Prognostic Value of COX-2, NF-κB, and Sp1 Tissue Expressions in Pancreatic Ductal Adenocarcinoma: A Systematic Review and Meta-analysis

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ABSTRACT

Background: *Pancreatic ductal adenocarcinoma (PDAC) is deadly cancer with a poor prognosis. Molecular prognostic markers are needed to predict the patient's survival. The cyclooxygenase-2 enzyme (COX-2) and its 2 major transcription factors—nuclear factor-kappa B (NF-κB) and specificity protein 1 (Sp1)—are activated during inflammation caused by neoplasia. Several studies have investigated the association between the COX-2, NF-κB, and Sp1 tissue expressions with the patient's overall survival. Therefore, we conducted this systematic review and meta-analysis to evaluate those studies.*

Methods: *We searched for relevant articles from the MEDLINE database through June 2020. Studies were eligible if they included dichotomized tissue protein expression status and the overall survival as the outcome. We used RevMan and ProMeta programs to perform the meta-analysis.*

Results: *We identified 11 eligible studies. The meta-analysis showed that COX-2 tissue expression was associated with decreased overall survival (crude HR = 1.35; 95% CI, 1.05-1.74), although the result was not significant when controlling for other covariates. The NF-κB tissue expression was associated with decreased overall survival (crude HR = 2.18; 95% CI, 1.49-3.18), although it was not significant when controlling for other covariates. The Sp1 tissue expression showed significantly decreased overall survival even when adjusted with other covariates (aHR = 3.47; 95% CI, 1.52-7.94). The limitations included searching only for English publications and the substantial heterogeneity among the studies.*

Conclusion: *COX-2, NF-κB, and Sp1 tissue expressions have the potential to be used as prognostic markers in PDAC. Further studies are still needed to clarify the associations.*

Keywords: *Cyclooxygenase-2, NF-kappa B, pancreatic neoplasms, prognosis, Sp1 transcription factor*

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is the seventh leading cause of cancer-related death worldwide.[1] Only 15-20% of PDAC patients are eligible for pancreaticoduodenectomy at diagnosis.[2] However, early recurrence after resection has remained high, and none of the available chemotherapy regimens has resulted in satisfactory treatment outcomes. Traditional clinicopathologic prognostic markers, such as tumor grade, resection margin, and vascular or neural invasion, are still insufficient, and molecular prognostic markers may be needed to account for all the observed PDAC clinical outcomes.[3]

Cyclooxygenase-2 (COX-2) is a rate-limiting enzyme for the synthesis of prostaglandin, a relevant substance in the development and progression of various cancers.[4] Notably, COX-2 is overexpressed in PDAC, and its increased expression has been associated with angiogenesis and tumor invasion.[5,6]

Specificity protein 1 (Sp1) and nuclear factor-kappa B (NF-κB) are transcription factors with DNA-binding protein which is sequence-specific to the proximal promoter regions of some genes, including COX-2.[7,8] Similar to COX-2, both Sp1 and NF-κB are also highly expressed in PDAC and are associated with poor clinical outcomes in the patients. Notably, Sp1 expression was reported to influence the aggressiveness of PDAC,[9] while NF-κB may be responsible for the chemotherapeutic resistance in PDAC.[10]

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To date, there have been many studies suggesting the valuable prognostic role of molecular biomarkers, such as COX-2-, Sp-1-, and NF-κB immunohistochemistry (IHC)-based expression in PDAC.[11] The meta-analysis by Wang et al.[12] in 2014 provided empirical evidence on the prognostic significance of COX-2 overexpression for PDAC patients. However, several new studies with conflicting results have been published after this meta-analysis.[12,13] Moreover, no meta-analysis on the role of Sp-1 and NF-κB in the prognosis of PDAC has been conducted. Therefore, we performed a meta-analysis of all available data to examine the prognostic significance of COX-2-, Sp-1-, and NF-κB IHC-based expression in PDAC.

MATERIALS AND METHODS

An ethics committee approval statement and verbal or written informed consent were not needed, since our work was a systematic review and meta-analysis. We conducted this systematic review and meta-analysis under the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) checklist.[14]

Eligibility Criteria

The review question based on the PICOTS[15] framework was *Population*: patients with pancreatic ductal adenocarcinoma; *Index prognostic factor*: tissue expressions of COX-2, NF-κB, and Sp1 based on immunohistochemistry; *Comparator prognostic factor*: include conventional prognostic factor such as age, gender, and tumor stage; *Outcome*: mortality; *Timing*: survival since resection; *Setting*: tertiary center to predict the course of the disease.

Based on the PICOTS[15] structure, the inclusion criteria were studies evaluating the difference in mortality based on the tissue expressions of COX-2, NF-κB, and Sp1 from immunohistochemical analysis, studies published up to June 2020, English language publications, studies with dichotomized protein expression status, and studies which included the hazard ratio and the corresponding confidence interval as the effect measure. The exclusion criteria were studies that only included pancreatic cell lines and those that did not have the overall survival as the outcome of the study, which was the main outcome of interest in our meta-analysis.

