Gastroenterology and Hepatology From Bed to Bench.
2024 **RIGLD**, Research Institute for Gastroenterology and Liver Diseases

SYSTEMATIC REVIEW

p-ISSN: 2008-2258 e-ISSN: 2008-4234

Differentiation of gastric adenocarcinoma and pancreatic adenocarcinoma using immunohistochemistry biomarkers: a systematic review and meta-analysis study

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ABSTRACT

Aim: This survey aimed to assess the differentiation of Gastric adenocarcinoma (GA) and pancreatic adenocarcinoma (PA) via immunohistochemistry biomarkers.

Background: GA and PA are two gastrointestinal malignancies with similarities in immunohistochemical features, making the diagnosis complex in some cases.

Methods: We searched international databases, including Google Scholar, Web of Science, PubMed, Embase, PROQUEST, and Cochrane Library, using appropriate keywords. The variance of each study was calculated using the binomial distribution formula, with all data analyzed by R version 16. Pooled odds ratios (OR), 95% confidence intervals (CI), and the I^2 test were calculated to evaluate the effectiveness of various immunohistochemistry biomarkers. Publication bias was assessed using funnel plots plus Begg's and Egger's tests.

Results: Based on the finding of our study, four potent biomarkers which can distinguish GA from PA were Cadherin 17 (CDH17) with pooled OR= 3.73 (95% CI 1.58 to 8.87), P value=0.003, and I2=55.5%; Caudal-type homeobox 2 (CDX2) with pooled OR=8.99 (95% CI 4.52 to 17.90), P value= <0.001, and I2=52.2%; CK7 with pooled OR= 0.15 (95% CI 0.04 to 0.57), P value= 0.005, and I2=56.6%; CK20 with pooled OR=2.06 (95% CI 1.38 to 3.08), P value= <0.001, and I2=0%.

Conclusion: Our study identified CDH17, COX-2, CK7, and CK20 as potent IHC biomarkers for differentiating PA and GA. Incorporating these biomarkers into routine diagnostics is essential for improving accuracy in challenging cases, ultimately aiding timely treatment decisions and improving patient outcomes.

Keywords: Gastric adenocarcinoma, Pancreatic adenocarcinoma, Immunohistochemistry, Biomarker, CDH17, COX-2, CK7, CK20.

(Please cite as: **Garousi B, Rezaei Z, Nazerian Y, Yasaghi Y, Alaei M, Bahrami Zanjanbar D, Tavasol A, Khoshrou A, Khademolhosseini S, Mirfakhraee H. Differentiation of gastric adenocarcinoma and pancreatic adenocarcinoma using immunohistochemistry biomarkers: a systematic review and meta-analysis study. Gastroenterol Hepatol Bed Bench 2024;17(4):325-337. https://doi.org/10.22037/ghfbb.v17i4.3057).**

Introduction

Gastrointestinal cancers account for a significant proportion of cancers worldwide, representing over 22% of cancer-related deaths annually (1). Several risk factors contribute to the development of these cancers. Chronic inflammation is a major risk factor, often associated with viral or bacterial infections; for instance, Helicobacter

Received: 07 July 2024 *Accepted*: 10 October 2024

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pylori infection is linked to gastric cancer, while hepatitis infection is associated with liver cancer. Additionally, autoimmune complications and disorders, such as inflammatory bowel disease, are significant risk factors for gastrointestinal malignancies, including colorectal cancers (2). Evidence also indicates that other risk factors include smoking, obesity, diet, and alcohol consumption (3).

Among the most important types of gastrointestinal cancers, gastric and pancreatic cancers are responsible for a considerable number of cancer-related deaths globally. Adenocarcinoma is a major type of cancer affecting organs with glandular epithelium, such as the lungs, gastrointestinal organs, breasts, prostate, and uterus. Over 80% of pancreatic malignancies, encompassing both the exocrine and endocrine components of the pancreas, are adenocarcinomas, which have a poor prognosis (4-6). Furthermore, most gastric malignancies consist of adenocarcinomas with unfavorable clinical outcomes (7). Thus, adenocarcinomas originating from the stomach and pancreas are of great importance, especially regarding their early diagnosis and treatment.

Although pancreatic cancer is the 12th most common cancer, its mortality rate exceeds 90%, with a 5-year survival rate of only about 9% (3, 5). This survival rate is negatively correlated with the clinical stage at diagnosis (5). Most cases are diagnosed at a late stage, which primarily contributes to the high mortality and low 5-year survival rate associated with this disease. Current diagnostic methods include CT scans, endoscopic ultrasound, ERCP, MRI, and MRCP. However, these techniques often lack effectiveness in providing accurate diagnoses due to their high cost as well as low sensitivity and specificity (3, 8). Additionally, gastric cancer ranks as the third leading cause of death related to overall cancer mortality (3, 9). Accurate detection of gastric adenocarcinoma (GA) is crucial for reducing disease-related mortality, as studies indicate that early detection is associated with better clinical outcomes (10, 11).

While most cancers manifest at their primary site, some invade other parts of the body and form metastatic lesions (12), which are predominantly adenocarcinomas (12-15). The management and prognosis of these secondary adenocarcinoma lesions depend on the original site of the tumor (13-15). If the primary site of the metastatic lesion is unknown, specific diagnostic tests should be conducted on the lesion (16).

