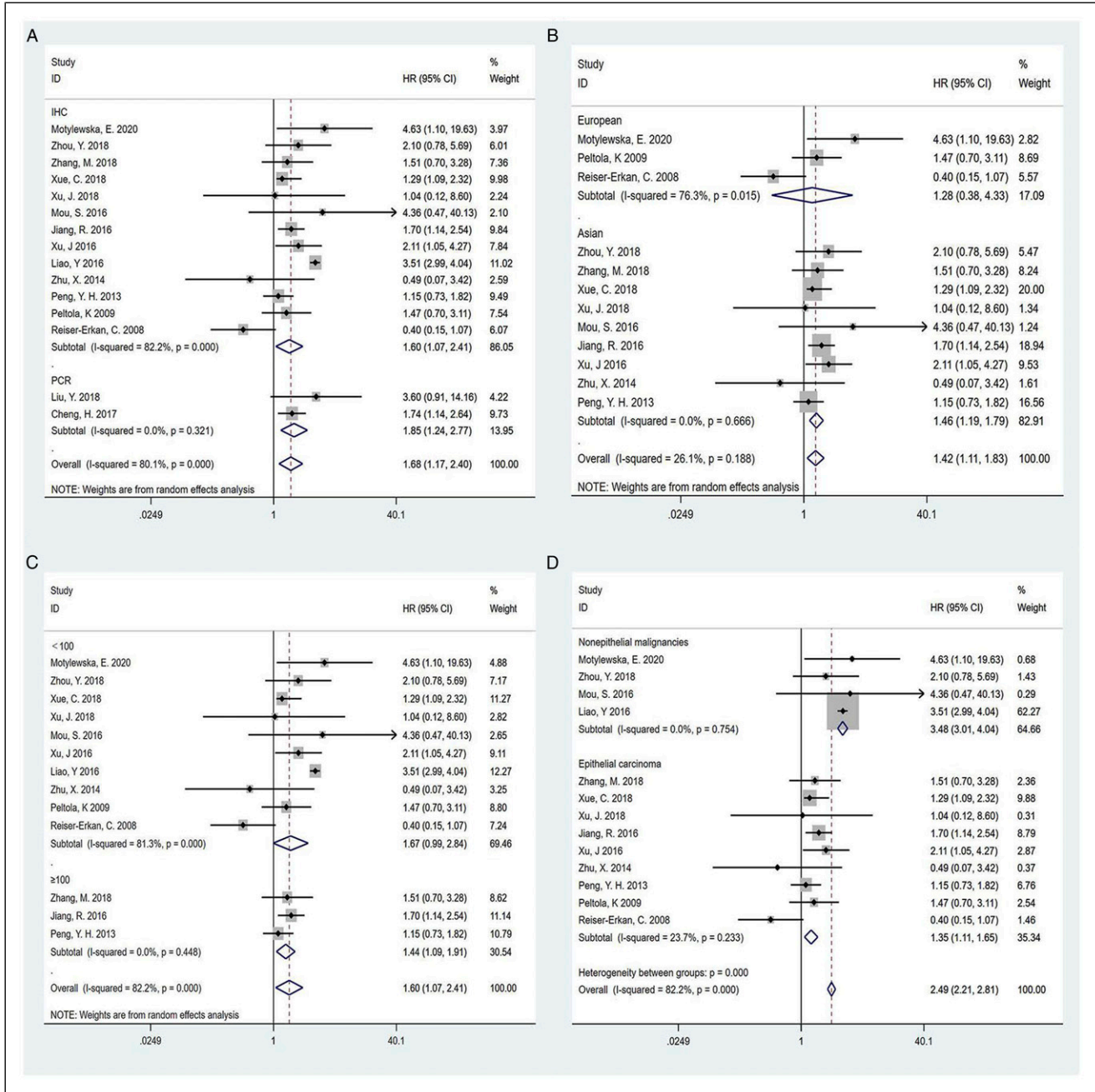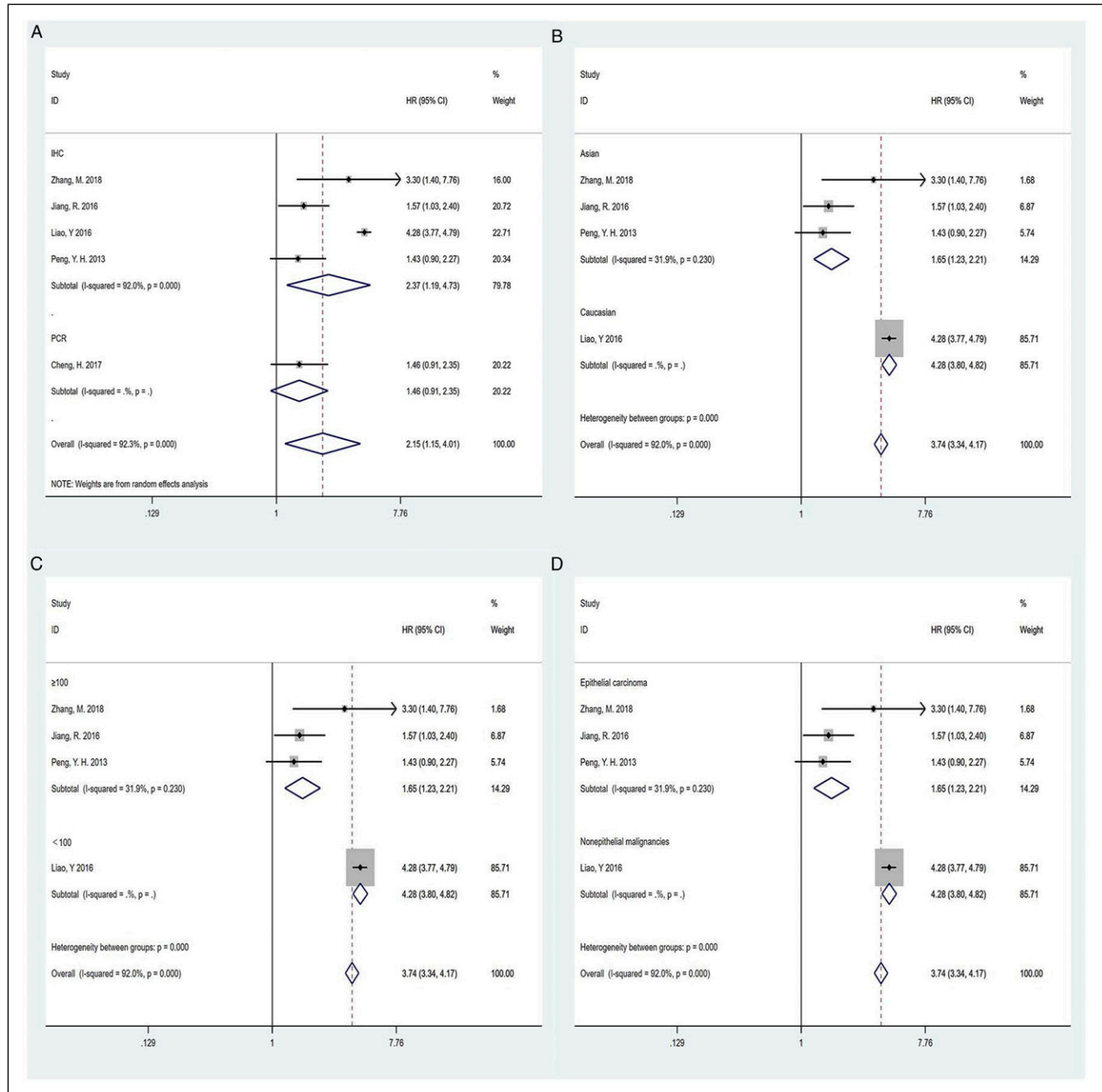
Figure 4. Forest plot for subgroup analysis of detection technique (4A), ethnicity (4B), sample size (4C), and histopathological origin (4D) in overall survival.

