SUPPLEMENTARY MATERIAL. TABLE OF CONTENTS

Liquid Biopsy Status After Resection Of Pancreatic Adenocarcinoma And Its Relation To Oncological Outcomes. Systematic Review And Meta-Analysis.

1. PRISMA checklist

1.1. Article Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	-
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	3
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	4
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	5

Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	4
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	4
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	5,6
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	5, 6
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	6,7
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	5
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	6
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	7
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	5
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	5
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	5
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	7

	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	7,8
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	7,8
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	-
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	6
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	7, 8
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	7, 8
Study characteristics	17	Cite each included study and present its characteristics.	8, 9, Table 1
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	6
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	8-9
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	8-9
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	8-9
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	8-9
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	8-9

Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	-
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	-
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	11-13
	23b	Discuss any limitations of the evidence included in the review.	14
	23c	Discuss any limitations of the review processes used.	14
	23d	Discuss implications of the results for practice, policy, and future research.	14-15
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	-
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	4-6
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	-
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	16
Competing interests	26	Declare any competing interests of review authors.	16
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Supplementary material

1.2. Abstract Checklist

Section and Topic	Item #	Checklist item	Reported (Yes/No)
TITLE			
Title	1	Identify the report as a systematic review.	Yes
BACKGROUND			
Objectives	2	Provide an explicit statement of the main objective(s) or question(s) the review addresses.	Yes
METHODS			
Eligibility criteria	3	Specify the inclusion and exclusion criteria for the review.	Yes
Information sources	4	Specify the information sources (e.g. databases, registers) used to identify studies and the date when each was last searched.	Yes
Risk of bias	5	Specify the methods used to assess risk of bias in the included studies.	No
Synthesis of results	6	Specify the methods used to present and synthesise results.	No
RESULTS			
Included studies	7	Give the total number of included studies and participants and summarise relevant characteristics of studies.	Yes
Synthesis of results	8	Present results for main outcomes, preferably indicating the number of included studies and participants for each. If meta-analysis was done, report the summary estimate and confidence/credible interval. If comparing groups, indicate the direction of the effect (i.e. which group is favoured).	Yes

DISCUSSION			
Limitations of evidence	9	Provide a brief summary of the limitations of the evidence included in the review (e.g. study risk of bias, inconsistency and imprecision).	Yes
Interpretation	10	Provide a general interpretation of the results and important implications.	Yes
OTHER			
Funding	11	Specify the primary source of funding for the review.	No
Registration	12	Provide the register name and registration number.	No

2. QUIPS

2.1. Definitions

Study participation	
Low	Diagnosis of PDAC is confirmed by histology. Location and period of recruitment are described. General features of the included population are detailed. The exclusion and inclusion criteria are adequately described.
Moderate	Diagnosis of PDAC is confirmed by histology. Incomplete information about exact location or period of recruitment or lack of included population features.
High	No histological confirmation of PDAC.
Study attrition	
Low	Reasons for participants losing follow-up are indicated. Adequate response rate (proportion of study sample that complements study and provides outcome data).
Moderate	Reasons for participants losing follow-up are not indicated or there is no adequate response rate.
High	Reasons for participants losing follow-up are not indicated and there is no adequate response rate.
Prognostic factor measurement	
Low	Liquid biopsy method is clearly defined and described. Same liquid biopsy method is used among all participants and it is analysed preoperatively and postoperatively.
Moderate	Liquid biopsy method is named but not described.
High	Liquid biopsy is not uniform among all participants or not defined.
Outcome measurement	
Low	Clear definition of outcome is provided. Outcome is uniform for all study participants.
Moderate	Unclear definition of outcome is provided. Outcome is uniform for all study participants.
High	Unclear definition of outcome is provided. Outcome not uniform for all study participants.

Study confounding	
Low	Confounders are defined and measured (TNM, age, comorbidities…). Stratification if needed is done
Moderate	Confounders are defined and measured (TNM, age, comorbidities…). Stratification is not done.
High	Confounders are not defined and measured.
Statistical analysis and reporting	
Low	There is sufficient presentation of data and the statistical model is correct.
Moderate	There is insufficient presentation of data or the statistical model is incorrect.
High	There is insufficient presentation of data and the statistical model is incorrect.

2.2. Global view of risk of bias of the meta-analysis performed with QUIPS tool.

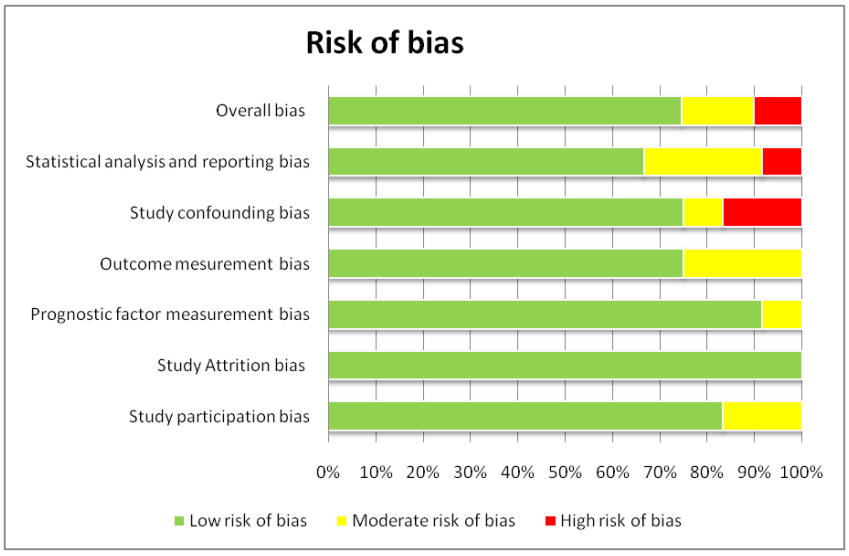

Figure S1: Global view of risk of bias of the meta-analysis performed with QUIPS tool.