MAIN POINTS

- *One of the critical indicators of pathogenesis in PDAC is inflammation, which involves the cyclooxygenase-2 (COX-2) enzyme and its 2 major transcription factors, nuclear factor-kappa B (NF-κB) and specificity protein 1 (Sp1).*
- *These proteins are overexpressed in PDAC and may have potential prognostic value.*
- *Our meta-analysis showed that COX-2, NF-κB, and especially Sp1 tissue expression have the potential to predict survival in PDAC patients.*
- *Further studies with larger sample sizes and studies investigating the prognostic value of COX-2, NF-κB, and Sp1 coexpressions should be encouraged.*

Search Strategy

We performed a literature search from the MEDLINE database using highly sensitive search terms, which included: ("pancreatic ductal adenocarcinoma" OR PDAC OR "pancreatic cancer") AND (cox-2 OR "cyclooxygenase" OR "prostaglandin G/H synthase 2" OR "PTGS-2" OR "PGHS-2") for studies of COX-2; AND ("specificity protein" OR Sp1) for studies of Sp1; AND ("nuclear factor kappa B" OR "NF-kB" OR RelA OR RelB OR c-Rel OR p65 OR p50 OR p52) for studies of NF-κB. PTGS-2 and PGHS-2 are the abbreviations for prostaglandin G/H synthase 2, and RelA to c-Rel, also known as p65 to p52, are REL-associated proteins involved in the heterodimer formation and the activation of NF-κB.[10]

From the search results, we screened the title and abstract to find potential eligible studies. Then, we read the full article to identify studies that fulfilled the inclusion and exclusion criteria. We also looked at the reference list of each study to identify additional potential studies. Two independent reviewers were involved in these processes. If there were any discrepancies, a third reviewer would be consulted.

Data Extraction

Two independent reviewers extracted the relevant information from the eligible studies. The data extraction template was based on the CHARMS-PF checklist.[15] The 2 reviewers also assessed each study's risk of bias by using the modified quality in prognostic studies (QUIPS)[15] and the reporting recommendations for tumor marker prognostic studies (REMARK) checklists.[16] Table 1 shows the risk-of-bias assessment indicators. If there were any discrepancies between the 2 reviewers' assessments, a third reviewer would be consulted.

Statistical Analysis

We used the Review Manager (RevMan) 5.3 program (Copenhagen, The Nordic Cochrane Centre, Denmark)[17] to conduct the meta-analysis. The primary effect

Table 1. Risk-of-Bias Indicators Based on the QUIPS Tool and REMARK Guideline[15,16]

No.	Domains	Indicators	Risk-of-Bias Ratings
1.	Study participation	- Details of the study population - Details of the period and place of study - Inclusion and exclusion criteria were provided	High = none present Moderate = some present Low = all present
2.	Study attrition	- Study participants with adequate response rate (>90%) - Cause of loss to follow-up was described	High = none present Moderate = some present Low = all present
3.	Prognostic factor measurement	- Description of immunohistochemistry methodologies (description of primary and secondary antibodies, the positive and negative controls) - Description of the scoring system (>1 independent scorer, blinding to the outcome, magnification and number of fields examined, the scoring system for staining intensity and % of stained cells, appropriate score cut-off)	High = none present Moderate = some present Low = all present
4.	Outcome measurement	- Description of the overall survival measurement	High = no definition provided Moderate = definition unclear Low = clear definition
5.	Adjustment for other prognostic factors	- Key covariates were included (age and some indicators related to the stage of the tumor)	High = no adjustment Moderate = some key prognostic factors adjustment Low = all key prognostic factors adjustment
6.	Statistical analysis and reporting	- Hazard ratio and the confidence interval are provided for both the univariable and multivariable analysis - Exact *P*-value provided - Details on the number of censored cases - Provide limitations of the study, the implications for future research, and the clinical value	High = none present Moderate = some present Low = all present

measure was the hazard ratio. For studies that included multivariate Cox regression analysis involving key covariates (age and some indicators related to the stage of the tumor), the adjusted hazard ratios were pooled. For studies without Cox regression analysis, the unadjusted HR were pooled separately. We calculated the logHR and its variance by imputing the HR and its confidence interval to the RevMan program. For studies that did not specify the hazard ratio (e.g., because of nonsignificant results), it would be estimated from other values if possible.[18] We assessed the heterogeneity by using the I^2 statistics. If the $I^2 > 50\%$, there was substantial heterogeneity,[19] and we used random effects analysis to calculate the pooled ratio. We detected the presence of publication bias by creating funnel plots through the RevMan and Egger's test through the ProMeta 3 (Internovi, Cesena, Italy)[20] program. Sensitivity analysis was also performed based on the number of patients (including studies with ≥ 50 patients only), type of pathologic specimens (whole section vs. tissue microarray), type of primary antibodies (monoclonal vs. polyclonal), and the detection method (polymer-based vs. avidin–biotin vs. streptavidin–biotin), excluding studies with estimated hazard ratio, and only including studies with both key covariates for the multivariate analysis (age and relevant parameters for tumor stage).

RESULTS

Study Selection

The initial search yielded 2275 records from the MEDLINE database using highly sensitive keywords and excluding the duplicates. We excluded 2252 records based on screening the title and abstracts. From the remaining 26 articles, we excluded 3 articles because they did not include the overall survival as one of the clinicopathological outcomes; 1 article was excluded because it was a meta-analysis, 2 articles were excluded because they contained the same population as one other study that had been included, and the other 9 studies only stated that the results were not significant and we were unable

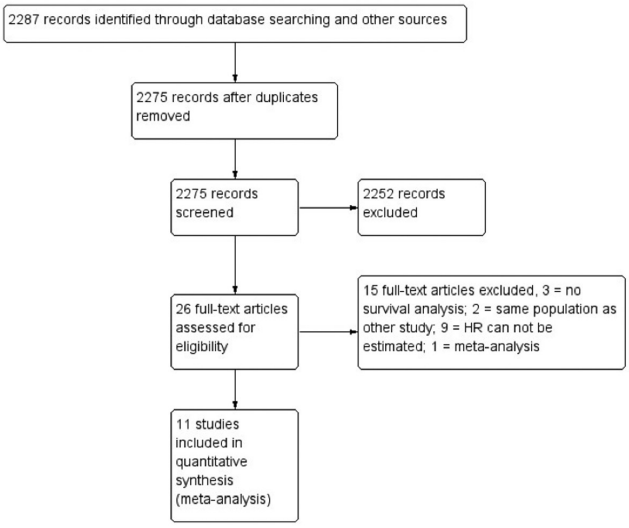

Figure 1. Flow diagram.

to estimate the hazard ratios using the methods proposed by Tierney et al.[18] We included 11 studies for the quantitative analysis. Figure 1 shows the flow diagram.