To date, several studies have examined various biomarkers crucial for the diagnosis and differentiation of GA and PA from other types of cancers (17). The significance of these biomarkers lies in their ability to enhance diagnostic accuracy, thereby improving patient outcomes. Key biomarkers, such as Cadherin-17 (CDH17), Caudal-type homeobox 2 (CDX2), Carcinoembryonic antigen (CEA), Cytokeratin 7 (CK7), Cytokeratin 20 (CK20), AT-rich binding protein-2 (SATB2), and Villin play a vital role in distinguishing GA and PA, facilitating timely and appropriate treatment strategies. Meanwhile, there are many similarities in immunohistochemical features of gastric and pancreatic adenocarcinoma (18). Therefore, despite significant advancements in identifying biomarkers for the diagnosis of GA and PA, challenges persist in accurately differentiating between these two malignancies, especially in cases where traditional imaging and histopathology yield inconclusive results. Current diagnosis methods often lack the sensitivity and specificity needed for reliable differentiation, particularly in metastatic cases where the primary tumor site is unknown.

As such, the capability of IHC biomarkers to distinguish between GA and PA is of great importance. This study aims to identify a specific panel of biomarkers—CDH17, CDX2, CK7, and CK20—which can more effectively differentiate these adenocarcinomas than previously studied markers. Incorporating these IHC biomarkers into routine diagnosis workflows could enhance early detection and the integration of this panel into clinical guidelines and pathology reporting could further standardize diagnostic approaches and improve patient outcomes.

Methods

Literature search strategy and screening

To identify studies that evaluated immunohistochemistry biomarkers for differentiating gastric adenocarcinoma (GA) and pancreatic adenocarcinoma (PA), the databases of Web of Science, PubMed, Embase, PROQUEST, and the Cochrane Library were systematically searched independently by two authors in July 2022. The following keywords, combinations, abbreviations, and MeSH terms were used for the systematic search: ((((gastric adenocarcinoma*) OR (pancreatic

adenocarcinoma*)) OR (gastrointestinal adenocarcinoma*)) OR (adenocarcinoma*)) AND ((((immunohistochemistry) OR (immunomarker)) OR (immunostaining)) OR (immunohistochemical)).

Relevant studies that aligned with our research aims were selected by assessing the titles and abstracts. These studies included publications that evaluated biomarkers using immunohistochemistry for the differentiation of GA and PA or adenocarcinomas of the gastrointestinal tract. The full texts of potentially eligible studies were then reviewed for data extraction.

Inclusion and exclusion criteria

Publications were included if they assessed immunohistochemistry (IHC) biomarkers for the differentiation of adenocarcinomas of the gastrointestinal tract, specifically gastric and pancreatic adenocarcinomas. In addition to the original sites of the cancers, metastatic lesions

