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Adequacy of EUS–guided fine-needle aspiration and fine-needle biopsy for next-generation sequencing in pancreatic malignancies: A systematic review and meta-analysis

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ABSTRACT

Background and Objectives: A majority of pancreatic malignancies are unresectable at the time of presentation and require EUS–guided fine-needle aspiration or fine-needle biopsy (EUS-FNA/FNB) for diagnosis. With the advent of precision therapy, there is an increasing need to use EUS-FNA/FNB sample for genetic analysis. Next-generation sequencing (NGS) is a preferred technology to detect genetic mutations with high sensitivity in small specimens. We performed a meta-analysis to evaluate the adequacy of EUS-FNA/FNB for NGS in pancreatic malignancies.

Methods: PubMed, Embase, Cochrane Library, and Web of Science were searched from database inception to November 11, 2023. The primary outcome was the proportion of sufficient sample acquired by EUS-FNA/FNB in pancreatic malignancies for NGS. Secondary outcomes were the proportion of sufficient sample for NGS in pancreatic ductal adenocarcinoma (PDAC) and the detection rates of mutations in KRAS, TP53, CDKN2A, and SMAD4 and actionable mutations in PDAC. The pooled proportions were calculated using a random-effects model. Potential sources of heterogeneity were investigated with subgroup analyses and meta-regression.

Results: Twenty studies with 881 samples were included. The pooled adequacy of EUS-FNA/FNB sample for NGS was 89.9% (95% CI, 80.8%–96.7%) in pancreatic malignancies and 92.0% (95% CI, 81.3%–98.8%) in PDAC. Screening sample suitability before NGS testing was associated with lower adequacy in subgroup analysis (79.7% *vs.* 98.4%, $P = 0.001$). The pooled prevalences of mutations in KRAS, TP53, CDKN2A, and SMAD4 in PDAC were 87.4% (95% CI, 83.2%–91.2%), 62.6% (95% CI, 53.2%–71.7%), 20.6% (95% CI, 11.9%–30.8%), and 19.4% (95% CI, 11.2%–29.1%), respectively. The pooled prevalence of potentially actionable mutations in PDAC was 14.5% (95% CI, 8.2%–22.0%).

Conclusions: In the majority of cases, EUS-FNA/FNB can acquire adequate sample for NGS and identify tumor-specific mutations in patients with pancreatic malignancies. Strict pre-analysis screening criteria may negatively impact the sample adequacy and the success rate for NGS.

Key words: Pancreatic neoplasms; Endoscopic ultrasound; Fine needle aspiration; Fine needle biopsy; High-throughput nucleotide sequencing

INTRODUCTION

Pancreatic malignancies are one of the most lethal malignant diseases with a 5-year survival rate around 13%, and its incidence has been increasing over recent years.[1] Pancreatic ductal adenocarcinoma (PDAC) accounts for over 90% of cancers in the pancreas.[2] The high mortality and poor prognosis of pancreatic malignancies are due to late detection and drug resistance. There is usually a lack of early symptoms in patients with pancreatic malignancies, and only 20% of patients are candidates for surgical resection at the time of diagnosis.[3] Other patients may receive chemotherapy, radiotherapy, or immunotherapy, but the efficacy remains unsatisfactory.

Personalized therapy is changing the practice of oncology. Targeted and immune-based therapies optimized for the molecular and immune landscapes of pancreatic malignancies have shown promising benefits.[4] Clinical trials are ongoing to evaluate targeted therapies for specific molecular alterations in PDAC, including KRAS, EGFR, BRAC1, BRAC2, and others.[4] Next-generation sequencing (NGS) enables multiple genetic analyses in a small amount of specimens and has been integrated into the standard management of advanced pancreatic malignancies.[5]

EUS–guided fine-needle aspiration or fine-needle biopsy (EUS-FNA/FNB) is a major technique for tissue sampling in advanced pancreatic malignancies with high diagnostic accuracy. However, the success rates of EUS-FNA/FNB sample for NGS testing have varied among different studies.[6] The feasibility and optimal

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methods of EUS-FNA/FNB to acquire sample for NGS remain uncertain. This study aimed to perform a meta-analysis of the adequacy of EUS-FNA/FNB for NGS in pancreatic malignancies and to investigate the influencing factors.

METHODS

This meta-analysis was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Table S1, http://links.lww.com/ENUS/A365).[7] The protocol was previously registered in the International Prospective Register of Systematic Reviews (PROSPERO) database (Protocal ID: CRD42024502296).

Search strategy

A systematic search was performed in PubMed, Embase, Cochrane Library, and Web of Science databases from database inception to November 11, 2023. The search included terms related to EUS-FNA/FNB, pancreas, and NGS (search strategy in Table S2, http://links.lww.com/ENUS/A365). The search was restricted to articles in English language. Only peer-reviewed original articles were included. Reviews, case reports, editorials, and conference abstracts were excluded. If the studies from the same center have overlapping study populations, the one with the larger sample size was selected. The primary search was supplemented by manually cross-checking the reference lists in the retrieved articles to identify additional studies. Citations from all databases were managed using EndNote version X9 (Clarivate, Philadelphia, PA), where duplicates were removed.