Study Characteristics

We extracted the data from each eligible study based on the CHARMS-PF and REMARK[15,16] checklists. The study characteristics are presented in Tables 2 and 3. We also assessed the risk of bias from each study based on the QUIPS tool.[15] Table 4 shows the risk-of-bias assessments.

META-ANALYSIS

COX-2

Eight studies were eligible for the meta-analysis. Among those, we were able to extract the crude HR in 7 studies. Only 4 studies presented the adjusted hazard ratio. Figures 2 and 3 show the forest plots for the crude and adjusted hazard ratios, respectively. The pooled crude hazard ratio showed that positive COX-2 tissue expression significantly decreased the overall survival in PDAC patients (HR = 1.35; 95% CI, 1.05-1.74). Sensitivity analysis for the crude hazard ratio outcome showed no significant differences, as shown in Table 5. The adjusted hazard ratio also showed a trend toward worse overall survival, but the result was not significant (aHR = 1.30; 95% CI, 0.80-2.13). For the adjusted hazard ratio outcome, including only studies that controlled for both key covariates (age and indicators for tumor stage) resulted in a significant pooled adjusted hazard ratio (aHR = 1.51; 95% CI, 1.22-1.87; I^2 = 18%). Figures 4 and 5 show the funnel plots for the crude and adjusted hazard ratios, respectively. Table 5 shows the results of the sensitivity analysis. Egger's test for the crude hazard ratio (P = .595) and adjusted hazard ratio (P = .933) showed no significant publication bias.

NF-κB: Two studies were eligible for the meta-analysis. Figures 6 and 7 show the forest plots for the crude and adjusted hazard ratios, respectively. The pooled crude hazard ratio showed that NF-κB tissue expression significantly decreased the overall survival in PDAC patients (HR = 2.18; 95% CI, 1.49-3.18). The pooled adjusted hazard ratio also showed a trend toward worse overall survival, but the result was not significant (aHR = 2.38; 95% CI, 0.68-8.25). The funnel plots for the crude hazard ratio and the adjusted hazard ratio are shown in Figures 8 and 9, respectively. Egger's tests could not be performed because only 2 studies were eligible for these outcomes.

Sp1: Three studies were eligible for the meta-analysis. Among those, we were able to extract the crude hazard ratio in 2 studies and the adjusted hazard ratio in 2 studies. Figures 10 and 11 show the forest plots for the crude and adjusted hazard ratios, respectively. The pooled crude hazard ratio showed that positive Sp1 tissue expression significantly decreased the overall survival in PDAC patients (HR = 2.50; 95% CI, 1.63-3.84). The adjusted hazard also showed that Sp1 tissue expression significantly decreased the overall survival in PDAC patients (aHR = 3.47; 95% CI, 1.52-7.94). Figures 12 and 13 show the funnel plots for the crude and adjusted hazard ratios, respectively. Egger's test for the adjusted hazard ratio (P = .111) showed no significant publication bias. Egger's test could not be performed for the crude hazard ratio because only 2 studies were eligible.

DISCUSSION

COX-2 is activated during an inflammatory process, which can be triggered by neoplasia such as PDAC. Previous immunohistochemical studies have also shown that there was an increased tissue expression of COX-2 in PDAC tissues compared with the normal pancreatic tissue, as reviewed in this study.[21] COX-2 converts arachidonic acid into prostaglandins. Prostaglandins, especially PGE_2, have been associated with the inhibition of apoptosis, promoting cellular growth leading to neoplasia. They are also associated with increased angiogenesis and cellular migration, promoting metastasis. Thus, it was

Table 2. General Study Characteristics

Study	Proteins Evaluated	Sample Size	Positive Expression	Negative Expression	Period of Recruitment	Evaluation of Survival	No. of Patients Receiving Adjuvant Therapy	Variables in Multivariate Analysis	Crude Hazard Ratio (95% CI)	Adjusted Hazard Ratio (95% CI)
Fagman 2019[23]	COX-2	32	16	16	1998-2005	- Start point: surgery - Follow-up duration not described	NS	Gender, age, T stage, primary tumor location, regional lymph node metastasis, EGFR status, COX-2 status	1.22 (0.59-2.51)	NS
Juuti 2006[5]	COX-2	128	46	82	1974-1998	NS	A few received postoperative chemotherapy	Histological grade, TNM stage, COX-2, tumor, tumor size, age	1.62 (1.12-2.34)[a]	1.6 (1.1-2.4)
Matsubayashi 2007[6]	COX-2	299	166	133	1998-2003	- Start point: date of surgery - Endpoint: date of death or last follow-up (January 2006) - Censored: alive at last follow-up, maximum 60 months	137 patients received adjuvant therapy	Size, number of positive nodes, tumor margins, grade, age	1.48 (1.14-1.92)	1.41 (1.08-1.84)
Pomianowska 2014[13]	COX-2	92	65	27	1998-2011	- Endpoint: death or censored after a maximum of 5 years	13 patients received adjuvant chemotherapy	R status, vascular invasion, perineural invasion, tumor size, COX-2 expression, lymph node ratio	0.64 (0.38-1.08)[a]	1.642 (1.01-2.68)
Schmid 2013[40]	COX-2	114	59	55	1996-2009	- Median follow-up = 18.2 months (range: 0.2-118.6 months)	NS	Stage, pM, grade, Ki-67 proliferation index, COX-2 status, c-FLIP status	1.55 (1.03 – 2.33)[a]	NS

Table 2. General Study Characteristics (*Continued*)