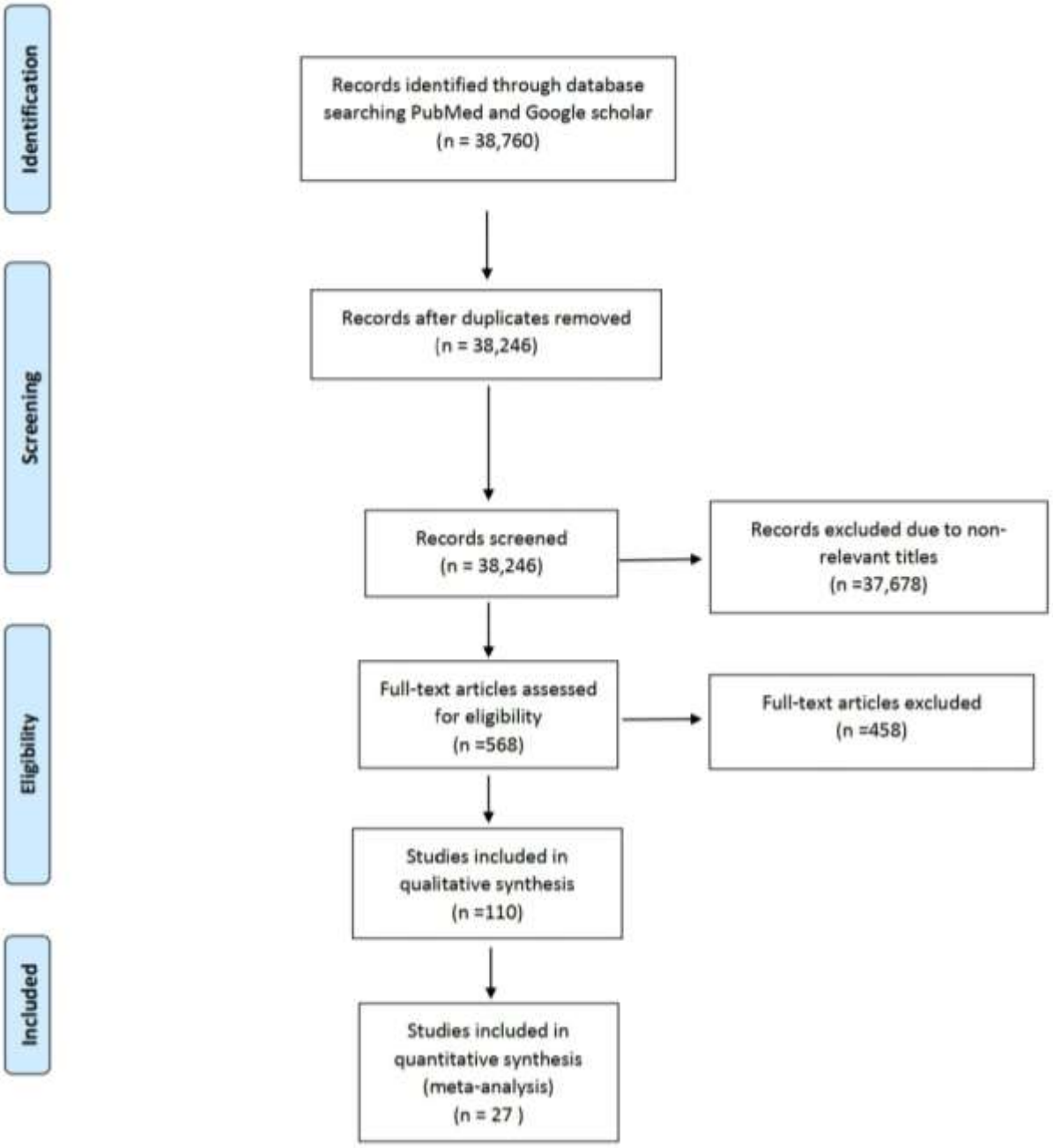

Figure 1. Flow chart of studies included in the present study

diagnosed as adenocarcinomas of gastrointestinal tract origin, including those of gastric and pancreatic origins, were also included. Publications with incomplete data and those that did not include both gastric and pancreatic adenocarcinomas together were excluded from the literature search, together with reviews, case reports, editorials, meta-analyses, as well as in vivo or in vitro studies.

Data extraction and eligibility

The full texts of the eligible studies were reviewed by two authors, with the extracted data organized in an Excel spreadsheet. The following data were collected from each study: first author's name, publication year, journal, biomarker(s), sample size (total and for individual gastric adenocarcinoma and pancreatic adenocarcinoma), and immunohistochemistry (IHC) staining positivity for GA and PA samples.

Quality assessment

The quality of included studies was assessed by an author using QUADAS criteria which is a quality assessment tool to evaluate the risk of bias and applicability of primary diagnostic accuracy studies in systematic reviews. The QUADAS questionnaire assesses the quality of the mentioned studies in four domains, including (1) patient selection, (2) index test(s), (3) reference standard, as well as (4) flow and timing.

Statistical analysis

The software used for statistical analyses was R. We compared the odds ratio (OR) with a 95% confidence interval (CI) between gastric adenocarcinoma (GA) and pancreatic adenocarcinoma (PA). Pooled ORs with 95% CI and the I^2 test for heterogeneity were calculated for biomarkers investigated in at least two publications. The interpretation of the I^2 heterogeneity test results is as

Table 1. Characteristics of included studies

First author (Reference)	Year of publication	Sample size	Biomarkers
Shahabinejad M (42)	2021	110	CK7, CK20
De Michele S (53)	2021	301	SATB2
Aasebø K (23)	2020	796	CDX2
Yu J (24)	2019	20	CDX2
Jiang XJ (19)	2019	160	CDH17
Baqar AR (36)	2019	623	CEA
Fei F (41)	2019	178	CK7
Seipel AH (33)	2016	99	CDX2, CK7, CEA, CK20, Villin
Moh M (52)	2016	149	SATB2
Lin F (20)	2014	18	CDH17, SATB2
Altree-Tacha D (21)	2014	884	CDH17, CDX2, CK20
Panarelli NC (22)	2012	777	CDH17, CDX2
Arango D (54)	2012	577	Villin
Bayrak R (28)	2012	91	CDX2, CK20, CK7
Mosnier JF (44)	2009	60	CK7
Park SY (25)	2007	314	CDX2
Mazziotta RM (26)	2006	183	CDX2, CK20, CK7
Moskaluk CA (30)	2006	745	CDX2
Vang R (31)	2006	90	CDX2, CK20
Kobayashi M (32)	2006	116	CDX2
Wang C (35)	2006	58	CDX2
Vang R (46)	2006	179	CK20, CK7
Dennis JL (34)	2005	27	CDX2, CK20, CK7
Kaimaktchiev V (27)	2004	121	CDX2
Werling RW (29)	2003	476	CDX2, Villin
Lau SK (45)	2002	107	CK7, CK20, Villin
Chu P (43)	2000	435	CK7, CK20
Tot T (48)	1999	93	CK20
Kaufmann O (50)	1996	328	CK20
Cheung PY (65)	1994	88	CK7
Moll R (49)	1992	93	CK20
Sheahan K (40)	1990	303	CEA
Pavelic ZP (39)	1990	217	CEA
Kahn HJ (38)	1989	86	CEA
Denk H (37)	1972	64	CEA

follows: 0% indicates total consistency between studies, while 100% indicates no consistency. Heterogeneity was considered significant if the p-value was < 0.1.

Results

Among 568 relevant manuscripts, 458 were excluded based on the inclusion and exclusion criteria. The full texts of 110 eligible publications from 1989 to July 2022 were reviewed (Figure 1). Overall, 81 biomarkers were identified; of these, 74 were investigated once or twice in studies that were excluded. Seven biomarkers, assessed in at least three studies, were obtained from the 27 included studies in our analyses. This selection is illustrated in Figure 1. The pooled odds ratios (OR) with 95% confidence intervals (CI) and calculated p-values for these biomarkers were analyzed. Ultimately, seven potential biomarkers that could differentiate gastric adenocarcinoma (GA) from pancreatic adenocarcinoma (PA) were discussed. The characteristics of the included studies are presented in Table 1.