3. Supplementary figures

3.1. Mortality in detectable and undetectable liquid biopsy status after surgery.

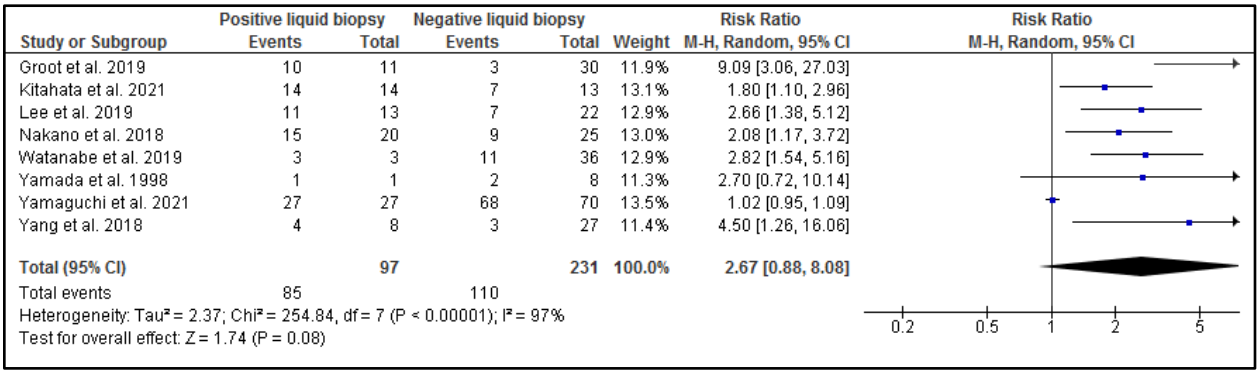

Figure S2 : Forest plot of mortality rates comparing positive vs negative liquid biopsy status after surgery in patients with resectable PDAC.**Only patients that went through surgery were included in this meta analysis from the Groot et al publication*.

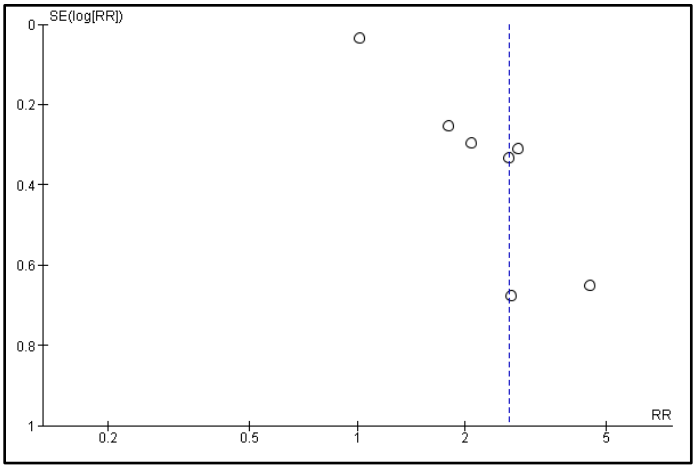

Figure S3: Funnel plot of publications analysed in the comparison of mortality between positive vs negative liquid biopsy status after surgery in patients with resectable PDAC.

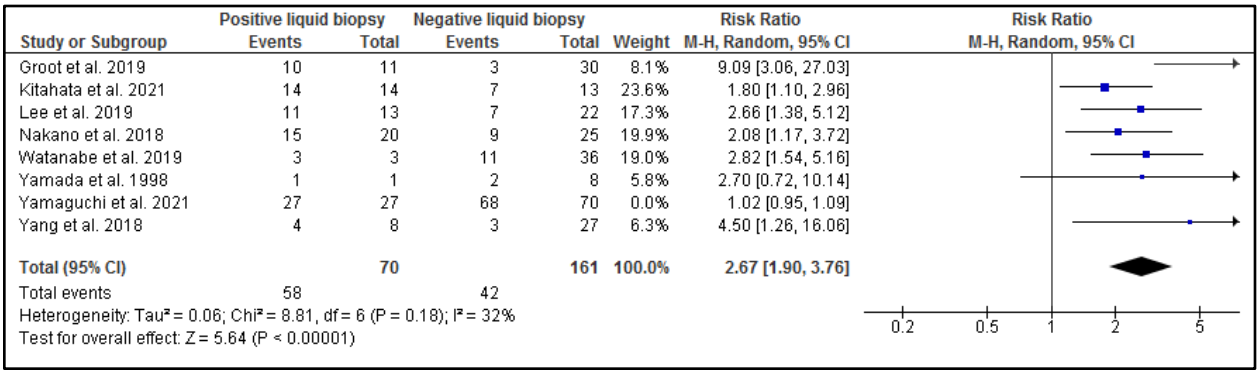

Figure S4: Forest plot of mortality rates comparing positive vs negative liquid biopsy status after surgery in patients with resectable PDAC excluding Yamaguchi et al.**Only patients that went through surgery were included in this meta analysis from the Groot et al publication*.

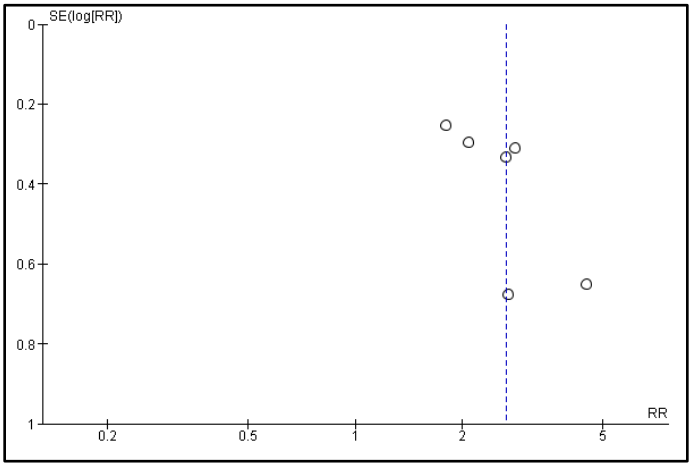

Figure S5: Funnel plot of publications analysed in the comparison of mortality between positive vs negative liquid biopsy status after surgery in patients with resectable PDAC, excluding Yamaguchi's et al.