Study	Proteins Evaluated	Sample Size	Positive Expression	Negative Expression	Period of Recruitment	Evaluation of Survival	No. of Patients Receiving Adjuvant Therapy	Variables in Multivariate Analysis	Crude Hazard Ratio (95% CI)	Adjusted Hazard Ratio (95% CI)
Tonini 2005[41]	COX-2	67	32	35	1986-2003	- Start point: the date of initial surgery - Median follow-up after surgery = 22 months (range: 3–100 months) - The minimum follow-up for patients without tumor recurrence = 9 months	58 received adjuvant therapy	Age, gender, T and N stage, adjuvant therapy, TUNEL staining, cytoplasmatic and nuclear Survivin expression, Cox-2 stain	1.14 (0.64-2.03)[a]	NS
Hang 2016[29]	COX-2	88	62	26	2009-2012	NS	NS	Gender, age, T stage, N stage, primary tumor location, lymphovascular invasion, nuclear grade, jaundice, abdominal pain, Sp1 status, COX-2 status, combined Sp1/COX-2 status	2.28 (1.18-4.42)	3.84 (1.08-13.71)
	Sp1		53	35					2.42 (1.35-4.33)	4.48 (1.14-17.62)
Hu 2016[42]	Sp1	77	38	39	2012-2014	- Start point: recruited from 2012-2014 - Endpoint: last follow-up visit on Feb 28, 2016	NS	Gender, age, TNM stage, location of the primary tumor, neurovascular invasion, nuclear grade, jaundice, abdominal pain, Sp1, and PLD1 status	2.60 (1.38-4.90)	NS

(*Continued*)

Table 2. General Study Characteristics (*Continued*)

Study	Proteins Evaluated	Sample Size	Positive Expression	Negative Expression	Period of Recruitment	Evaluation of Survival	No. of Patients Receiving Adjuvant Therapy	Variables in Multivariate Analysis	Crude Hazard Ratio (95% CI)	Adjusted Hazard Ratio (95% CI)
Jiang 2008[9]	Sp1	42	17	25	2000-2006	- Start point: date of tumor resection - Endpoint: date of death or censored as the date of last follow-up for survivors	NS	Tumor differentiation, tumor stage, Sp1 status	2.99 (1.06-8.46)[a]	2.995 (1.060-8.466)
Weichert 2007[43]	Nuclear NF-κB	82	37	45	1991-2000	- Median follow-up for patients who died = 11.5 months - Median follow-up for patients who were still alive at the end of the study = 44.0 months	NS	Number of positive nodes, grade, cytoplasmic or nuclear RelA expression	1.86 (1.14-3.03)[a]	1.47 (0.90-2.40)[a]
Yang 2013[44]	Nuclear NF-κB	68	22	46	1995-2007	- Start point: after surgery or chemotherapy - End point: December 31, 2010	68 received adjuvant therapy	tumor site, the effect of chemotherapy, stage, RelA/p65 expression, Gli1 expression, age, gender, chemotherapy type, CEA value, Ca 19-9 value, Shh expression	2.76 (1.51-5.04)[a]	5.501 (1.311-23.084)

[a]Estimated HR; NS, not specified; PDAC, pancreatic ductal adenocarcinoma; COX-2, Cyclooxygenase-2; Sp1, Specificity Protein 1; NF-κB, nuclear factor – kappaB.

Table 3. Study Characteristics by Immunohistochemistry Methods

Study	Pathologic Specimen	Primary Antibody	Detection Method	Scoring System	Controls
Fagman 2019[23]	Whole section	Polyclonal rabbit antiCOX2 (cytosolic detection), ab15191, Abcam, Cambridge, UK, dilution 1 : 200	Biotin-free polymer-based	- COX-2 high grade = >median score - Score = average intensity score x average percentage of tumor cells showing positive staining	Negative control = sections stained by mouse IgG1 (X0931, Agilent Technologies) and rabbit IgG1 (X0903, Agilent Technologies) diluted in 5% non-fat milk and TBS to the same protein concentration as the primary antibody
Juuti 2006[5]	Whole section	Mouse antihuman anti-COX-2 monoclonal antibody (160112;5 Cayman Chemical, Ann Arbor, Michigan, USA) dilution 1 : 200	Avidin–biotin–peroxidase system	COX-2 (+) = >5% cells stained with moderate intensity	- External positive control: a colon sample containing adenocarcinoma cells stained >50%, and adjacent epithelial non-neoplastic cells stained 5-0% - Internal positive control: pancreatic islet cells that consistently expressed COX-2 - Negative control: Sections with phosphate buffered saline or nonimmune antibody
Matsubayashi 2007[6]	Tissue Microarray	Anti-COX-2 monoclonal antibody (Cayman Chemical, Ann Arbor, MI) dilution 1 : 100	NS	COX-2 (+) = staining intensity was moderate (++) to strong (+++) and the extent was ≥10% in the neighboring fibroblasts	NS
Pomianowska 2014[13]	Whole Section	Monoclonal anti-COX-2 antibodies (Thermo Fischer Scientific rabbit), dilution not specified	Biotin-free polymer-based	- COX-2 (+) = immunoscore >1.4 - Immunoscore = the average numeric scores of 5 high-power fields from each case to be considered positive in intensity scoring. - 1 = < 10% cells stained, 2 = 10-50% and 3 > 50%	- Positive control = Islets of Langerhans and duodenum mucosa - Negative control = identical sections without the primary antibody

(*Continued*)

Table 3. Study Characteristics by Immunohistochemistry Methods (*Continued*)