Study selection

The titles and abstracts were independently screened, and the full texts of potentially eligible studies were independently assessed by 2 reviewers. Disagreements were resolved by a third reviewer. Studies were included according to the following criteria: 1) adult patients with suspected pancreatic malignancies who underwent EUS-FNA/FNB; 2) diagnosis of malignancies confirmed by EUS-FNA/FNB; 3) sample obtained by EUS-FNA/FNB were sent for NGS; and 4) sample adequacy for NGS was reported. Adequacy was defined as the capability of a sample for successful NGS testing. Studies were excluded if 1) mainly evaluating pancreatic cyst lesions; 2) NGS performed in patients with inconclusive FNA/FNB results; 3) sample obtained from tumor deposits other than pancreas, such as lymph nodes or liver; and 4) sample obtained by other methods, such as surgery or computed tomography guided biopsy. If studies reported outcomes of sample obtained from other deposits or by other methods, only outcomes for pancreas and EUS-FNA/FNB were included.

Data extraction

Data were independently extracted by 2 reviewers using a predesigned Excel form. Disagreements were resolved by a third reviewer. The following data were extracted: author, year, country, design, study population, cancer type, needle type and size, number of passes, use of rapid on-site evaluation, specimen type, sequencing method, number of samples, number of adequate samples for NGS, and proportion of mutations.

Data analysis

The primary outcome was the proportion of sufficient sample acquired by EUS-FNA/FNB in pancreatic malignancies for NGS. Secondary outcomes were the proportion of suitable sample for NGS in PDAC and the detection rates of mutations in KRAS, TP53, CDKN2A, and SMAD4 and actionable mutations in PDAC. The pooled proportions were calculated using the DerSimonian-Laird random-effects model with a Freeman-Tukey double-arcsine transformation. Point estimates of proportions with corresponding 95% confidence intervals (CIs) were calculated and displayed in forest plots. Heterogeneity was assessed by the I^2 statistic and the Cochran's Q test. Potential sources of heterogeneity were investigated with subgroup analyses, and meta-regression was used to examine the differences between subgroups. The quality of included studies was independently assessed by 2 reviewers using a modified version of the Newcastle-Ottawa Scale.[8,9] Discrepancies were resolved by a third reviewer. A sensitivity analysis was performed by excluding studies with low quality. Publication bias was examined with funnel plots and Egger test. All analyses were performed using Stata version 18 (StataCorp, College Station, TX). P values less than 0.05 were considered statistically significant.

RESULTS

Literature search

The search yielded 815 studies. After title and abstract screening, 57 articles were reviewed in full text. Of these, 37 studies were excluded for the use of other diagnostic specimen, duplicate patient population, no NGS adequacy data, or no data on pancreatic malignancies. Finally, 20 studies met the inclusion criteria for the primary meta-analysis [Figure 1].

Study characteristics

The characteristics of the included studies are summarized in Table 1. A total of 881 samples from 879 patients with pancreatic malignancies were included. The sample sizes across studies varied greatly, and 3 studies included fewer than 20 samples.[10,13,22] Ten studies had a prospective study design,[10–13,15,18–20,22,28] and the remaining 10 had a retrospective study design.[14,16,17,21,23–27,29] Eight studies were from Asian countries,[16–20,23–25] and 12 were from Western countries.[10–15,21,22,26–29] Thirteen studies utilized samples from PDAC,[10–13,15,16,19,20,22,25–28] whereas 7 studies might include a few samples from other types of pancreatic malignancies besides PDAC,[14,17,18,21,23,24,29] such as adenocarcinoma not otherwise specified or acinar cell cancer. Nine studies conducted NGS testing on all the sample,[11,13,15,16,19,20,22,23,28] and the other 11 screened sample suitability regarding tumor cellularity or DNA quantity prior to NGS testing.[10,12,14,17,18,21,24–27,29] Thirteen studies used recently obtained specimens for NGS testing,[10–13,15,18–20,22,24–26,28] whereas the other 7 used sample archived for a duration of over 6 months.[14,16,17,21,23,27,29]

Adequacy of EUS-FNA/FNB sample for NGS

The pooled proportion of sufficient EUS-FNA/FNB sample in pancreatic malignancies for NGS was 89.9% (95% CI, 80.8%–96.7%; I^2 = 91.9%) [Figure 2]. In 14 studies describing sample from PDAC patients, the pooled adequacy for NGS was 92.0% (95% CI, 81.3%–98.8%; I^2 = 91.0%) (Figure 3), not significantly different from pancreatic malignancies.