3.2. Recurrence in detectable and undetectable liquid biopsy status after surgery.

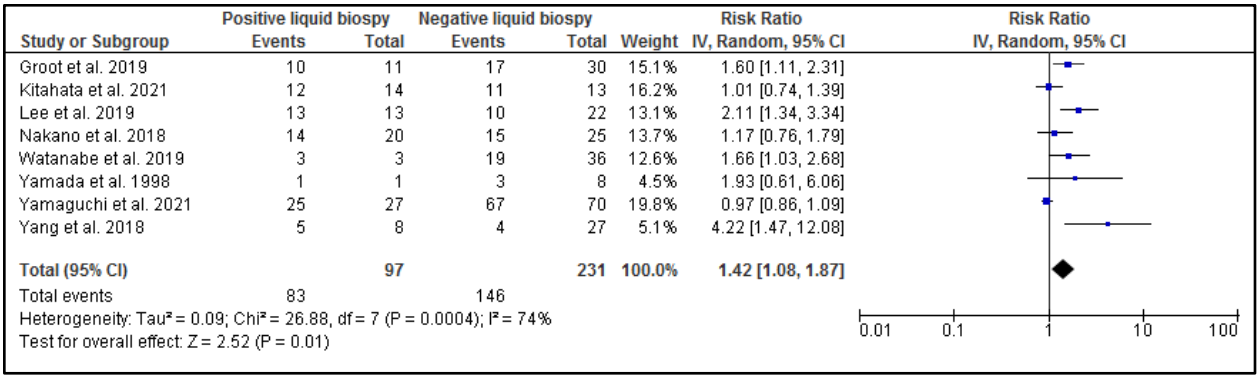

Figure S6: Forest plot of recurrence rates comparing positive versus negative liquid biopsy status after surgery in patients with resectable PDAC.

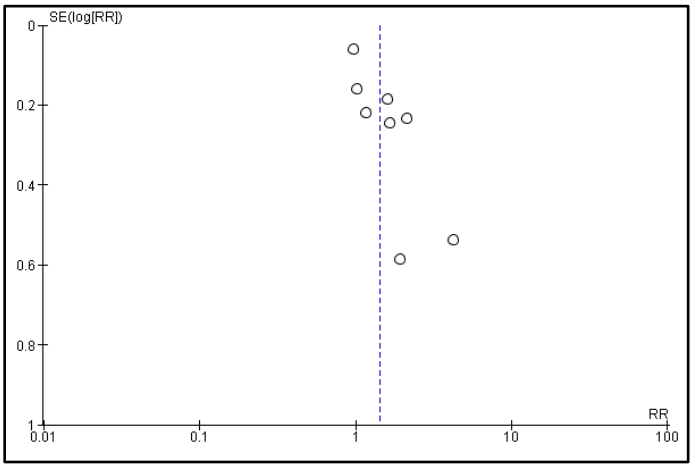

Figure S7: Funnel plot of publications analysed in the comparison of recurrence rate between positive versus negative liquid biopsy status after surgery in patients with resectable PDAC.

3.3. Effect of surgery on liquid biopsy (ctDNA) dynamics

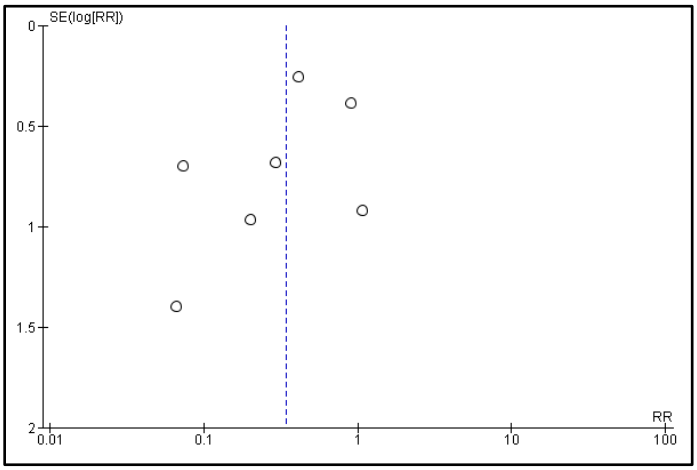

Figure S8: Funnel plot of publications analysed comparing ctDNA shift after surgery in patients with resectable PDAC.

3.4. Survival analysis

3.4.1. Survival analysis according to liquid biopsy status before surgery

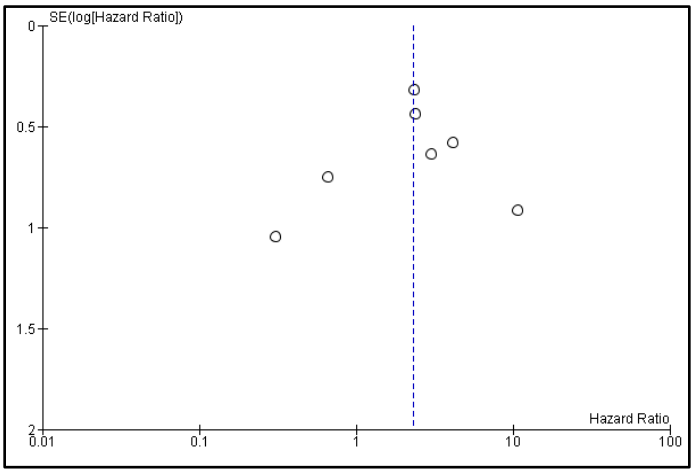

Figure S9: Funnel plot of publications analysed in the comparison of OS between positive versus negative ctDNA status before surgery in patients with resectable PDAC.

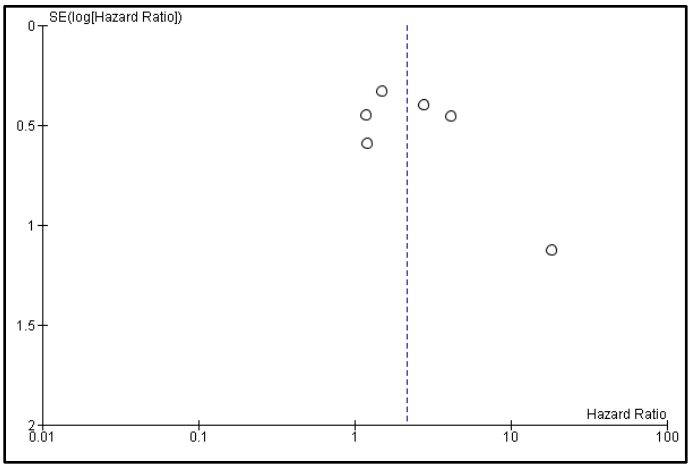

Figure S10: Funnel plot of publications analysed in the comparison of DFS between positive versus negative ctDNA status before surgery in patients with resectable PDAC.