Meta-analysis

In the process of the analysis, the OR was

Table 2. Results of 7 biomarkers included in this study

Biomarkers	Pooled OR (GA/PA)	95% CI	I^2	P value of pooled OR (calculated)
CDH17	3.73	1.58 to 8.87	55.5%	0.003
CDX-2	8.99	4.52 to 17.90	52.2%	<0.001
CEA	2.05	0.54 to 7.78	16.2%	0.288
CK7	0.15	0.04 to 0.57	56.6%	0.005
CK20	2.06	1.38 to 3.08	0%	<0.001
SATB2	2.08	0.12 to 34.03	44.4%	0.608
Villin	1.962	0.52 to 7.33	21.6%	0.316

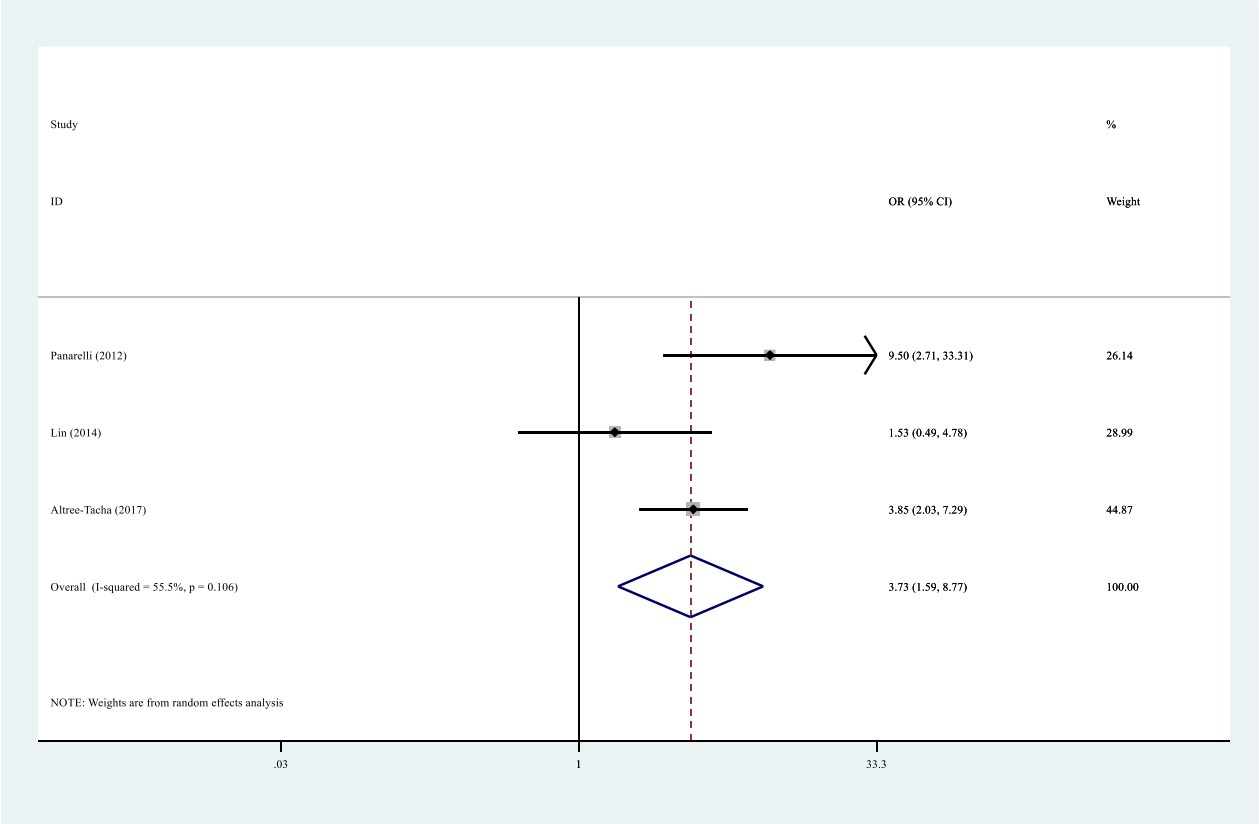

Figure 2. Forest plot of the CDH17 as potential biomarker that could differentiate GA from PA

calculated as the odds of each IHC biomarker in GA compared to PA (OR GA/PA). Studies with mathematically intangible OR were excluded. The ability of these IHC biomarkers to differentiate GA from PA was shown by significant (P<0.05) OR (CI=95%). Therefore, a significant OR>1 for a biomarker reveals that it is stained more in GA, while on the other hand, a significant OR<1 means it is stained more in PA. Among the analyzed biomarkers, CDH-17, CDX-2, CK-7, and CK-20 had significant OR values meaning that they can be used as biomarkers for differentiating GA and PA (Table 2).

CDH17

CDH17 is a member of the cadherin protein family, which is crucial for cellular adhesion and maintaining cellular structure. Dysregulated expression of cadherins can lead to cancer initiation and metastasis. CDH17, specifically, has been found to play a significant role in the progression of various cancers by disrupting normal cell-cell adhesion and promoting invasive as well as metastatic behavior (19). The total sample size obtained from studies (20-22) that evaluated CDH-17 as an IHC biomarker was 601 (GA=419 and PA=182). Further, 37% of GAs and 27.4% of PAs were positive for CDH-17 IHC staining. The significant (P=0.003) pooled OR calculated for this biomarker was 3.732 (CI=1.587-8.773) (Figure 2).

CDX2

CDX2 is a nuclear transcription factor that is specifically expressed in intestinal tissues. It plays a pivotal role in regulating cell proliferation and differentiation, which are essential processes for maintaining the normal function and structure of the intestinal epithelium. CDX2 is crucial for the development and maintenance of the intestinal lining, influencing the differentiation of cells within the intestinal tract (23). CDX2's involvement in