Subgroup analysis and meta-regression

Subgroup analyses were conducted according to prespecified study-level characteristics. The pooled adequacy of prospective

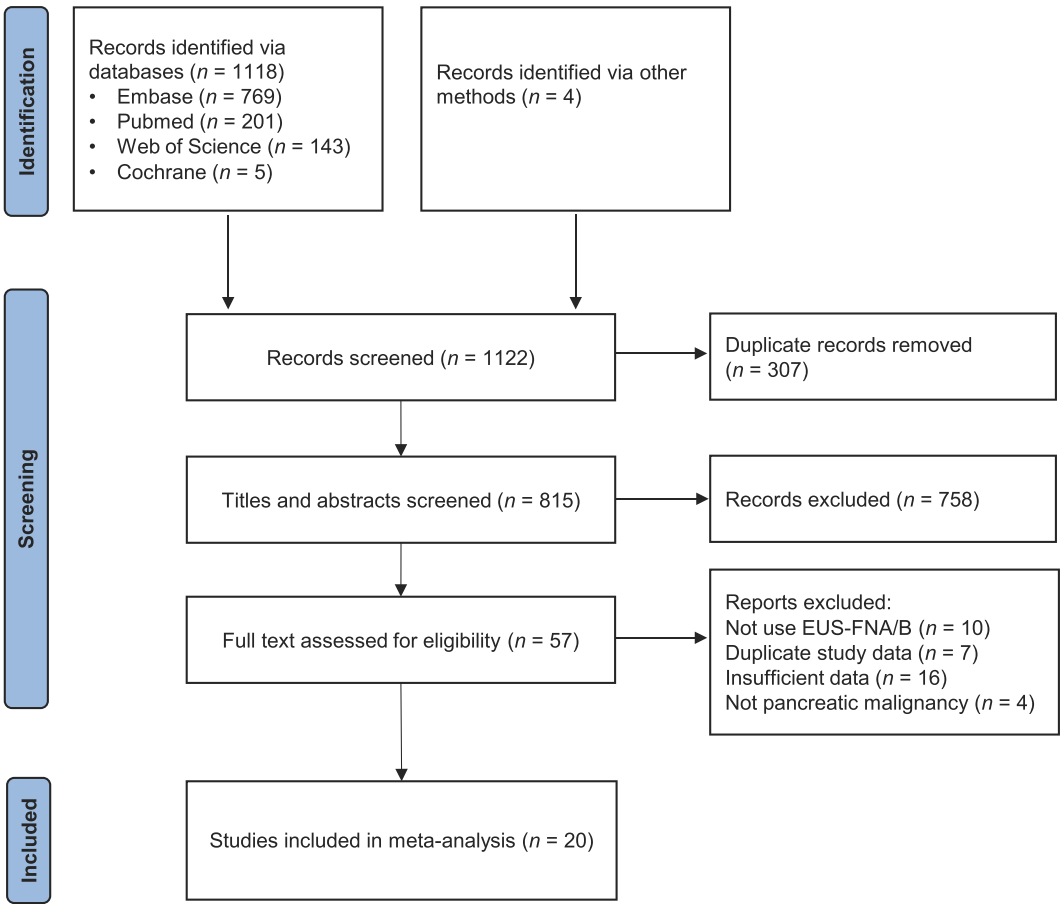

Figure 1. Preferred Reporting Items for Systematic reviews and Meta-Analyses flow diagram.

studies (97.2%; 95% CI, 90.4%–100.0%; I^2 = 72.0%) was higher than retrospective studies (82.1%; 95% CI, 68.8%–92.5%; I^2 = 92.9%; P = 0.030) [Figure 4A]. The pooled adequacy was lower in studies that screened sample suitability based on tumor cellularity or DNA quantity before NGS testing (79.7%; 95% CI, 67.3%–90.0%; I^2 = 90.2%) compared with those that performed NGS testing without prior screening (98.4%; 95% CI, 93.2%–100.0%; I^2 = 69.9%; P = 0.001) (Figure 4B). Pooled proportions were higher in studies using end-cutting needles (97.1%; 95% CI, 88.5%–100.0%; I^2 = 75.0%) than FNA (89.1%; 95% CI, 73.8%–98.9%; I^2 = 87.7%) or Procore needles (83.4%; 95% CI, 22.3%–100.0%; I^2 = 97.4%) (P = 0.372), in studies using ≤22-gauge needles (87.4%; 95% CI, 73.6%–97.1%; I^2 = 90.5%) than 25-gauge needles (51.2%; 95% CI, 4.6%–96.6%; I^2 = 94.9%, P = 0.130), and in studies with application of rapid on-site evaluation (ROSE) (99.1%; 95% CI, 94.0%–100.0%; I^2 = 62.1%) than those without (88.7%; 95% CI, 66.5%–99.9%; I^2 = 94.32%, P = 0.183), but the differences did not reach statistical significance (Figures S1–S3, http://links.lww.com/ENUS/A364). Pooled proportions were comparable in other subgroup analyses, including region, sample freshness, and type of specimen pathology (Figures S4–S6, http://links.lww.com/ENUS/A364). Univariate meta-regression analysis did not reveal the year of publication or the number of targeted genes as significant effect moderators for NGS adequacy (Table S3, http://links.lww.com/ENUS/A365).

Next, a bivariate meta-regression was performed incorporating study type and sample screening covariates [Table 2]. The pooled adequacy remained lower in studies that screened sample suitability than those that did not (–0.5160; 95% CI, –0.9486 to –0.0834). The study type did not demonstrate a significant association with the sample adequacy. Given the limited number of studies, further multiple meta-regression was not carried out.