3.4.2. Survival analysis according to liquid biopsy status after surgery

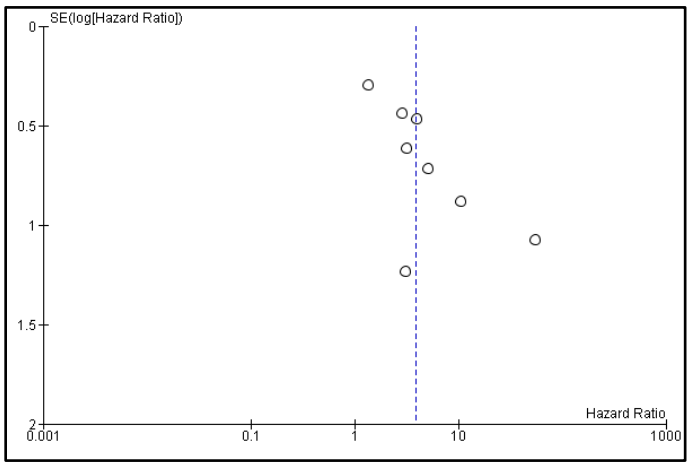

Figure S11: Funnel plot of publications analysed in the comparison of OS between positive versus negative ctDNA status after surgery in patients with resectable PDAC.

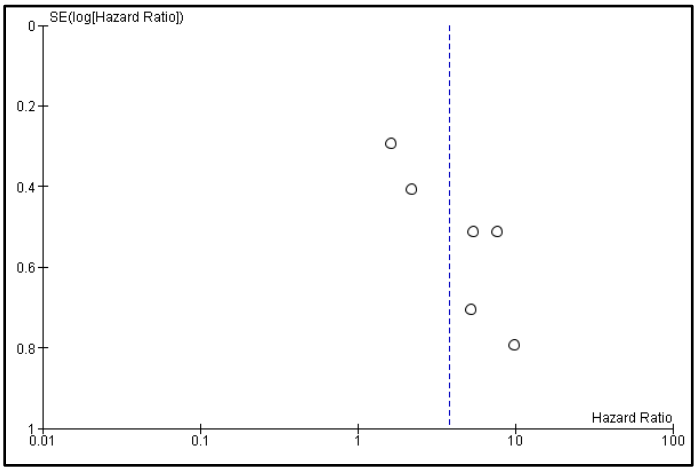

Figure S12: Funnel plot of publications analysed in the comparison of DFS between positive versus negative ctDNA status after surgery in patients with resectable PDAC.

3.4.3. Survival analysis according to the shift dynamics after surgery.

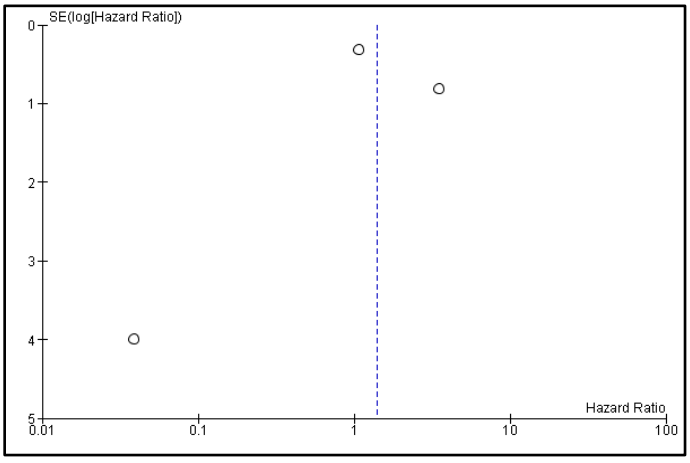

Figure S13: Funnel plot of publications analysed in the comparison of OS in patients with ctDNA shift *positive-to-negative* versus those patients who

stayed positive or shifted *negative-to-positive* after surgery.

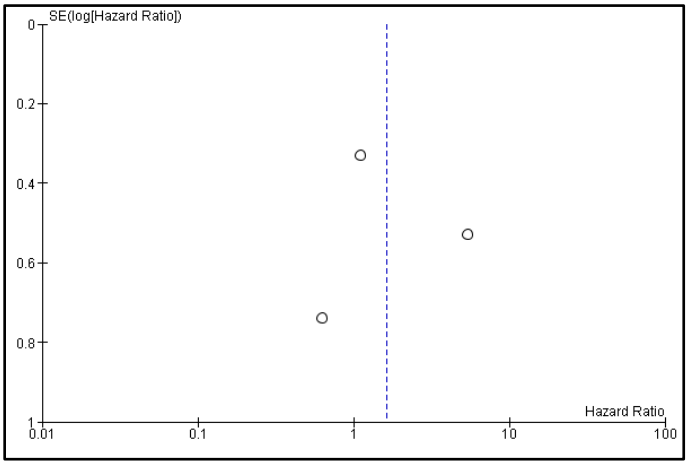

Figure S14: Funnel plot of publications analysed in the comparison of DFS in patients with ctDNA shift *positive-to-negative* versus those patients who stayed positive or shifted *negative-to-positive* after surgery.

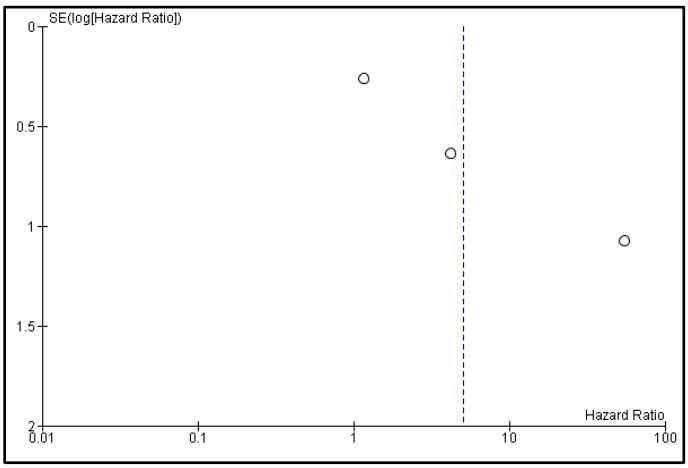

Figure S15: Funnel plot of articles that evaluate OS between patients with ctDNA shift *negative-to-positive* versus those patients who stayed negative or shifted *positive-to-negative* after surgery.