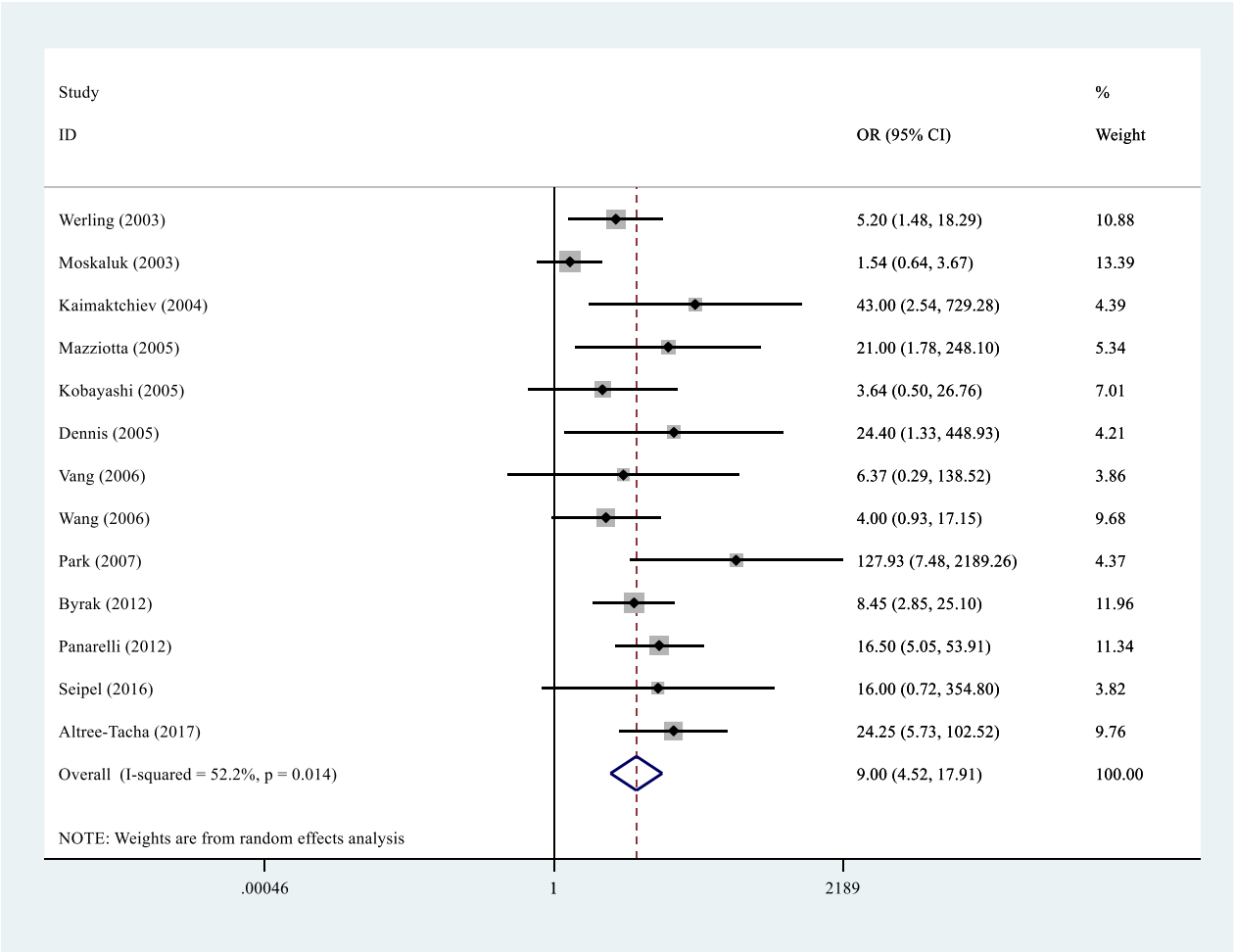

Figure 3. Forest plot of the CDX2 as potential biomarker that could differentiate GA from PA

tumorigenesis has been extensively studied, particularly in gastrointestinal cancers. Its expression can be dysregulated in cancer, which contributes to the abnormal growth and spread of cancer cells. In many gastrointestinal malignancies, CDX2 acts as a tumor suppressor, meaning its loss or diminished expression is often associated with the progression of cancer. Conversely, its presence is indicative of a more differentiated state, which can be associated with less aggressive tumor behavior (24). A large sample size was extracted from the data represented in studies (21, 22, 25-35) (Total sample size=1112), in which 52.9% of GA samples (395 of 747 samples) were positive, whereas only 14.2% of PA samples were stained with CDX-2. The pooled OR was 9 (CI=4.523-17.906), which was statistically significant (P<0.001) (Figure 3).

CEA

CEA is a glycoprotein involved in cell adhesion, originally identified in fetal gut tissue, but also expressed in certain cancers and normal adult tissues. It is a well-established tumor marker, particularly for colorectal cancers. It is often used as a biomarker to Monitor Treatment Response and Detect Recurrence (36). In the included studies (33, 37-40), CEA was stained in 80% (48 of 60) of GAs and 71% (28 of 39) of PAs. The total OR calculated for this biomarker was 2.058 (CI=0.544-7.785), which was comparable to the differentiation of GA and PA (P=0.288).

CK7

CK7 is a type of protein known as a cytokeratin, which forms part of the cytoskeleton within cells and is characterized by its specificity to certain types of epithelial cells. These cells include those lining the respiratory tract, the urogenital system, and parts of the gastrointestinal tract. Structurally, cytokeratins such as CK7 form filamentous structures within cells, contributing to their overall shape and integrity (41, 42). Analysis of the data obtained from studies (26, 28, 33,