Mutation rates in PDAC

Eleven studies reported mutation rates of commonly altered genes in 416 EUS-FNA/FNB samples from PDAC successfully analyzed by NGS (Table S4, http://links.lww.com/ENUS/A365).[11–13,15,19,20,25–29] Four studies reported frequencies of alterations that could potentially be targeted by drugs.[12,26–28] KRAS, TP53, CDKN2A, and SMAD4 were 4 commonly mutated genes that characterize PDAC.[30] The pooled prevalences of mutations in them were 87.4% (95% CI, 83.2%–91.2%; I^2 = 20.4%), 62.6% (95% CI, 53.2%–71.7%; I^2 = 70.4%), 20.6% (95% CI, 11.9%–30.8%; I^2 = 79.9%), and 19.4% (95% CI, 11.2%–29.1%; I^2 = 78.5%) (Figure S7, http://links.lww.com/ENUS/A364). The pooled prevalence of potentially actionable mutations was 14.5% (95% CI, 8.2%–22.0%; I^2 = 21.6%) [Figure 5].

Quality assessment and publication bias

Studies were assessed for quality through an adapted version of the Newcastle-Ottawa Scale (Table S5, http://links.lww.com/ENUS/A365).

Table 1

Characteristics of studies included in the primary analysis

Study	Country	Study Type	Patients, *n*	Samples, *n*	PDAC, *n*	Other type, *n*	Targeted genes, *n*	Tumor cellularity criteria	DNA quantity criteria	Sample archived	Specimen type	Needle type	Needle size, G	ROSE
Bruno et al.,[10] 2021	Italy	Prospective	3	4	4	0	2	≥10%	N/A	No	Histology	FNA	18/21/22	N/A
Carrara et al.,[11] 2021	Italy	Prospective	33	33	33	0	161	N/A	N/A	No	Histology	End-cutting FNB	22	No
Dreyer et al.,[12] 2019	UK	Prospective	36	36	36	0	N/A	N/A	>50 ng	No	Histology	N/A	N/A	N/A
Fulmer et al.,[13] 2020	USA	Prospective	15	15	15	0	143	N/A	N/A	No	Cytology	FNA	N/A	N/A
Gleeson et al.,[14] 2016	USA	Retrospective	47	47	N/A	N/A	160	≥20%	≥5 ng/μL	Yes	Cytology	FNA	N/A	N/A
Habib et al.,[15] 2021	USA	Prospective	52	52	52	0	9	N/A	N/A	No	Histology	FNA	N/A	N/A
Ishikawa et al.,[16] 2023	Japan	Retrospective	42	42	42	0	324	N/A	N/A	Yes	Histology	End-cutting FNB	19/22	No
Ishizawa et al.,[17] 2020	Japan	Retrospective	26	26	N/A	N/A	409	N/A	20 ng	Yes	Cytology	Reverse-bevel FNB	22	Yes
Iwaya et al.,[18] 2023	Japan	Prospective	31	31	30	1	28	≥10%	N/A	No	Histology	End-cutting FNB	22	No
Kamata et al.,[19] 2023	Japan	Prospective	25	25	25	0	50	N/A	N/A	No	Histology	End-cutting FNB	22	No
Kameta et al.,[20] 2016	Japan	Prospective	20	20	20	0	50	N/A	N/A	No	Histology	FNA	N/A	Yes
Larson et al.,[21] 2018	USA	Retrospective	61	61	60	1	324	≥20%	N/A	Yes	Histology	FNA/FNB	19/22/25	N/A
Murphy et al.,[22] 2021	USA	Prospective	11	11	11	0	N/A	N/A	N/A	No	Histology	FNA/End-cutting FNB	22	Yes
Ohyama et al.,[23] 2022	Japan	Retrospective	42	43	N/A	N/A	60	N/A	N/A	Yes	Histology	End-cutting FNB	22/25	Yes
Okuno et al.,[24] 2023	Japan	Retrospective	81	81	N/A	N/A	324/114	≥20%	N/A	No	Histology	FNA/End-cutting FNB	19/22	N/A
Park et al.,[25] 2020	Korea	Retrospective	190	190	190	0	83	≥30%	≥50 ng	No	Histology	FNA/Reverse-bevel FNB	19/22/25	No
Razzano et al.,[26] 2022	USA	Retrospective	43	43	43	0	134/146	≥10%	N/A	No	Histology	FNA/FNB	22/25	N/A

(*continued*)

Table 1
(continued).

Study	Country	Study Type	Patients, *n*	Samples, *n*	PDAC, *n*	Other type, *n*	Targeted genes, *n*	Tumor cellularity criteria	DNA quantity criteria	Sample archived	Specimen type	Needle type	Needle size, G	ROSE
Redegalli et al.,[27] 2023	Italy	Retrospective	76	76	76	0	161	≥10%	≥10 ng	Yes	Cytology	FNA/ Reverse-bevel FNB	25	Yes
Semaan et al.,[28] 2021	USA	Prospective	23	23	23	0	WES	N/A	N/A	No	Cytology	FNA	N/A	Yes
Young et al.,[29] 2013	USA	Retrospective	22	22	18	4	287	≥20%	≥50 ng	Yes	Histology	FNA	N/A	N/A

PDAC: pancreatic ductal adenocarcinoma; ROSE: rapid on-site evaluation; N/A: data not available; WES: whole exome sequencing; FNA: fine needle aspiration; FNB: fine needle biopsy.

