INPLASY

Diagnostic Accuracy of High Resolution Melting Curve Analysis for Discrimination Oncology-Associated EGFR Mutations: A Systematic Review and Meta-Analysis

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ADMINISTRATIVE INFORMATION

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Review Stage at time of this submission - Completed but not published.

Conflicts of interest - None declared.

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Amendments - This protocol was registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) on 16 September 2024 and was last updated on 16 September 2024.

INTRODUCTION

Review question / Objective High-resolution melting (HRM) curve analysis is a simple, PCR-based method for rapidly detecting epidermal growth factor receptor (EGFR) gene mutations by measuring melting temperature changes. We aimed to investigate the diagnostic value of HRM analysis for oncology-associated EGFR mutations by meta-analysis.

Rationale EGFR mutations are located on exons 18, 19, 20, and 21 of the EGFR, and most are in-frame deletion of codons 746 to 750 in exon 19 and a missense mutation at codon 858 in exon 21. An activating mutation in EGFR can be found in a high incidence in non-smokers, women, those with adenocarcinoma, and individuals of Asian ethnic background. Currently, some genotypic methods for screening gene mutations have been developed, as well as expanding the knowledge of the drug-gene relationships, such as DNA sequencing, single-strand conformation polymorphism (SSCP), denaturing high-performance liquid chromatography (DHPLC), allele-specific PCR (AS-PCR), array analysis, pyrosequencing, and high-resolution melting (HRM) curve analysis. Some of these methodologies require sample separation on a gel or matrix; others require expensive fluorescently labeled probes or special instruments. However, HRM is performed in a closed-tube system that protects the amplified DNA from cross-contamination, which is a main advantage of HRM and has proven to be a rapid, cost-effective method with few or no probes.As an alternative molecular testing platform for genotyping of polymorphisms, it has been applied to various diseases, such as oncological, infectious, and inherited diseases.

Condition being studied Whether epidermal growth factor receptor (EGFR) activates signaling pathway plays an important role in developing tumor-associated diseases. The EGFR gene with tyrosine kinase activity is a member of the human epidermal growth factor receptor (HER) family composed of HER1 (erbB1, EGFR), HER2 (erbB2, NEU), HER3 (erbB3), and HER4 (erbB4). Over-expression of EGFR is critical for lung, breast, and gastric cancer and squamous cell carcinoma of the head and neck. Activation of EGFR launches a series of cellular signaling pathways that promote cancer proliferation, invasion, and metastasis and protects carcinoma cells from apoptosis via an anti-apoptosis pathway. Tyrosine kinase inhibitors (TKIs), such as gefitinib and erlotinib, can inhibit this pathway and consequently offer efficacy for patients with an EGFR mutation. Certainly, EGFR gene mutational status is the most sensitive target for TKI therapy selection.

METHODS

Search strategy We performed this meta-analysis following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.32 Excerpta Medica Database (EMBASE), Medline (using PubMed as the search engine), and the Web of Science were searched to identify relevant publications in English until September 12, 2024, using 'epidermal growth factor receptor' or 'EGFR' or 'EGF-R' or 'EGF-receptor' or 'EGF receptor' or 'receptor, epidermal growth factor' or 'transforming growth factor alpha receptor' or 'ERBB-1 proto-oncogene protein' or 'receptor, transforming-growth factor alpha' or 'receptor, transforming growth factor alpha' or 'C-ERBB-1 protein' or 'receptors, epidermal growth factor' or 'receptor, EGF' or 'urogastrone receptor' or 'TGF-alpha receptor' or 'epidermal growth factor receptor kinase' or 'epidermal growth factor receptor protein-tyrosine kinase' or 'epidermal growth factor receptor protein tyrosine kinase' AND 'HRMA' or 'HRM' or 'HRMCA' or 'HRMC' or 'high resolution melting analysis' or 'high resolution melting' or 'high resolution melting curve analysis' or 'high resolution melting curve'. We also carried out manual research for additional eligible studies.

Participant or population There were 34 subsets from 26 published studies and 6,089 samples assayed to evaluate HRM's diagnostic accuracy to identify EGFR mutations.

Intervention No.

Comparator No.

Study designs to be included We searched Embase, PubMed, and Web of Science for HRM and EGFR mutation detection articles. We drew 34 subsets from 26 published studies and assayed 6,089 samples to systematically evaluate HRM's diagnostic accuracy in detecting EGFR mutations. Data were processed with Meta-Disc (version 1.4) and STATA 12.1 software. Degrees of heterogeneity were evaluated with a Chi-squared test of heterogeneity (Cochran's Q statistical test) and an inconsistency index (I-square). Publication bias was determined using Deek's Funnel Plot Asymmetry Test.

Eligibility criteria We performed this meta-analysis following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

Information sources Excerpta Medica Database (EMBASE), Medline (using PubMed as the search engine), and the Web of Science were searched to identify relevant publications in English until September 12, 2024, using 'epidermal growth factor receptor' or 'EGFR' or 'EGF-R' or 'EGF-receptor' or 'EGF receptor' or 'receptor, epidermal growth factor' or 'transforming growth factor alpha receptor' or 'ERBB-1 proto-oncogene protein' or 'receptor, transforming-growth factor alpha' or 'receptor, transforming growth factor alpha' or 'C-ERBB-1 protein' or 'receptors, epidermal growth factor' or 'receptor, EGF' or 'urogastrone receptor' or 'TGF-alpha receptor' or 'epidermal growth factor receptor kinase' or 'epidermal growth factor receptor protein-tyrosine kinase' or 'epidermal growth factor receptor protein tyrosine kinase' AND 'HRMA' or 'HRM' or 'HRMCA' or 'HRMC' or 'high resolution melting analysis' or 'high resolution melting' or 'high resolution melting curve analysis' or 'high resolution melting curve'. We also carried out manual research for additional eligible studies.