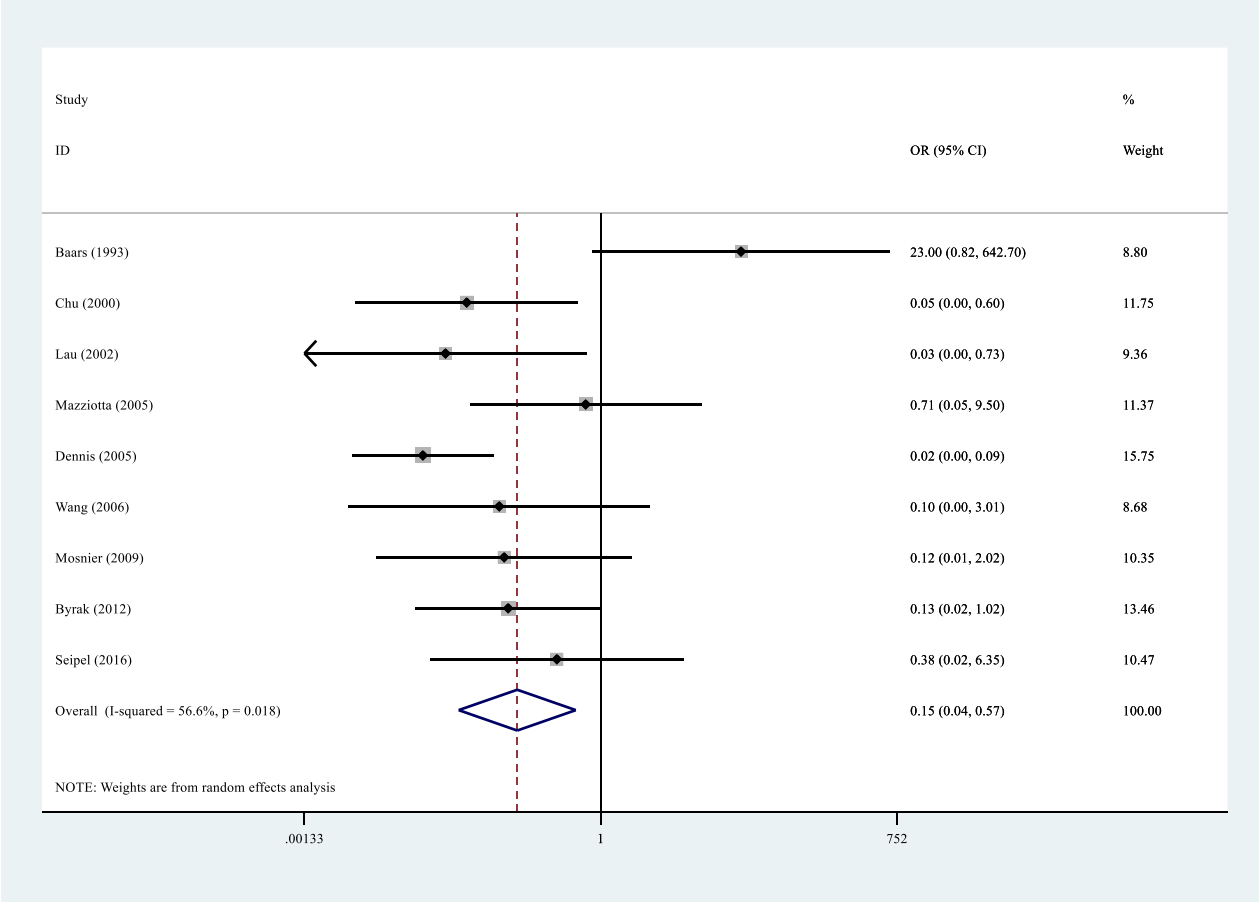

Figure 4. Forest plot of the CK7 as potential biomarker that could differentiate GA from PA

34, 43-47) revealed that the pooled OR of CK-7 for distinguishing GA from PA was 0.155 (CI=0.042-0.572), which is statistically significant (P=0.005). Compared to ~71% positive staining for GA samples, 93.2% of PA samples were positive for CK-7 (Figure 4).

CK20

CK20 is a type of protein belonging to the cytokeratin family, which forms part of the cytoskeleton within cells. CK20, similar to CK7, is specific to certain types of epithelial cells and is often used as a marker in cancer diagnosis, either alone or in combination with other markers such as CK7 (42). A significant (P<0.0001) OR=2.068 (CI=1.386-3.084) was calculated for this biomarker in our analysis. With was calculated for this biomarker in our analysis. With the data gathered from studies that evaluated CK-20 (21, 26, 28, 31, 33, 34, 43, 45, 46, 48-50), it was determined that 38.4% of GA samples are positive compared to ~32.8% of positive Pas (Figure 5).

SATB2

SATB2 is a gene expression regulator working as a transcriptional co-factor and a modulator of the chromatin structure, which is found to be highly expressed in several cancers with an important role in cancer formation and metastasis (51). Of the three studies which evaluated SATB2 (20, 52, 53), one was excluded due to an intangible calculation of OR. The pooled OR of this biomarker was comparable to the differentiation of GA from PA (P=0.608) and was equal to 2.08 (CI=0.127-34.039).

Villin

Villin is an actin-binding protein modulated by calcium and is one of the important components of microvilli structures that is mainly expressed in the brush border of the intestinal epithelium and proximal renal tubule epithelium (54). Similar to SATB2, only