A total of 11 studies were graded as low-quality studies, getting 0 star in any 1 of 5 areas on the modified Newcastle-Ottawa Scale. A lack of representativeness of the exposed cohort was the major determinant of low quality. Excluding studies with low quality demonstrated a similar pooled proportion of adequate sample for NGS in pancreatic malignancies of 92.7% (95% CI, 75.8%–100.0%;

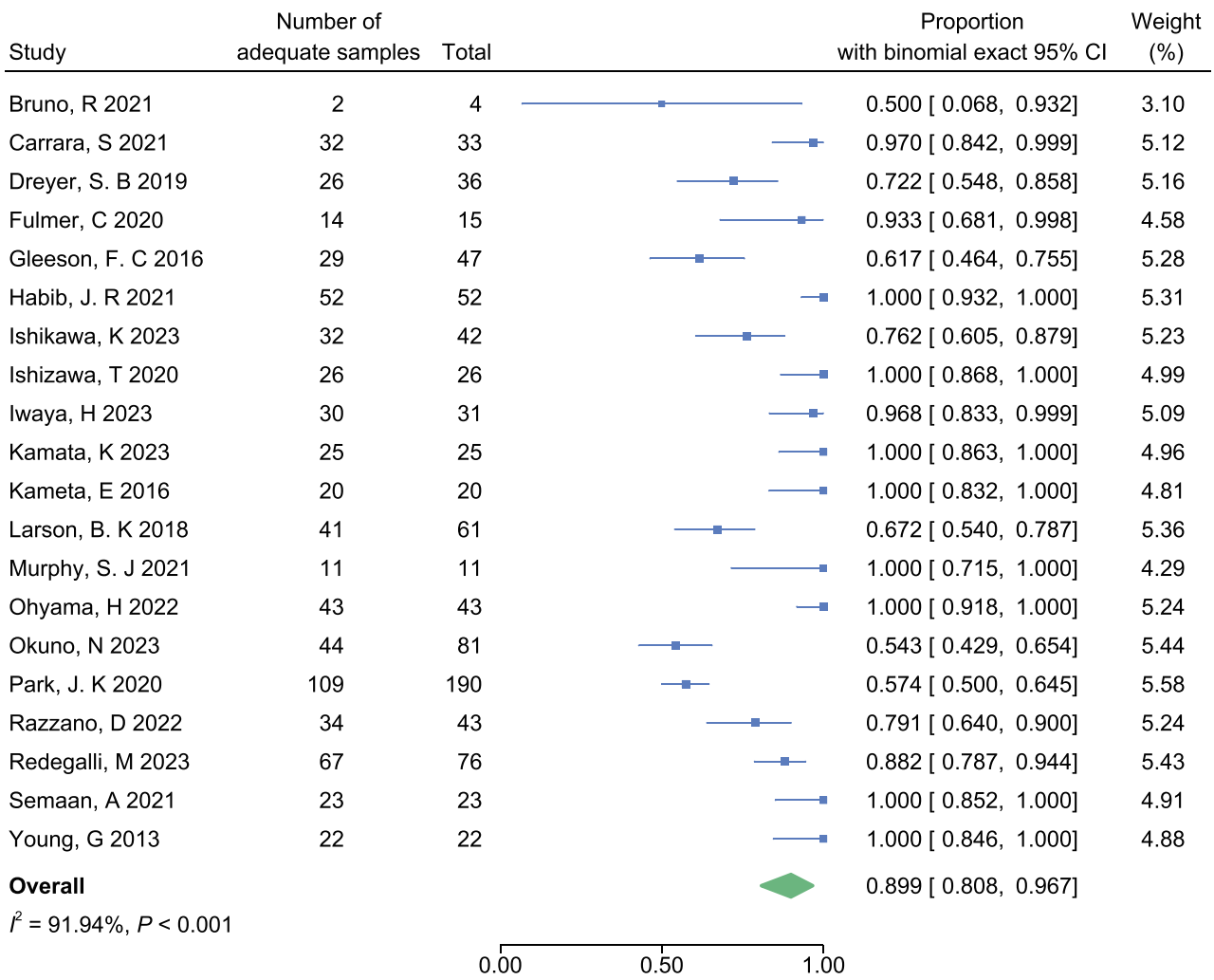

Figure 2. Forest plot of pooled proportion of adequate EUS-FNA/B samples for NGS in pancreatic malignancies.