Study	Pathologic Specimen	Primary Antibody	Detection Method	Scoring System	Controls
Schmid 2013[40]	Whole section	Mouse monoclonal anti-COX-2 antibody (clone CX220; Cayman Chemical, Ann Arbor, Mich) dilution 1 : 200	Streptavidin–biotin–peroxidase system	- COX-2 (+) = detectable staining	- Negative Control: incubating the sections without primary antibody - Internal positive control: immunoreactivity of the pancreatic islets
Tonini 2005[41]	Whole section	Goat polyclonal anti-COX-2 (Santa Cruz Biotechnologies, Santa Cruz, CA, USA), dilution not specified	Streptavidin–biotin–peroxidase system	- COX-2 (+) = score ≥+1 (positive staining)	- Negative control: sections with rabbit or goat preimmune serum
Hang 2016[29]	Tissue Microarray	Anti-COX-2 (Cell Signaling Technology, Beverly, MA, USA), dilution not specified	NS	- COX-2 or Sp1 (+) = score ≥ 4 - Score = staining intensity (0-3) x % of cells stained (1-4)	NS
		Anti-Sp1 (Cell Signaling Technology, Beverly, MA, USA), dilution not specified			NS
Hu 2016[42]	Tissue Microarray	Anti-Sp1 (CST, Beverly, MA, USA) dilution 1 : 1000	NS	- Sp1 (+) = score > 6 - Score = staining intensity (0-3) x % of cells stained (1-4)	NS
Jiang 2008[9]	Whole section	Mouse monoclonal anti-Sp1 at 2 μg/mL	Biotin-free polymer-based	- Sp1 (+) = >20% cells stained	- Negative Control = sections stained with nonimmune mouse IgG (vector) at a concentration of 2 μg/mL
Weichert 2007[43]	Whole section	Monoclonal RelA-antibody (sc-8008, Santa Cruz Biotechnology, Santa Cruz, CA, USA) dilution 1 : 250	Streptavidin–biotin–peroxidase system	Nuclear NF-κB (+) = stained nuclei of tumor cells representing the expression of RelA	NS
Yang 2013[44]	Whole section	Anti-NF-κBp65 (sc-109, Santa Cruz Biotechnology) dilution 1 : 50	Avidin–biotin–peroxidase system	Nuclear NF-κB (+) = > 10% of tumor cells had positively stained nuclei	NS

NS, not specified; PDAC, pancreatic ductal adenocarcinoma; COX-2, Cyclooxygenase-2; Sp1, specificity protein 1; NF-κB, nuclear factor-kappaB.

Table 4. Risk-of-Bias Assessment Results

No.	Study	Study Participation	Study Attrition	Prognostic Factor Measurement	Outcome Measurement	Adjustment for Other Prognostic Factors	Statistical Analysis and Reporting
1.	Juuti 2006[5]	Moderate	Low	Low	Moderate	Low	Moderate
2.	Matsubayashi 2007[6]	Low	Low	Moderate	Low	Low	Low
3.	Schmid 2013[40]	Moderate	Low	Moderate	High	Low	High
4.	Pomianowska 2014[13]	Moderate	Low	Moderate	Moderate	Low	Moderate
5.	Hang 2016[29]	Moderate	Low	Moderate	High	Low	Low
6.	Hu 2016[42]	Moderate	Low	Moderate	Low	Low	Moderate
7.	Jiang 2008[9]	Moderate	Low	Moderate	Low	Low	Moderate
8.	Weichert 2007[43]	Moderate	Low	Moderate	High	Low	High
9.	Yang 2013[44]	Low	Low	Moderate	Low	Low	Moderate
10.	Fagman 2019[23]	Moderate	Low	Moderate	Moderate	Low	Moderate
11.	Tonini 2005[41]	Low	Low	Moderate	Moderate	Low	High

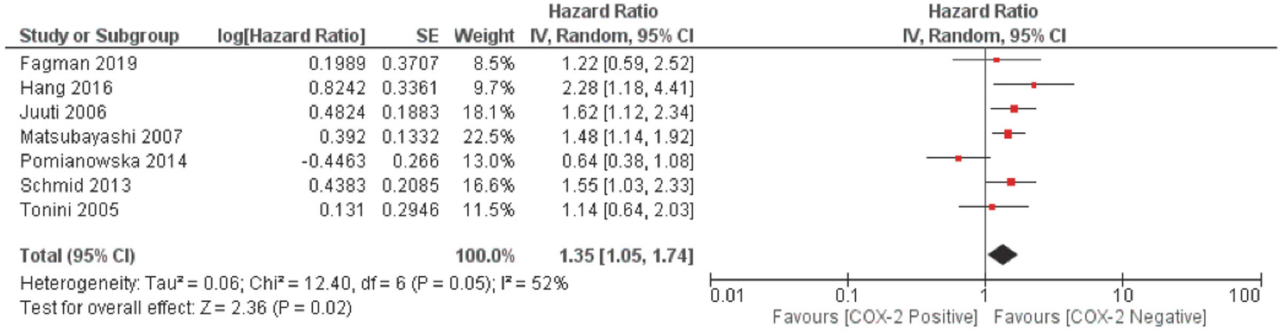

Figure 2. Forest plot for COX-2 expression crude hazard ratio.

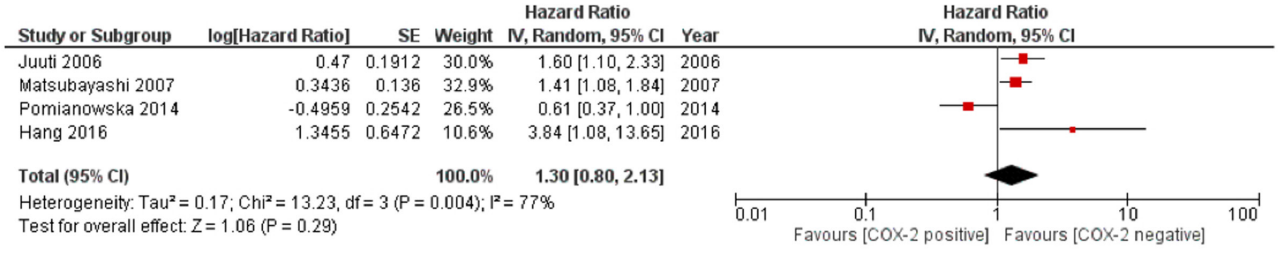

Figure 3. Forest plot for COX-2 expression adjusted hazard ratio.

hypothesized that increased COX-2 expression could lead to a more aggressive PDAC associated with a worse prognosis.[22]

The results of our meta-analysis showed that positive COX-2 tissue expression detected by immunohistochemistry was associated with decreased overall survival. This is in line with the previous meta-analysis, with a similar crude hazard ratio (HR = 1.48; 95% CI, 1.12-1.85 in Wang et al.[12]). However, there are differences in the studies included in both analyses. We did not include 3 studies from Wang et al.[12] because we were not able to retrieve the articles in English. On the other hand, some eligible articles in our study were not included in their study, which might be due to differences in the inclusion criteria. Moreover, we also pooled the adjusted HR from 4 studies.