ORs were not affected, and the results were credible and stable (Figures 7A-7F).

Publication Bias

Funnel plot was used to estimate the publication bias of the included 15 studies, and there was no significant publication bias by Begg's test (P=.138) (Figure 8).

Discussion

In this paper, we involved a total of 15 studies and 1651 patients, and systematically evaluated the prognostic value of Pim-1 expression in human cancer tissues and the correlation of clinicopathological features in malignant tumors for the first time through meta-analysis. The results indicate that patients with high Pim-1 expression have unfavorable OS and DFS.

Figure 5. Forest plot for subgroup analysis of detection technique (5A), ethnicity (5B), sample size (5C), and histopathological origin (5D) in disease-free survival.

Subgroup analysis suggests that heterogeneity comes from different techniques for detecting Pim-1 expression products, of which 1 result have high homogeneity in studies using qPCR techniques. There was significant heterogeneity in other 2 results of IHC techniques, and subfraction analysis further showed that ethnicity, sample size and tissue origin were considered to be the factors affecting heterogeneity. Most effect size results were similar to those of the overall study population. In addition, we also analyzed the correlation between Pim-1 expression and clinicopathological factors that

may affect survival outcome. According to the pooled results, high Pim-1 expression was positively associated with lymph node metastasis, distant metastasis, and clinical stage III-IV. Moreover, sensitivity analysis showed that the pooled results for each effect size were robust and reliable, and Begg's test suggested no significant publication bias. This indicated the reliability of our combined results.

In our study, high Pim-1 expression reflects unfavorable prognosis, also announced a high risk of metastasis and advanced clinical stage. This is undoubtedly consistent with the
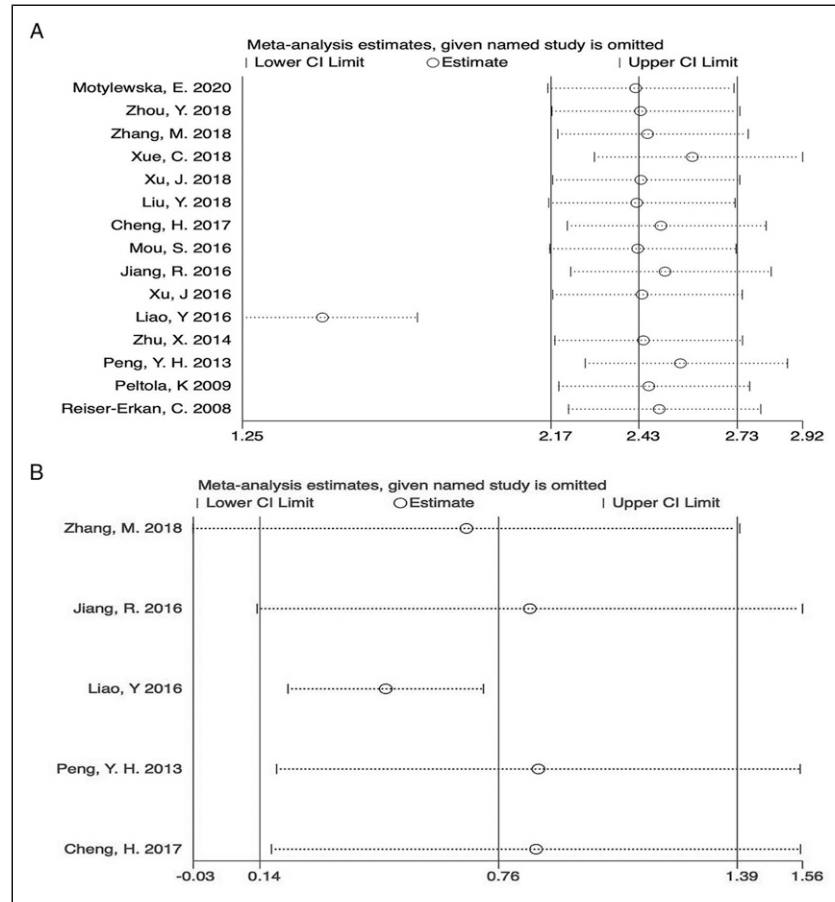
Figure 6. Sensitive analysis for correlation between Pim-1 and overall survival (6A), disease-free survival (6B).

malignant phenotypes of promoting tumor cell proliferation, cycle progression and metastasis and inhibiting apoptosis caused by the activation of serine-threonine kinase Pim-1.[44] Although individual studies have indicated that high Pim-1 was positively related with PSA-free survival in prostate cancer[45,46] and overall survival in pancreatic cancer,[38] the prognostic significance of Pim-1 above is controversial. It also reflects the need for us to find features that may lead to heterogeneous results. Differences in ethnicity, sample size, tissue origin and detection methods for pim-1 may all contribute to the different results.