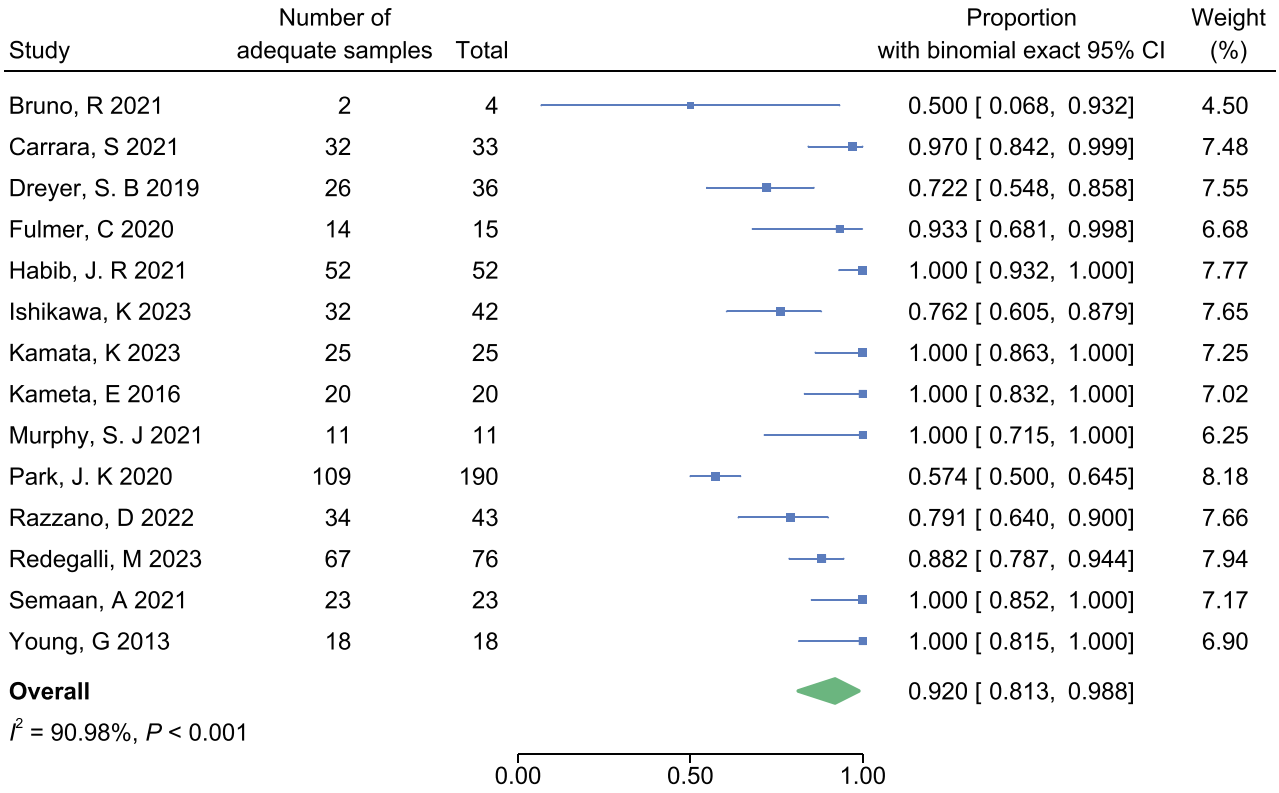

Figure 3. Forest plot of pooled proportion of adequate EUS-FNA/B samples for NGS in PDAC. NGS, next-generation sequencing; PDCA, pancreatic ductal adenocarcinoma.

I^2 = 93.3%) (Figure S8, http://links.lww.com/ENUS/A364). Also, excluding the study with less than 10 samples demonstrated a similar pooled proportion of 90.5% (95% CI, 81.5%–96.9%; I^2 = 92.3%) (Figure S9, http://links.lww.com/ENUS/A364). The funnel plot and the Egger's test to examine publication and small study bias suggested that the risk of missing some studies was