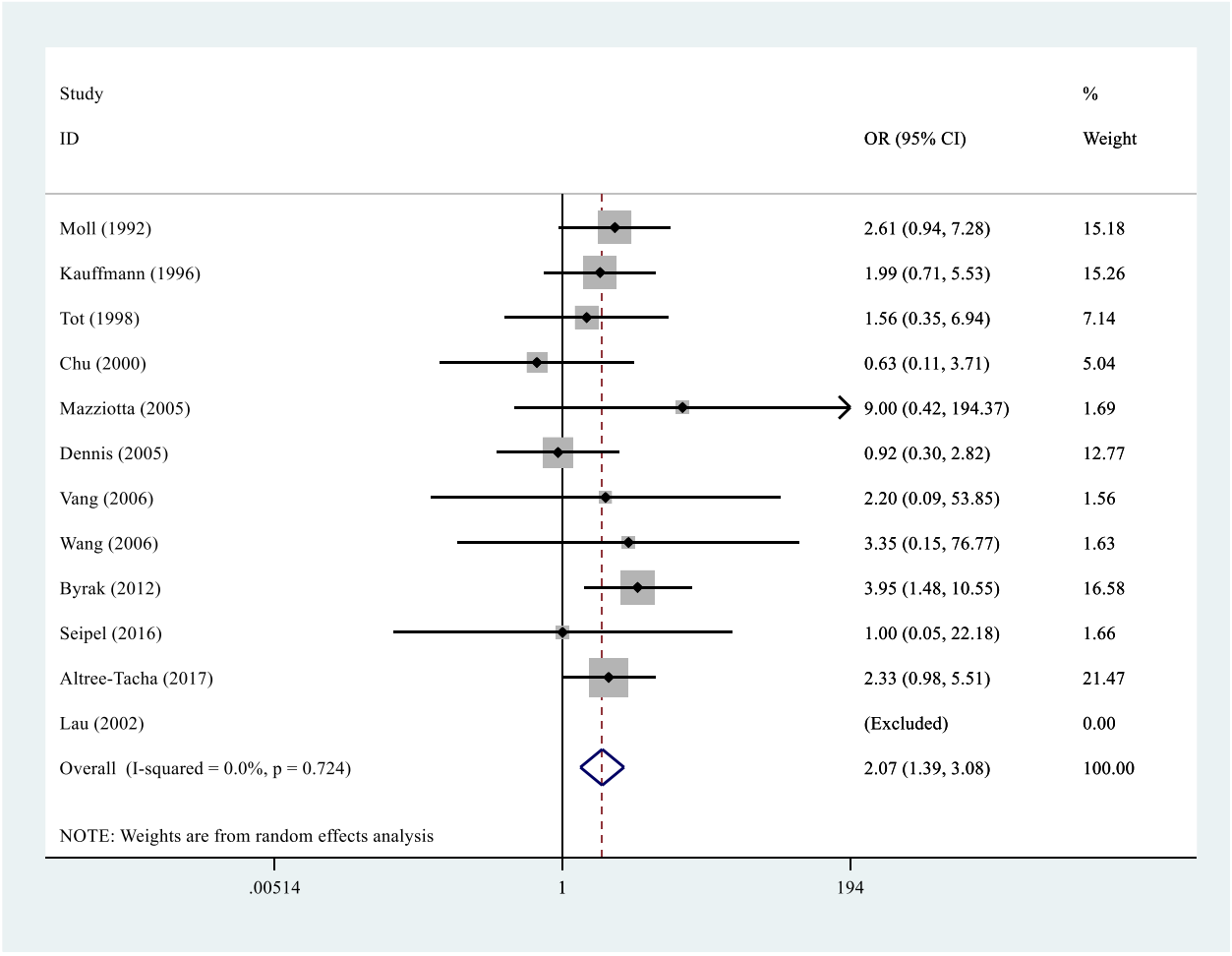

Figure 5. Forest plot of the CK20 as potential biomarker that could differentiate GA from PA

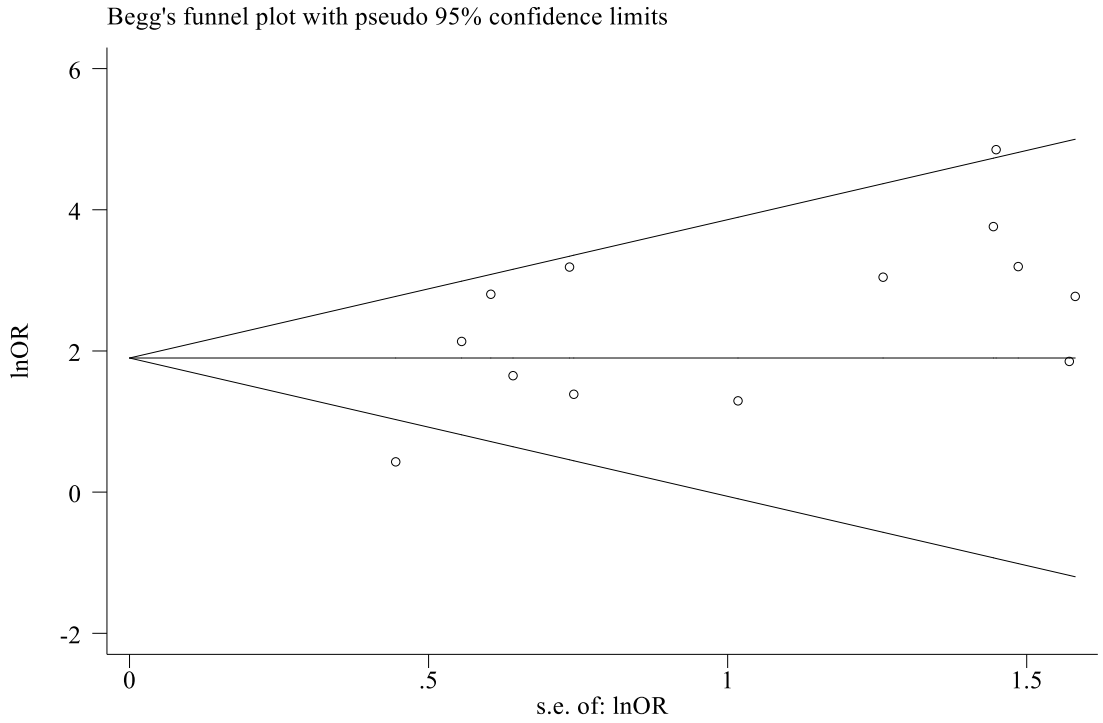

Figure 6. Publication bias test using Begg's funnel plot test

three studies were found to have evaluated villin as an IHC biomarker to differentiate GA from PA (29, 33, 45). The calculated pooled OR was 1.962 (CI=0.525-7.332), which was insignificant (P=0.316) for 42.4% (14/33) positive GAs compared to 28.6% (12/42) positive PA samples.