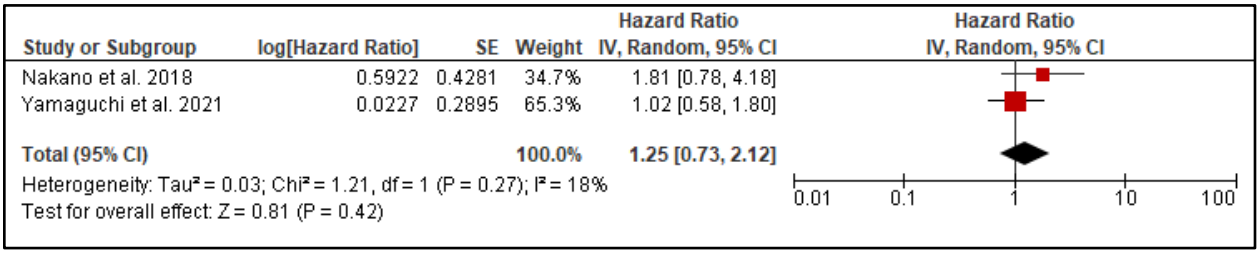

Figure S16: Forest plot of DFS in patients with ctDNA *shift negative-to-positive* versus those who stayed positive or shifted negative-to-positive after surgery.

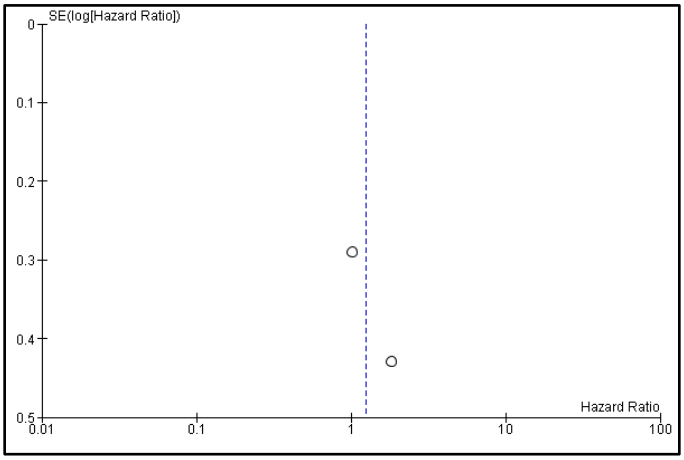

Figure S17: Funnel plot of articles that evaluate DFS between patients with ctDNA shift *negative-to-positive* versus those patients who stayed negative or shifted *positive-to-negative* after surgery.

3.5. Comparison between non-touch techniques (NTIT) versus standard technique.

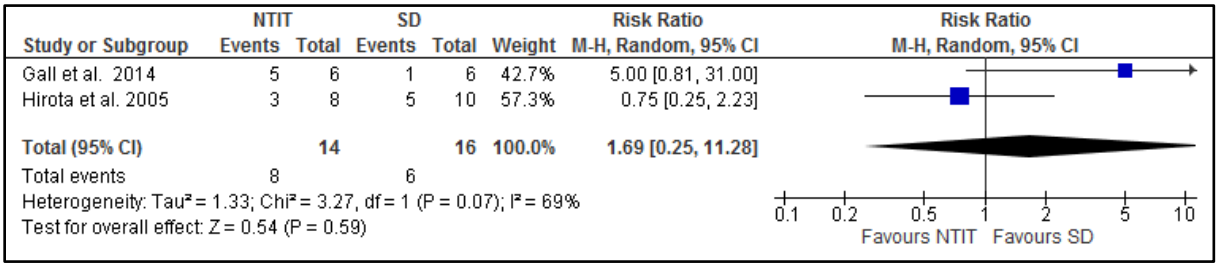

Figure S18: Forest plot of comparison of liquid biopsy negativization between NTIT and standard technique in patients with resectable PDAC.

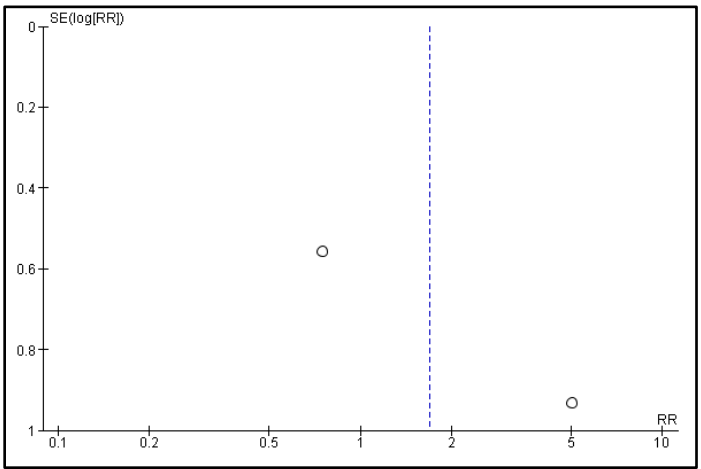

Figure S19: Funnel plot of articles that evaluate DFS between patients with ctDNA shift negative-to-positive versus patients with a shift positive-to-negative or that stay negative.

3.6. Sub-analysis excluding studies with metastatic patients

3.6.1. *Overall survival according to liquid biopsy status before surgery.*

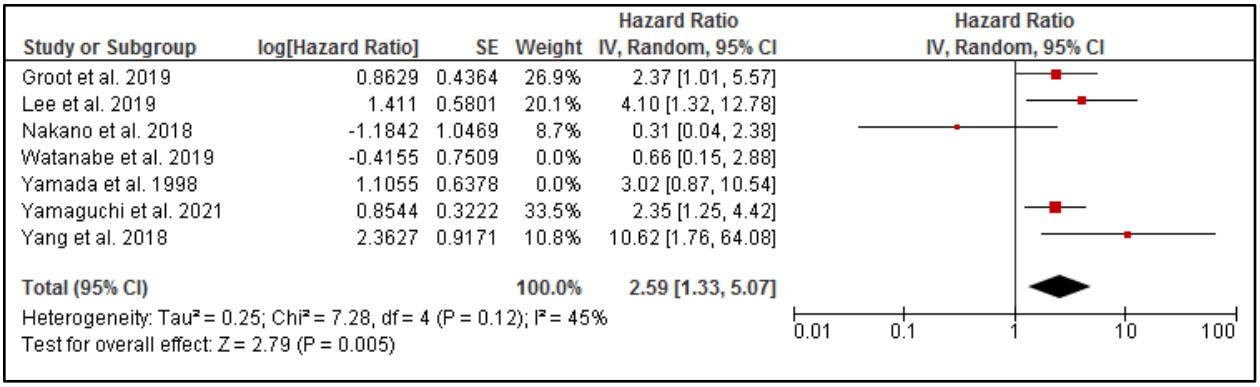

Figure S20: Forest plot of overall survival comparing positive versus negative ctDNA status before surgery, excluding studies with metastatic patients.