Table 5. Results of the Sensitivity Analysis for COX-2 Tissue Expression

Sensitivity Analysis	Pooled Crude Hazard Ratio (95% CI)	Pooled Adjusted Hazard Ratio (95% CI)
No. of patients (≥50)	1.36 (1.03-1.80) I^2 = 59%	-
Pathologic Specimen: TMA	1.57 (1.23-2.00) I^2 = 30%	-
Pathologic Specimen: Whole section	1.21 (0.86-1.70) I^2 = 57%	1.20 (0.90-1.61) I^2 = 84%
Monoclonal antibody only	1.40 (1.02-1.92) I^2 = 66%	-
Polyclonal antibody only	1.17 (0.74-1.84) I^2 = 0%	-
Polymer-based method	0.84 (0.45–1.57) I^2 = 50%	-
Streptavidin–biotin–peroxidase method	1.40 (1.00-1.95) I^2 = 0%	-
Excluding estimated hazard ratio	1.53 (1.22-1.93) I^2 = 0%	-
Including all key covariates (age and some indicators related to the stage of the tumor)	-	1.51 (1.22-1.87) I^2 = 18%

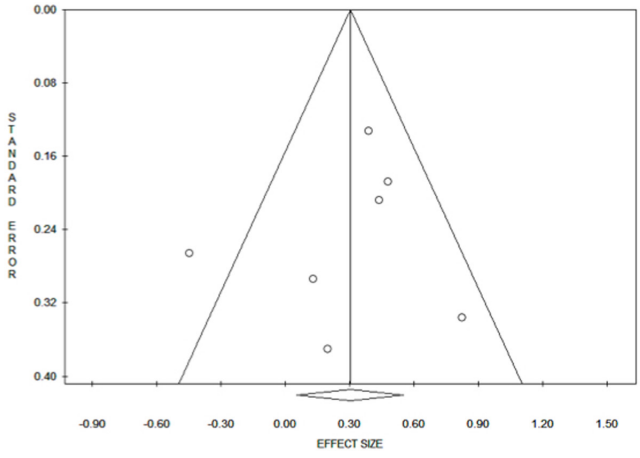

Figure 4. Funnel plot for COX-2 expression crude hazard ratio.

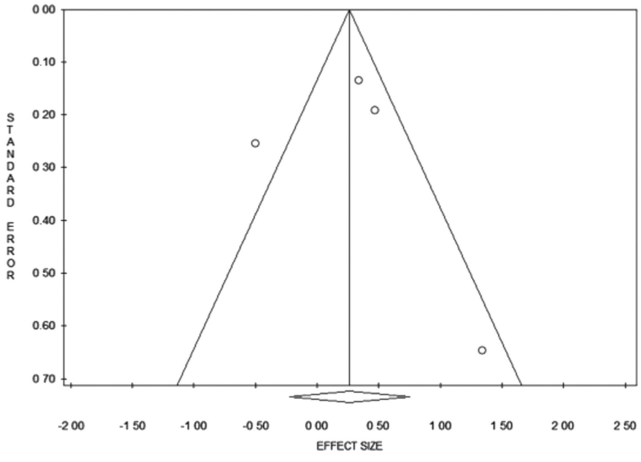

Figure 5. Funnel plot for COX-2 expression adjusted hazard ratio.

While the pooled adjusted HR showed a trend for worse prognosis, it was not statistically significant. Results from our sensitivity analysis showed that it might be caused by the difference in the covariates.

NF-κB is a major transcription factor for COX-2. KRAS mutation in PDAC has been linked to an increased activity of NF-κB, leading to the synthesis of various cytokines and pro-inflammatory molecules such as COX-2. Activation of NF-κB is also associated with increased tumor growth, angiogenesis, metastasis, and chemoresistance in PDAC patients, which leads to reduced survival.[24] Our meta-analysis of 2 studies showed that NF-κB tissue expression from immunohistochemistry was associated with decreased overall survival. Similar to COX-2, the adjusted HR was also not statistically significant, which might be due to differences in the scoring system, the length of follow-up, and the methodologies in the sample processing. In addition, the paucity of studies meant that further studies were needed before we could draw a conclusion.

Sp1 is a member of the Sp/Krupel-like family of transcription factors, which are ubiquitously expressed and play an important role in various basic cellular functions, such as proliferation, differentiation, and growth.[25] Overexpression of Sp1 is also found in various cancers, including PDAC. Expression of Sp1 in PDAC has been linked to increased risk of metastasis through epithelial-to-mesenchymal transition, angiogenesis through increased VEGF expression,[26] and increased mucin expression, leading to decreased survival.[27] Our meta-analysis of 2 studies showed that positive Sp1 tissue

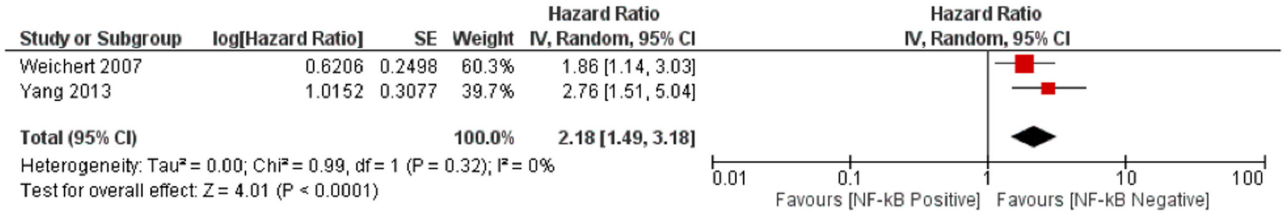

Figure 6. Forest plot for NF-κB expression crude hazard ratio.