Publication bias

Following the evaluation, according to Begg's test, there was no publication bias. The results of Begg's test and Begg's funnel plot are presented in Figure 6.

Discussion

Differentiation between adenocarcinomas of the pancreatic, biliary system, and gastrointestinal tract in surgical pathology is a diagnostic challenge, especially when metastases occur. IHC biomarkers can contribute to the detection of PA from GA by biological differentiation (17). In a systematic study conducted by Liu et al., more than 20 IHC biomarkers were reported as diagnostic tools for PA (55). Also, in a systematic review conducted by Ansari et al., more than 70 IHC biomarkers were reported as a targeted prognostic biomarkers of PA (56).

Expression patterns of IHC biomarkers, such as CDH17, CK7, and CK20, etc., have been well investigated in the pancreas, stomach, lung, colon, and breast cancers (43, 57, 58). However, these findings only were limited to one or two organs. Further, most of the previous studies involved a limited number of cases, and some of them revealed contradictory results (43, 48, 57-59).

This study aimed to assess the differentiation of GA and PA by immunohistochemistry biomarkers. The results indicated that four potent biomarkers which can distinguish GA from PA are: CDH17, CDX2, CK7, and CK20. In contrast to our results, Wang et al. (60) reported that CK immunostainings are not helpful in differentiating GA from PA since the former shows variable profiles of CK 7 and CK 20.

Consistent with our results, the findings of Ma et al. (61) study revealed that CDH17 and CDX2 expression was higher in small intestinal adenocarcinoma (73.1% and 65.4%) than in pancreatic (14.3% and 2.9%) and bile duct (41.2% and 23.5%) cancers, respectively. Also, CK20 expression was relatively high in small intestinal adenocarcinoma but low in 78 tumor tissues

(42.3%). Gastrointestinal epithelium lacks CK7, while CK7 is found in glandular epithelium and epithelial malignancies of the lung, ovary, endometrial, and breast (57). Meanwhile, CK20 is primarily expressed in normal glands, GI tract epithelial cancers, urothelium, and Merkel cells. Since primary and metastatic tumors frequently preserve the cytokeratin profiles of the epithelium from which they originate, the cytokeratin 7/20 profile of a specific tumor has proven to be a helpful tool in the differential diagnosis of carcinomas (57). Adenocarcinomas from the small bowel, appendix, and colorectum almost always include CK7-/CK20+ epithelium, which helps to differentiate them from adenocarcinomas of many other source sites. In several series, the CK7-/CK20+ pattern was found in anywhere between 65% and 95% of colorectal adenocarcinomas. On the other hand, less than 10% of pancreatic and about one-third of gastric adenocarcinomas also exhibit this pattern (43). The homeobox gene family contains the human CDX2 protein, which encodes a transcription factor unique to the intestine. In both embryonic and postnatal life, this protein, which controls intestinal development, is expressed in the nuclei of intestinal epithelial cells. The intestinal epithelium has been demonstrated to express CDX2 mRNA in a highly limited manner (62).

CDX2 expression was analyzed across 476 samples of human cancers by Werling et al. (63), who concluded that it is a highly accurate indicator of adenocarcinomas developing in the GI tract, particularly the duodenum and colon.

CDX2 was expressed in 114 of 118 (97%) colorectal, 36 of 59 (61%) gastric, and 5 of 32 (16%) pancreatic adenocarcinomas in the Bayrak et al. (64) investigation. They claimed that CDX2 expression could be a helpful supplemental test for the diagnosis of intestinal adenocarcinomas, especially when more reliable markers such as CK7 and CK20 generate conflicting results.

The study was limited by the small number of investigated biomarkers and the few studies available for each biomarker, which affects the generalizability of the results. There is also heterogeneity in the reference standards among the reviewed studies, along with potential publication bias, as negative studies may remain unpublished. The analysis involving diverse patient populations that may influence biomarker expression. Additionally, many of the studies included were retrospective, necessitating prospective validation. Finally, the lack of longitudinal data limits understanding of the prognostic implications of the biomarkers.

Therefore, the incorporation of these immunohistochemical biomarkers into routine diagnostic workflows can significantly enhance the sensitivity and specificity of distinguishing GA from PA. This improved diagnostic accuracy facilitates tailored treatment approaches, allowing clinicians to choose the most effective therapies based on the tumor type, which can lead to better patient outcomes. Additionally, understanding the origin of the adenocarcinoma has prognostic implications, aiding in patient counseling and management. Accurate tumor classification is also crucial for surgical planning and the development of clinical guidelines, ensuring timely and appropriate care. Overall, these findings underscore the potential of these biomarkers to improve clinical decision-making and patient outcomes in GA and PA.

Conclusion

To sum up, the findings of our study revealed that CDH17, CDX2, CK7, and CK20 are potent IHC biomarkers for the differentiation of GA and PA cases. Thus, in the future, these biomarkers can be employed for early detection and differentiation of these adenocarcinomas. Future research should focus on multicenter trials with diverse populations, longitudinal studies to assess prognostic value, and integration into standard diagnostic practices. Comparative studies with established biomarkers will enhance understanding of their clinical utility. By pursuing these avenues, we can strengthen and optimize the application of these biomarkers in future of medicine.

Conflict of interests

There is no conflict of interest for authors of this article.

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