3.6.2. Overall survival according to liquid biopsy status after surgery.

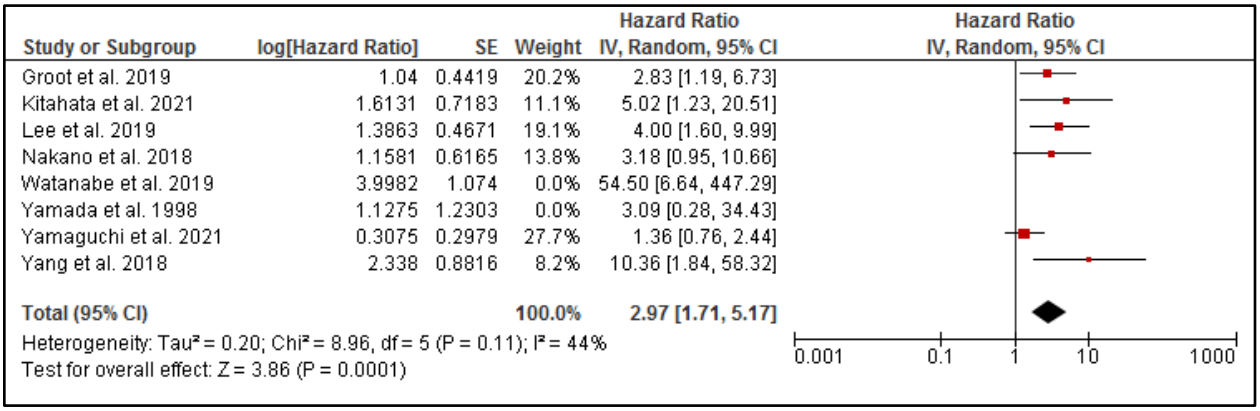

Figure S21: Forest plot of overall survival comparing positive versus negative ctDNA status after surgery, excluding studies with metastatic patients.

3.6.3. Disease-free survival according to liquid biopsy status before surgery.

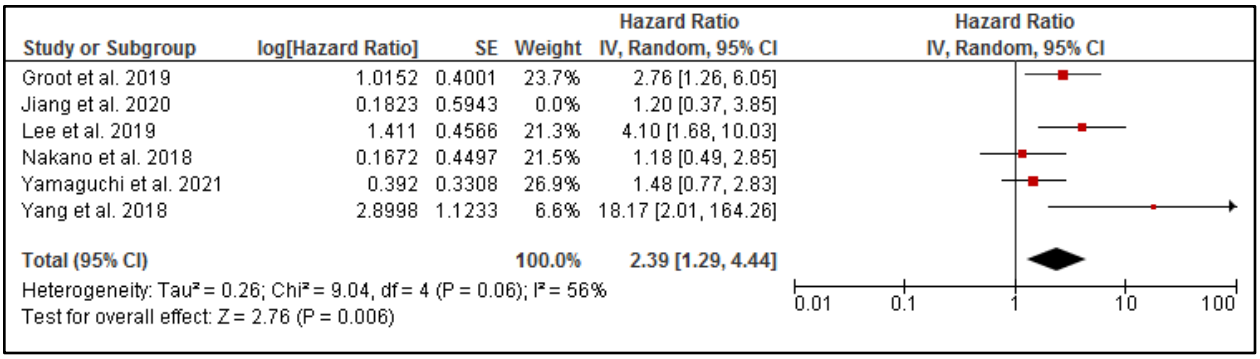

Figure S22: Forest plot of disease-free survival comparing positive versus negative ctDNA status before surgery, excluding studies with metastatic patients.

3.6.4. Disease-free survival according to liquid biopsy status after surgery.

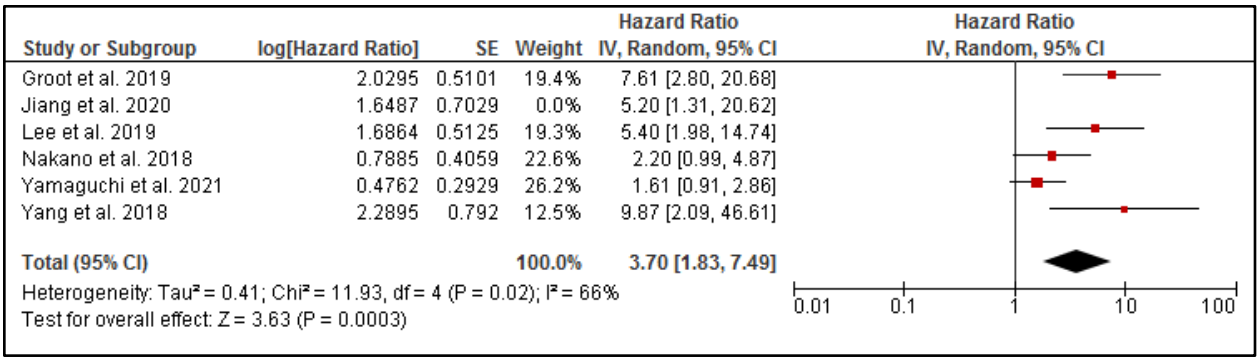

Figure S23: Forest plot of disease-free survival comparing positive versus negative ctDNA status after surgery, excluding studies with metastatic patients.