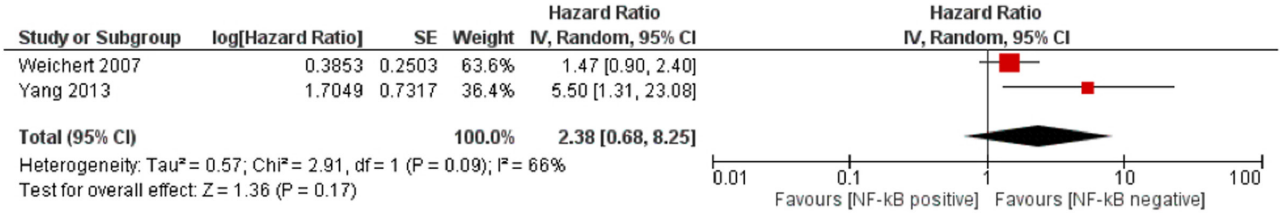

Figure 7. Forest plot for NF-κB expression adjusted hazard ratio.

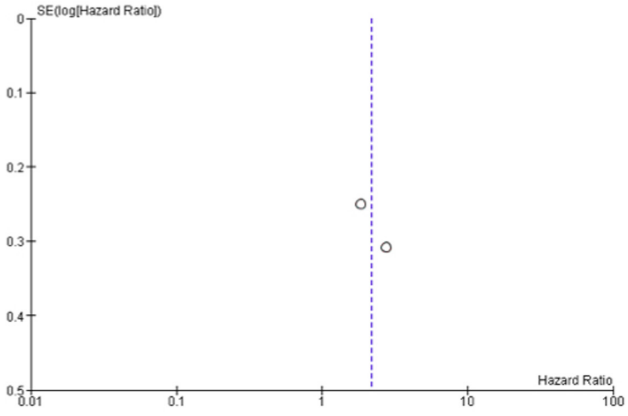

Figure 8. Funnel plot for NF-κB expression crude hazard ratio.

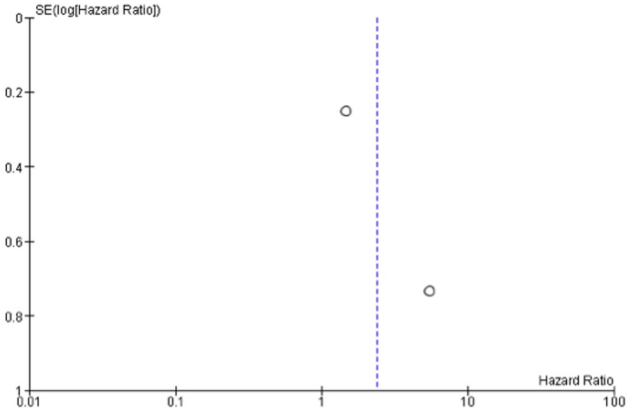

Figure 9. Funnel plot for NF-κB expression adjusted hazard ratio.

expression by immunohistochemistry was associated with decreased overall survival in PDAC patients. Unlike COX-2 and NF-κB, this result was still significant after adjustment to key covariates. Sp1 binding sites are also found in the COX-2 gene,[28] and 1 study showed that coexpression of Sp1 and COX-2 was associated with the worst prognosis in that study population.[29] Despite that, further studies are still needed, as our literature search only identified 2 eligible articles.

Overall, the findings of this meta-analysis support the role of inflammation in the progression of PDAC. One of the hallmarks of cancer is the presence of tumor-promoting inflammation.[30] The increased expressions of COX-2, NF-κB, and Sp1 are therefore not specific to PDAC and can be noted in other types of cancers such as breast cancer,[31,32] lung cancer,[33,34] and colorectal cancer.[35] In those cancers, the tumor microenvironment surrounding the cancer plays an essential role in maintaining a pro-inflammatory state.

The tumor microenvironment includes inflammatory cells such as macrophages. The macrophages, alongside other types of cells such as the fibroblasts, can secrete pro-inflammatory cytokines, including COX-2, into the tumor.[36] In PDAC, the pancreatic stellate cells create a dense stroma surrounding the cancer cells, promoting tumor growth, metastasis, and resistance to chemotherapy.[37] A study has shown that there is also an increase in the COX-2 expression in the pancreatic stellate cells.[38] Thus, COX-2 and its transcription factors,

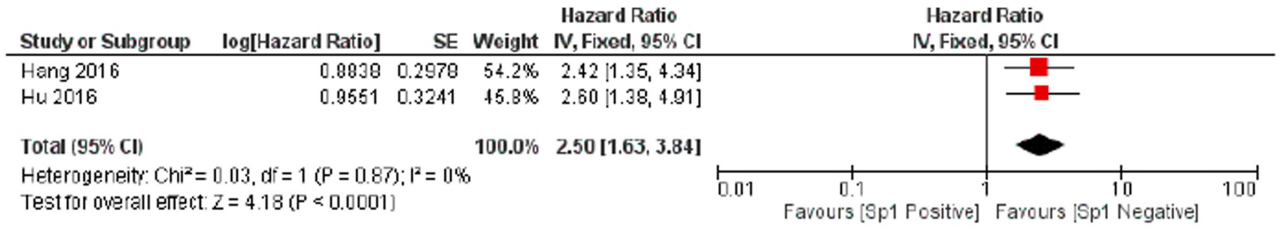

Figure 10. Forest plot for Sp1 expression crude hazard ratio.