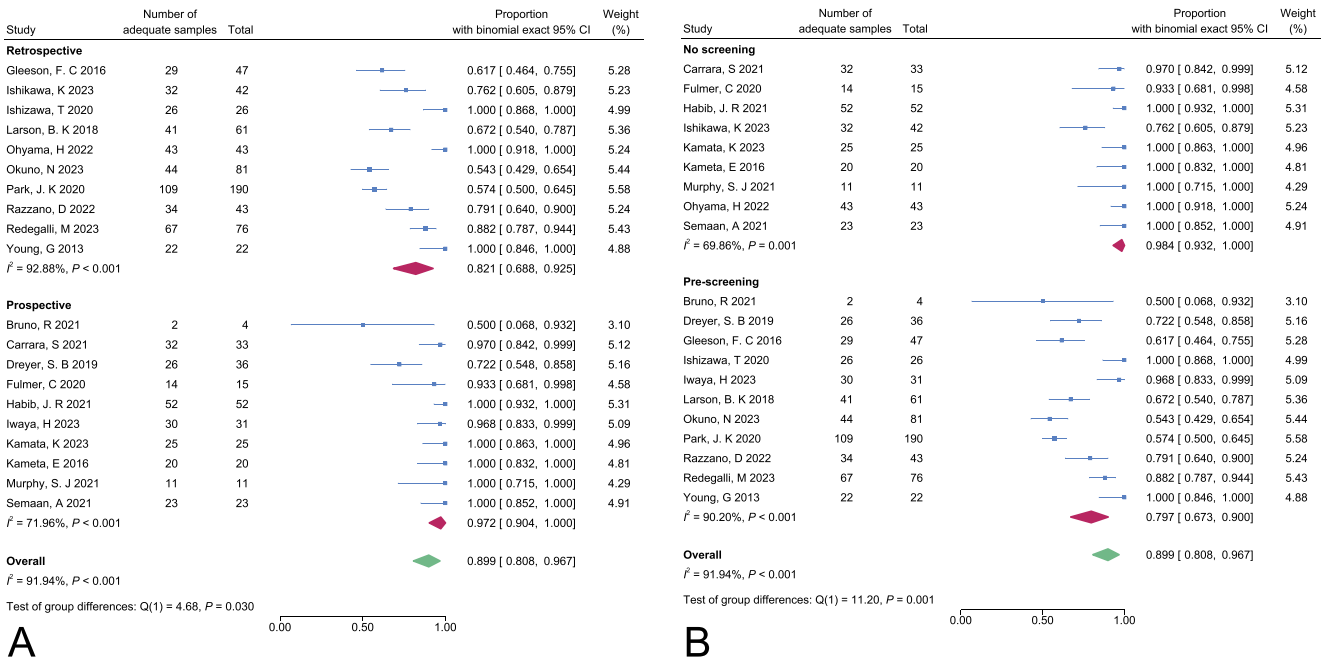

Figure 4. Forest plot of adequacy according to (A) study type subgroup and (B) sample screening subgroup.

Table 2

Bivariate meta-regression of adequacy of EUS-FNA/FNB samples for NGS

Covariate	Coefficient	Standard Error	z	P	95% CI Lower	95% CI Upper	I^2 (%)	R^2 (%)
Study type (Retrospective = 1)	0.1605	0.2198	0.73	0.465	−0.2702	0.5913	85.40	48.08
Sample screening (Yes = 1)	−0.5160	0.2207	−2.34	0.019	−0.9486	−0.0834		
Constant	2.6557	0.2176	12.21	0.000	2.2293	3.0822		

NGS, next-generation sequencing.

not statistically significant (P = 0.282) (Figure S10, http://links.lww.com/ENUS/A364).

DISCUSSION

EUS-FNA/FNB is a minimally invasive approach for diagnosing advanced pancreatic malignancies and takes an important part in directing treatment plans. This systematic review and meta-analysis found that the diagnostic sample obtained through EUS-FNA/FNB was suitable for NGS analysis, with a pooled adequacy rate of 89.9% in pancreatic malignancies and 92.0% in PDAC. There was substantial heterogeneity among studies. The subgroup analysis found significant differences according to study type and use of sample suitability screening. When both factors were incorporated in the bivariate meta-regression, the use of sample suitability screening was still related to a lower adequacy.

In the studies using sample screening, different sample screening criteria were established based on the gene panel test sensitivity to reduce false-negative detections.[31,32] These criteria assessed factors such as tissue amount, tumor cellularity, or DNA quantity. Pancreatic malignancies contain a high stromal component and low tumor cellularity, which challenges EUS-FNA/FNB specimens to fulfill strict pathologic screening criteria.[6] The detection sensitivity of gene panel tests can be influenced by the number of targeted genes, sequencing depths, and data processing methods.[33] High-sensitivity targeted genomic sequencing platforms were able to pick out mutations, even those with an allele frequency as low as 1%, in a sample containing a minimal amount of tumor material.[33] As a result, the studies employing high-sensitivity NGS platform had a high sample adequacy for successful NGS testing without suitability screening. In the centers where a high proportion of sample failed suitability screening, optimizing gene panel tests and screening criteria for EUS-FNA/FNB specimens would be essential to enhance the success rate of NGS testing.[34]

Several other factors could also contribute to some of the heterogeneity. End-cutting FNB needles showed higher histology yield and diagnostic performance than reverse-bevel FNB and FNA needles.[35,36] Besides, 22-gauge needle size or larger was reported to be superior in tissue core procurement than 25-gauge needle size.[21,25,36,37] Therefore, the use of end-cutting FNB needles with 22-gauge size or larger could lead to higher specimen adequacy for genomic profiling. In the present study, the subgroups of end-cutting needles and ≤22-gauge needles exhibited a trend toward increased adequacy, though no statistical significance was reached. Notable heterogeneity compromises the interpretation of the results. It is recommended to select relatively larger FNB needles that retain maneuverability for cases planned for NGS analysis.[34,38] Randomized, controlled trials are needed to determine the optimal needle for successful NGS analysis.

ROSE was introduced in the process of EUS-FNA to provide timely feedback on sample adequacy and optimize the number of needle passes performed. The benefit of ROSE for diagnostic adequacy and accuracy was controversial. Some data suggested that the effectiveness of ROSE might be confined to the learning phase and the centers with a low adequacy rate (<90%).[39] Other data indicated that ROSE could still have a role when reverse-bevel FNB and FNA needles are used, as compared to end-cutting FNB needles.[36,40] Our analysis did not find a significant correlation between ROSE and NGS adequacy. Ideally, the implementation of ROSE could optimize the triage of limited specimens for multiple ancillary tests, including NGS analysis.[41] Additional research is required to investigate the impact of ROSE under specific conditions.