3.6.5. ctDNA shift after surgery

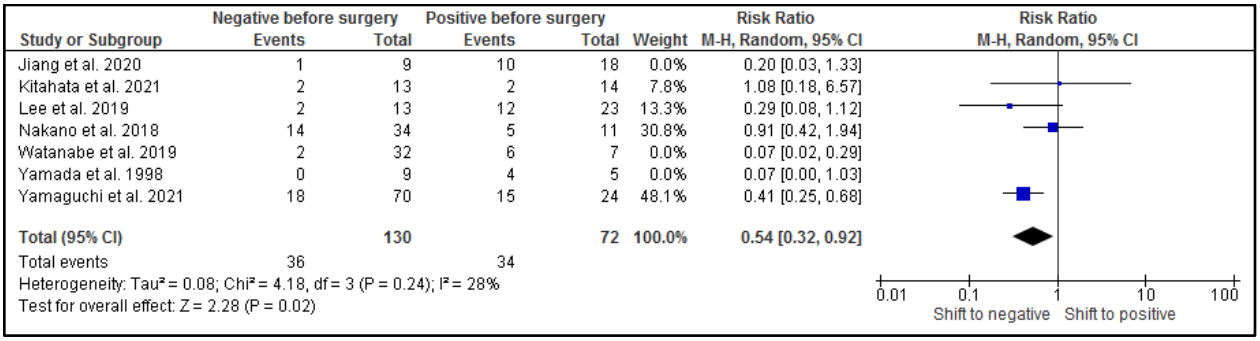

Figure S24: Forest plot of articles comparing ctDNA shift after surgery, excluding studies with metastatic patients.

3.7. Sub-analysis excluding studies carried out during the 1990s.

3.7.1. *Mortality rate according to liquid biopsy status after surgery.*

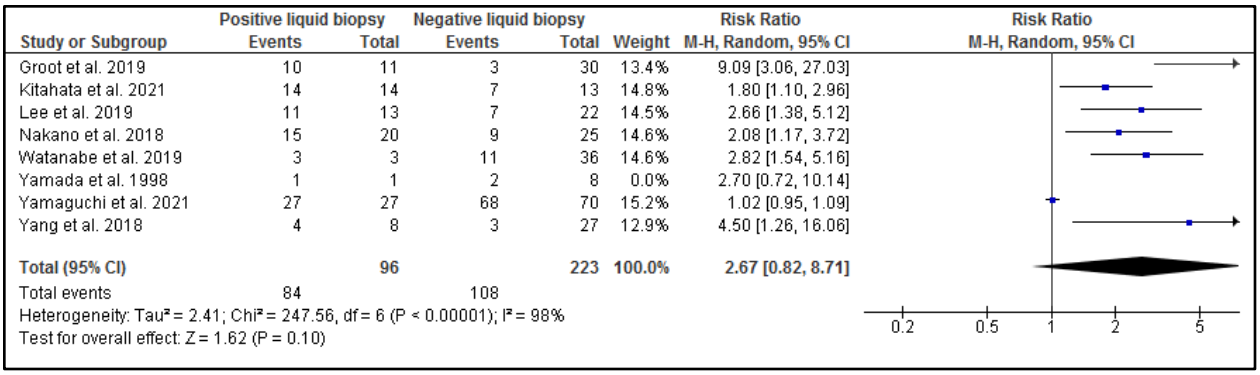

Figure S25: Forest plot of mortality rates comparing positive versus negative liquid biopsy status after surgery in patients with resectable PDAC, excluding studies carried out during the 1990s.

3.7.2. Overall survival according to liquid biopsy status before surgery.

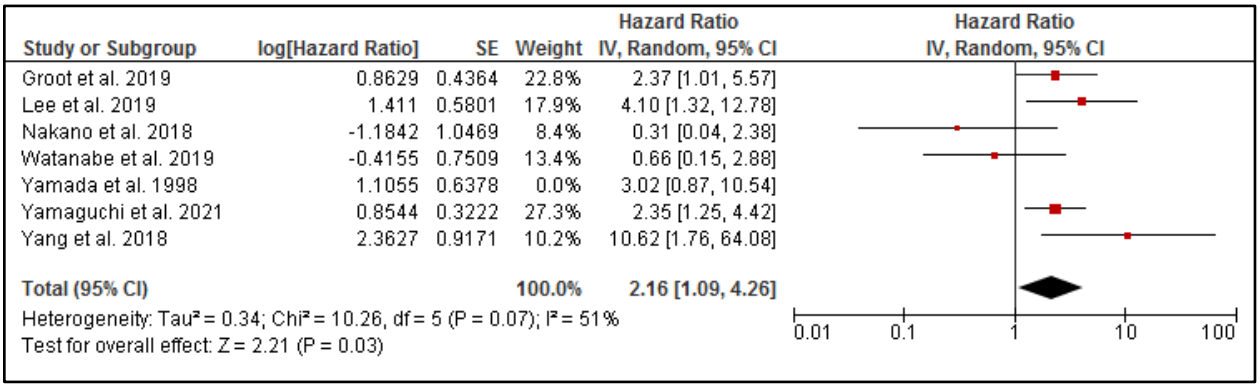

Figure S26: Forest plot of overall survival comparing positive versus negative ctDNA status before surgery in patients with resectable PDAC, excluding studies carried out during the 1990s.

3.7.3. Overall survival according to liquid biopsy status after surgery.

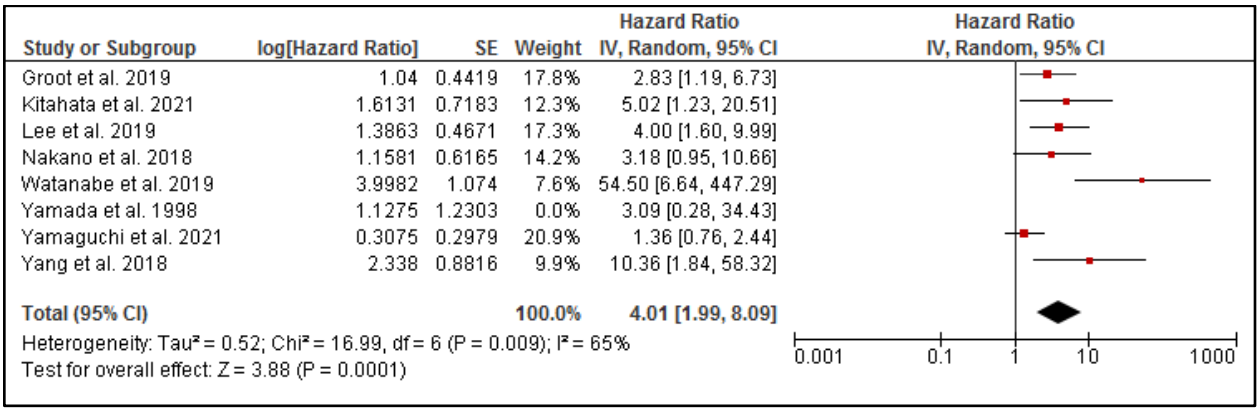

Figure S27: Forest plot of OS comparing positive versus negative ctDNA status after surgery in patients with resectable PDAC, excluding studies carried out during the 1990s.