As anti-tumor treatment tends to be personalized and precise, new molecular targets are constantly being developed, but it is challengeable for application in clinical practice.[47] It has been reported that off-target toxicity from Pim-1 kinases inhibitors can lead to cancer cell death and Pim-1 gene can be knocked out by CRISPR/Cas9 with no apparent effect on cell survival.[48] However, our study mainly focuses on the prognostic value of Pim-1 and its correlation with clinicopathological features in cancer, and do not involve the guidance of personalized therapy. In addition, CRISPR/Cas9 is an emerging gene-editing technology for the personalized therapy research of cancer. Many defects such as specificity and

safety of gene editor have not been solved[49,50] and dependability and accuracy of genome editing technology need constant refinement, including CRISPR/Cas9.[51,52] There is no denying that the overall direction of developing promising precision targets and relying on precision therapy for cancer has not changed. The off-target effect involving Pim1 also reminds us that the researches on anticancer drug targets have a long way to go.

In this study, although the pooled results are robust, the study results should be interpreted with caution. Firstly, the expression of Pim-1 in most of the literatures was detected by IHC. Although IHC is a widely used protein detection technique, it is not strictly quantitative and there is no uniformly adhered scoring system. The interpretation of their staining results varies from person to person, which may lead to some degree of heterogeneity. In the included cohort, the cut-off for high Pim-1 expression was defined differently, and the investigators arbitrarily defined IRS based on the proportion of positive cells and the degree of positive staining. In addition, the sensitivity of IHC may depend on antibody selection, antibody dilution ratio, specimen preparation, fixation method, and storage time. The primary antibody used for IHC is diverse across studies, and the dilution ratio of
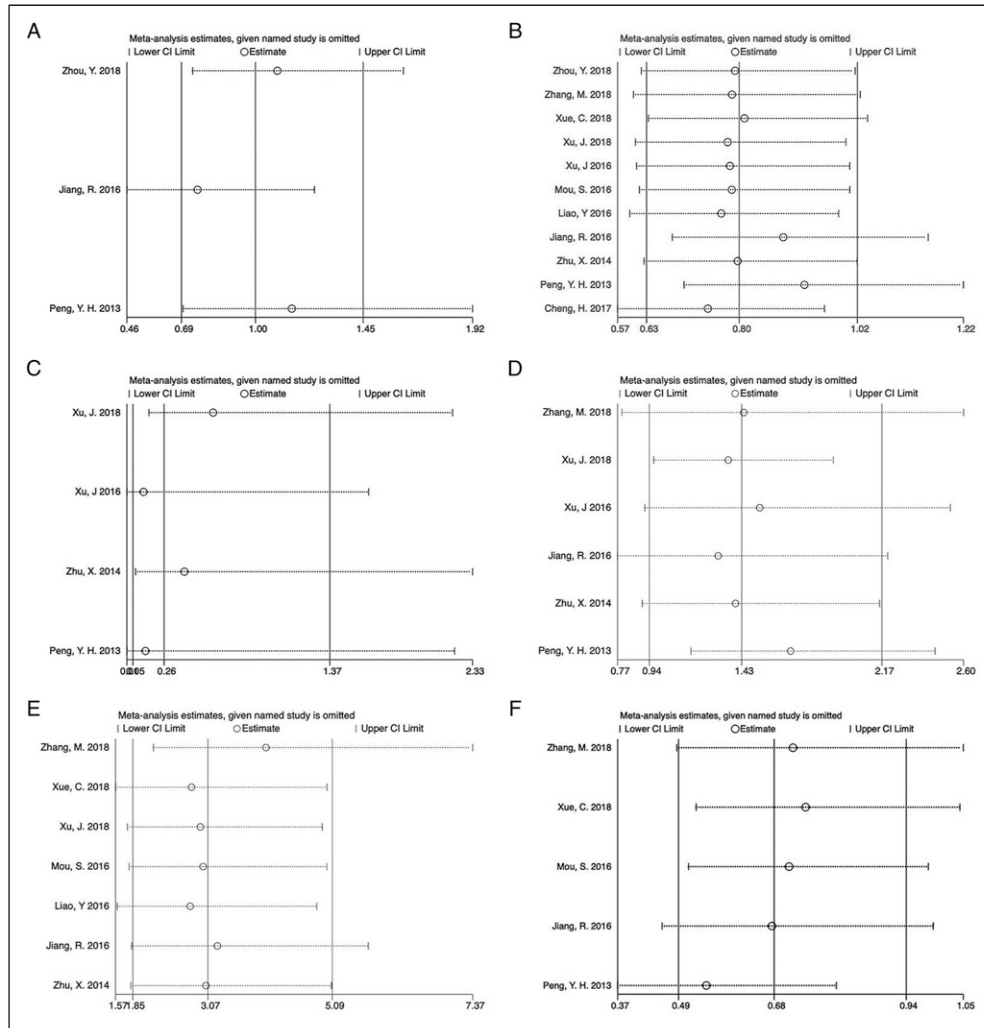
Figure 7. Sensitive analysis for correlation between Pim-1 and age (7A), gender (7B), T stage (7C), N stage (7D), M stage (7E), clinical stage (7F).
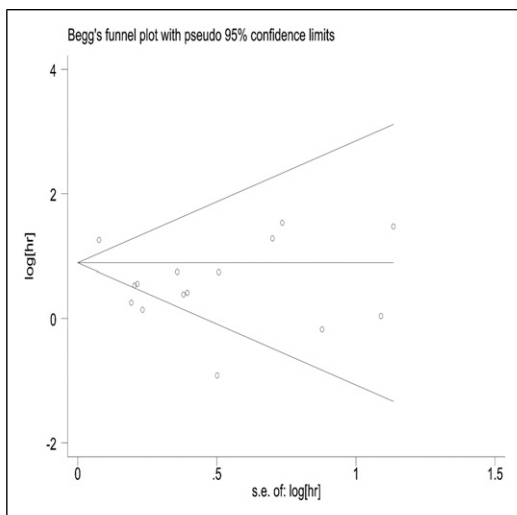