We observed no significant differences between subgroups based on sample freshness or type of specimen pathology. Variable factors in sample preparation and preservation influence DNA quality, thereby affecting the applicability of NGS analysis.[42] As nucleic acid quality diminishes over extended storage periods, it is advisable to utilize recently prepared sample as much as possible. Specimens not initially planned for genomic profiling can be stored

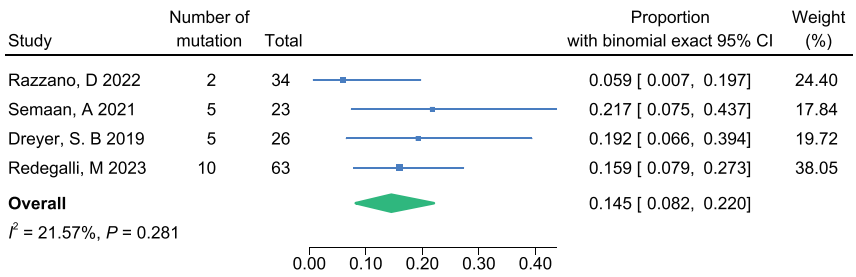

Figure 5. Forest plot of pooled prevalence of potentially actionable mutations.

for up to 3 years when genomic testing is considered.[42] The storage time was mostly less than 3 years in the archived group, which probably resulted in the minimal differences between the sample freshness subgroups. Conventionally, formalin-fixed, paraffin-embedded tissue sample is used for genomic testing, but there is a growing need for cytological specimens. Cytological specimens, with an appropriate cell count, could offer better nucleic acid quality preservation than histological specimens.[43] Therefore, both histological sample and cytological sample are suitable for NGS analysis. Institutions should customize their workflows for specimen preparation and preservation according to their specific conditions and experience.

In our study, the pooled prevalences of KRAS, TP53, CDKN2A, and SMAD4 were comparable with those reported in PDAC surgical specimens.[44,45] Pancreatic malignancies are highly heterogeneous, and EUS-FNA/FNB sample may not represent the comprehensive cancer profile. Several studies indicated that EUS-FNA/FNB mutation profiles largely matched surgical specimens, differing only in a few low-frequency allele alterations.[15,27,46] Therefore, EUS-FNA/FNB sample could be appropriate specimens for NGS in unresectable pancreatic malignancies. The pooled frequency of actionable mutations in 146 PDAC samples from 4 studies was 14.5%. However, the definition of actionable mutations and the standards for NGS data analysis varied across different studies. Further investigation is important to create reliable criteria for actionable mutations.

There are some limitations to our study. First, considerable heterogeneity was found in the analysis. The study methodology and sample population varied in the included studies, and many studies had limited sample size. Some potential sources of heterogeneity were explored through prespecified analysis. Other potential confounding factors, such as tumor size, tumor location, number of passes, and puncture technique, were not included because of a paucity of data from the original studies.

Second, only samples with malignant pathological diagnosis were analyzed in this meta-analysis. The diagnostic sensitivity of pancreatic malignancies with EUS-FNA/FNB is supposed to be over 85%[47]; thus, the NGS adequacy should be high for malignant patients in well-qualified centers. Plus, NGS was reported to improve diagnosis and change treatment plans in patients with uncertain diagnosis.[48–50] More research is needed to illustrate the value of NGS for undiagnostic sample.

Third, our study focused on DNA sequencing other than RNA sequencing. RNA is more susceptible to degradation and harder to extract than DNA. As a result, RNA sequencing of EUS-FNA/FNB specimen is more difficult, and related reports are limited.[51–55] Because transcriptomic subtypes reveal varied prognostic and chemotherapeutic responses,[56] the necessity for RNA sequencing is on the rise. Therefore, future investigations should give attention to extracting higher-quality RNA as well as DNA.

Finally, a cost-effectiveness analysis of NGS testing in EUS-FNA/FNB sample was not performed, which was beyond the scope of this study. NGS analysis using EUS-FNA/FNB specimens identifies the majority of mutations and could be complementary with blood-based NGS.[57] A main challenge arises from the insufficient data supporting targeted therapies guided by genomic profiling.[58] With evidence accumulating on personalized treatment, more patients will benefit from NGS testing.

In conclusion, EUS-FNA/FNB is a suitable technique to acquire adequate sample for NGS and identify tumor-specific mutations in patients with pancreatic malignancies. The implementation of strict sample screening criteria prior to actual analysis appears to adversely affect specimen adequacy and success rate for NGS. Further studies are needed to investigate optimal puncture methods and analysis workflow in clinical practice.

Ethical Approval

Not applicable.

Informed Consent

Not applicable.

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Conflicts of Interest

The authors declare no conflict of interest.

Author Contributions

Y.P., T.R., X.Z., C.Z., and D.Z. did the conception and design. Y.P. and T.R. performed data collection, data analysis, and drafting of the manuscript. X.Z., X.Q., and Y.Z. participated in data curation and analysis. C.Z. and D.Z. performed critical revision of the article for important intellectual content. All authors read and approved the final manuscript.

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