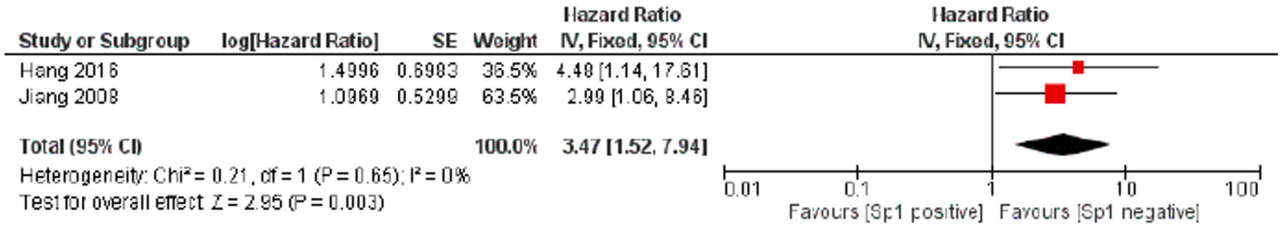

Figure 11. Forest plot for Sp1 expression adjusted hazard ratio.

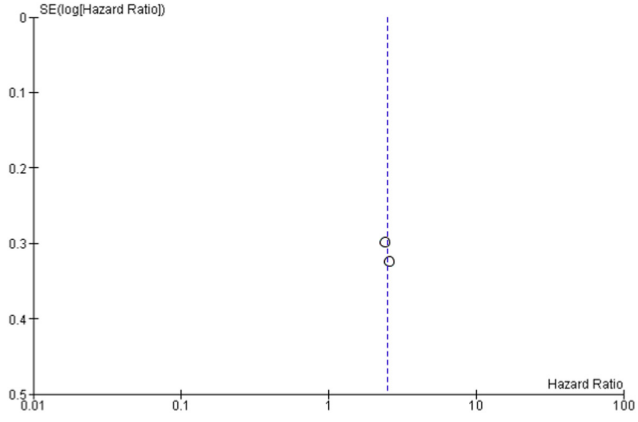

Figure 12. Funnel plot for Sp1 expression crude hazard ratio.

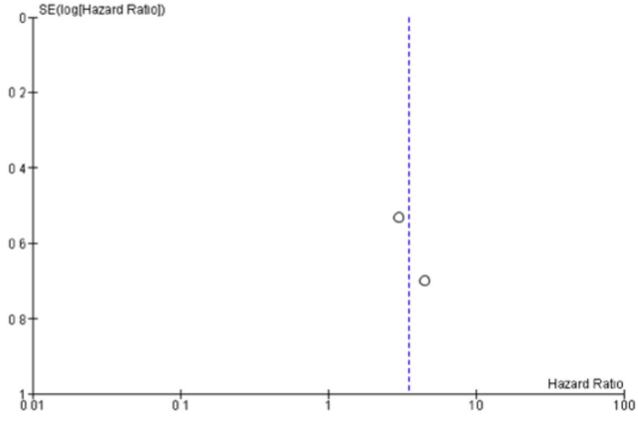

Figure 13. Funnel plot for Sp1 expression adjusted hazard ratio.

including NF-κB, and Sp1, can also be found both in the tumor cells and the cells in the tumor microenvironment.

The increased expression of COX-2 can also be found in non-cancerous inflammatory lesions such as chronic pancreatitis and pancreatic intraepithelial neoplasia (PanIN), which is the precursor lesion of PDAC. However, the expression of COX-2 is higher in PDAC compared with chronic pancreatitis and PanIN.[21,39] Therefore, although COX-2, NF-κB, and Sp1 expressions are not specific to cancer, the increased expressions compared to precancerous lesions suggest that they can still be utilized as prognostic markers.

There were several limitations to our study. We included articles published in English, which might lead to publication bias. Moreover, we did not seek unpublished results, which might lead to publication bias. The pooled adjusted HR might also be inaccurate because studies usually did not perform multivariate analysis if the result of the univariate analysis was not significant, which might lead to bias. There was also substantial heterogeneity between the studies. However, the advantage of our study compared to the previous meta-analysis was that we tried to estimate the unpublished HR by using the methods included in the Tierney et al.[18] paper. This resulted in more eligible studies in our meta-analysis, which reduced the risk of publication bias. Besides, to our knowledge, this was the first study to perform a meta-analysis of NF-κB and Sp1 tissue expressions with overall survival in PDAC patients.

In conclusion, the results of our study have shown that COX-2, NF-κB, and especially Sp1 tissue expressions by immunohistochemistry have the potential to be prognostic markers in PDAC patients. However, further studies with larger sample sizes are needed because of the heterogeneity of the studies. Moreover, studies that investigate coexpression between the 3 protein expressions are needed to identify whether it could lead to improved prognostic ability.

Ethics Committee Approval: An ethics committee approval statement was not needed since our work was a systematic review and meta-analysis.

Informed Consent: Verbal or written informed consent was not needed since our work was a systematic review and meta-analysis.

Peer-review: Externally peer-reviewed.

Author Contributions: All authors contributed to the study conception and design. Supervision – M.S., N.R., D.R.H., D.M.; Data Collection and/or Processing – K.R., M.S., A.W., F.S.; Analysis and/or Interpretation – K.R., M.S., N.R., D.R.H., D.M.; Literature Search – K.R., N.R., A.W., F.S.; Manuscript Writing – K.R., D.R.H., A.W., F.S.; Critical Review – all authors have given constructive inputs on the previous version of the manuscript, read the revised version, and approved the final manuscript.

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