Main outcome(s) Twenty-six articles were obtained from 416 references. The overall diagnostic sensitivity and specificity were high at 0.96 [95% confidence interval (CI), 0.94–0.97] and 0.99 (95% CI, 0.99–0.99), respectively. The value of other indicators, including the pooled positive likelihood ratio, negative likelihood ratio, and diagnostic odds ratio, was 118.76 (95% CI: 59.69–236.29), 0.07 (95% CI: 0.04–0.13), and 2249.61 (95% CI: 1137.93–4447.32), respectively. The summary SROC from our data showed that the Q value was 0.977, while the area under the curve (AUC) was 0.996. The typical "shoulder-arm" pattern in the SROC suggested a threshold effect.

Additional outcome(s) Our DOR was 2249.61 (95% CI: 1137.93 – 4447.32). As a global indicator for assessing diagnostic performance, AUC under SROC also indicated a high accuracy of HRM, with a Q value of 0.977 and an AUC close to 1 (0.996). DOR and AUC data indicate high overall accuracy of HRM for EGFR mutation screening.

Data management All data and information can be found in the articles and attachments.

Quality assessment / Risk of bias analysis We extracted data: author's name, publication year, country of origin, specimen sources, mutation prevalence, instruments, disease types, sample number, amplicon length, dye types, and disease-associated mutations. Outcome parameters such as TN, FN, TP, and FPs were calculated based on 'PCR amplicons', not based on tissue or blood samples. Two authors and disagreements performed data collection were resolved by discussion or consensus with a third author. We assessed the quality of each study based on a Quality Assessment for Studies of Diagnostic Accuracy (QUADAS-2),33 which includes four primary domains to evaluate bias and applicability of included studies by assessing patient selection methods, an index test, reference standards, and patient flow through studies. We measured the accuracy for each study by standard methods Meta-Disc (version 1.4) and STATA 12.1 software. Sensitivity, specificity, positive- and negative-likelihood ratios (PLRs, NLRs), and a diagnostic odds ratio (DOR) from studies and corresponding 95% confidence intervals (CIs) were computed with fixed or random effects models depending on significant heterogeneity. Degrees of heterogeneity were evaluated with a Chi-squared test of heterogeneity (Cochran's Q statistical test) and an inconsistency index (I-square). Alternatively, to quantify the effect of heterogeneity, significant heterogeneity was defined as a Q test with a p 50%. The threshold effect was performed by summary receiver operating characteristic (SROC) for each study to ascertain the presence of a "shoulder-arm" pattern, which would suggest a threshold effect. Spearman correlation coefficient between the logit of sensitivity and logit of 1-specificity for each study was also calculated to assess any threshold effect. A positive correlation ($p < 0.05$) would suggest a threshold effect. Publication bias was determined using Deek's Funnel Plot Asymmetry Test and STATA 12.1 software (Stata Corp., College Station, TX).

Strategy of data synthesis We measured the accuracy for each study by standard methods Meta-Disc (version 1.4) and STATA 12.1 software. Sensitivity, specificity, positive- and negative-likelihood ratios (PLRs, NLRs), and a diagnostic odds ratio (DOR) from studies and corresponding 95% confidence intervals (CIs) were computed with fixed or random effects models depending on significant heterogeneity. Degrees of heterogeneity were evaluated with a Chi-squared test of heterogeneity (Cochran's Q statistical test) and an inconsistency index (I-square). Alternatively, to quantify the effect of heterogeneity, significant heterogeneity was defined as a Q test with a p 50%. The threshold effect was performed by summary receiver operating characteristic (SROC) for each study to ascertain the presence of a "shoulder-arm" pattern, which would suggest a threshold effect. Spearman correlation coefficient between the logit of sensitivity and logit of 1-specificity for each study was also calculated to assess any threshold effect. A positive correlation ($p < 0.05$) would suggest a threshold effect. Publication bias was determined using Deek's Funnel Plot Asymmetry Test and STATA 12.1 software (Stata Corp., College Station, TX).

Subgroup analysis Meta-regression analysis was performed to explore heterogeneity sources using Meta-Disc (version 1.4) software. A multivariable regression model was applied, and a backward stepwise algorithm with covariates including disease type, specimen source, instruments, and dye type was used; variables were retained in the regression model if $p < 0.05$. Subgroup analysis was performed if reasons for heterogeneity could be found.

Sensitivity analysis Diagnostic sensitivity and specificity were 0.96 [95% CI: 0.94–0.97] and 0.99 (95% CI: 0.99–0.99), respectively (Figure 2a and 2b). As shown in Figure 2c and 2d, a high PLR of 118.76 (95% CI: 59.69–236.29) and a low NLR of 0.07 (95% CI: 0.04–0.13) indicated that HRM had an excellent ability to identify the presence of EGFR mutation. Additionally, the DOR supported that HRM was effective for EGFR mutation screening (Figure 3a). Chi-square and I2 tests for heterogeneity confirmed significant heterogeneity for specificity and sensitivity in the pooled results. The SROC is shown in Figure 3b. The SROC from our data showed that the Q value was 0.977, while the area under the curve (AUC) was 0.996, further indicating a high overall accuracy of HRM.

Language restriction No.

Country(ies) involved China.

Other relevant information All information is in the article and attached materials.

Keywords High-resolution melting curve; EGFR mutation; diagnostic accuracy; oncology-associated diseases; systematically evaluate.

Dissemination plans Article awaiting publication.

Contributions of each author

Author 1 - Shu Yu conceptualized and designed experiments,and wrote the main part of manuscript.
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