Figure 8. Funnel plot of Begg's test.

antibodies is heterogeneous. Secondly, the articles included in this paper are all retrospective studies and lack uniform follow-up time. Therefore, there may be some bias in the meta-analysis, such as selection bias, classification error and information bias. Thirdly, the HR values and 95% CIs of some of the included articles were obtained by indirect extraction from the Kaplan-Meier curves in the text and may not have been as reliable as the values provided directly in the literature. Fourthly, the samples tested for Pim-1 in the study were all derived from tissues, and only one article with serum samples tested for Pim-1 was not included. Fifthly, we failed to focus on the prognostic value of Pim-1 in a specific tumor due to the lack of studies on each cancer species.

Conclusion

In conclusion, we used a comprehensive and detailed search strategy combined with predetermined inclusion and

exclusion criteria to provide convincing evidence that high expression of Pim-1 predicts poor OS and DFS in cancer and is closely related to lymph node metastasis, distant metastasis, and advanced clinical stage. This may emerge the possibility of exploring more unknown biological functions of Pim-1 related cancers, and enables important baseline features such as detection technique, ethnicity, sample size and tissue origin to be considered in the design of future Pim-1 related clinical trial. More carefully designed studies need to be carried out to further verify these data in the future.

Author Contribution

Xiaodong Zhu: study conception and design. Lin Lai, Xinyu Chen, and Yuelan
Qin: literature screening and quality assessment. Lin Lai, Ge Tian, Xinyu Chen, Xishan
Chen and Kaihua
Chen: data extraction and data analysis. Lin Lai, Ge Tian, Xinyu Chen and Renba
Liang: manuscript writing and manuscript revision. All authors: manuscript review and final approval of manuscript.

Declaration of Conflicting Interests

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Ethical Approval

Ethical Approval is not applicable for this meta-analysis.

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Supplemental Material

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