3.7.4. Recurrence in detectable and undetectable liquid biopsy status after surgery in potentially resectable PDAC patients.

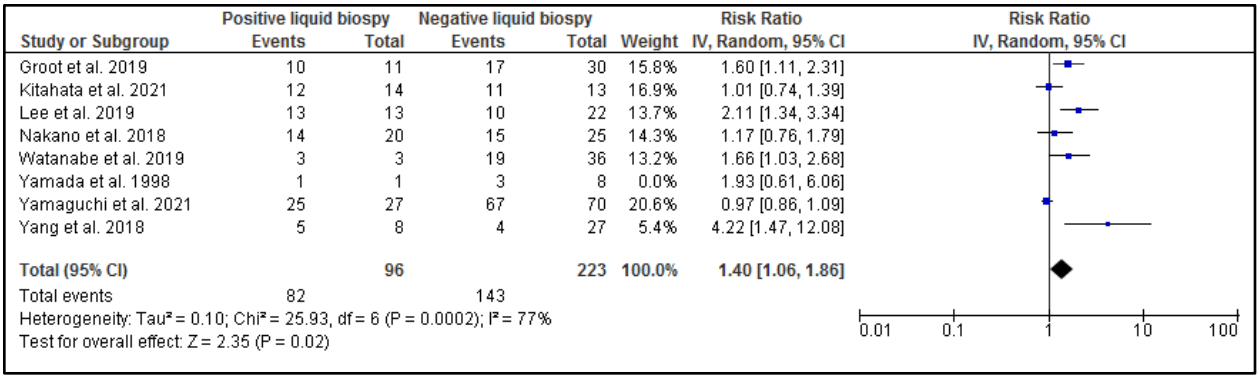

Figure S28: Forest plot of recurrence rates comparing positive versus negative liquid biopsy status after surgery in patients with resectable PDAC, excluding studies carried out during the 1990s.

3.7.5. ctDNA shift after surgery

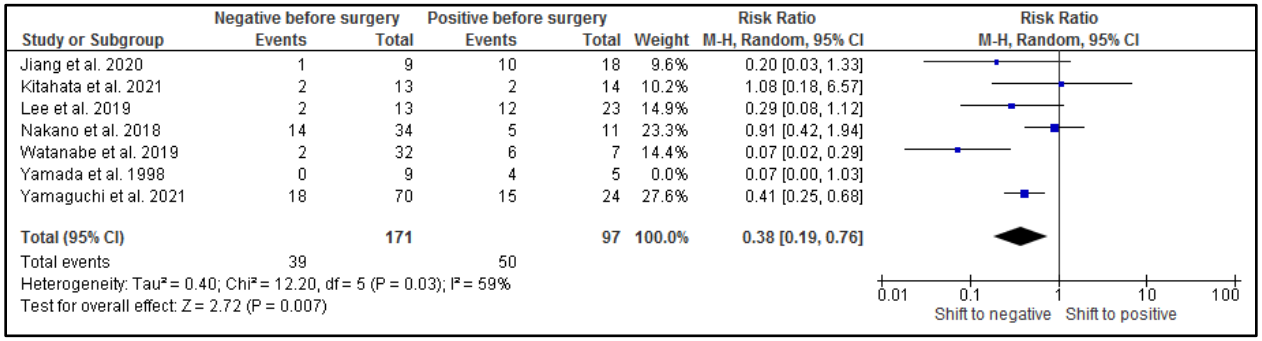

Figure S29: Forest plot of publications analysed comparing ctDNA shift after surgery in patients with resectable PDAC, excluding studies carried out during the 1990s.

4. Supplementary table

4.1. Table of liquid biopsy techniques and samples characteristics

Study	Sample	Detection method	Technology/ Assay	Marker	Targets	LoD	Tube	Time until processing
Gall et al. [26]	Blood	Fluorescence microscopy & scanning	Cell Search System (Veridex)	CTCs	counts	-	CellSave Preservative Tubes	< 24 hours
Groot et al. [36]	Plasma	Digital PCR	RainDrop Digital PCR system (RainDance Technologies)	ctDNA	KRASm (G12V, G12D, G12R, Q61H)	1/10000	EDTA	< 6 hours
Hirota et al.[27]	Blood (PV)	Real-time RT-PCR	LightCycler (Roche Biochemicals)	ctRNA	CEA	-	-	-
Jiang et al. [37]	Plasma	NGS	Custom hybrid-capture panel	ctDNA	1017 cancer susceptibility genes	-	-	-
Kitahata et al. [38]	Plasma	Digital PCR	QX200 Droplet Digital PCR system (Bio Rad)	ctDNA	KRAS multiplex assays (G12A, G12C, G12D, G12R, G12S, G12V, G13D)	2/10000	EDTA	< 2 hours
Lee et al. [39]	Plasma	NGS	SafeSeqS (Illumina)	ctDNA	KRAS (G12, G13, G61)	1/10000	-	-
Nakano et al. [40]	Serum	Real-time PCR	Peptide nucleic acid (PNA)-directed PCR clamping	ctDNA	KRAS (codon 12 and 13)	1/1000	Sodium Citrate	Immediately
Nomoto et al. [41]	Blood	PCR/RFLP & Sanger sequencing	Amplification KRAS mutant and WT and selective enzym digestion WT	ctDNA	KRAS (codon 12)	1/10000	Dodecyltrimet hylammonium bromide	Immediately
Watanabe et al. [42]	Plasma	Digital PCR	QX200 Droplet Digital PCR system (Bio Rad)	ctDNA	KRAS (G12V, G12D, G12R, Q61H)	-	EDTA	-
Yamada et al. [43]	Plasma	Real-time PCR	Mutant allele-specific amplification (MASA-PCR)	ctDNA	KRAS (1st & 2nd nucleotide in codon 12)	-	Sodium Citrate	Immediately
Yamaguchi et al. [44]	Plasma	Digital PCR	QX200 Droplet Digital PCR system (Bio Rad)	ctDNA	KRAS (G12V, G12R, G12D)	1/1000-1/10000	EDTA	< 1 hour
Yang et al. [45]	Plasma	Digital PCR	QuantStudio™ 3D Digital PCR System (Thermo Fisher Scientific)	ctDNA	KRAS (G12V, G12D, G12R)	-	EDTA	< 2 hours