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Application of meta-analysis for determining cancer biomarkers

Kanser biyobelirteçlerinin belirlenmesi için meta-analizin uygulanması

Halil İbrahim Pazarbaşı[1,2], Athanasia Pavlopoulou[1,2]

[1]Izmir Biomedicine and Genome Center (IBG), Izmir, Türkiye
[2]Izmir International Biomedicine and Genome Institute, Genomics and Molecular Biotechnology Department, Dokuz Eylül University, Izmir, Türkiye

ABSTRACT
The health care professionals are facing the challenge to combine and translate the findings from a plethora of, often conflicting, clinical trials or clinical studies in order to reach an evidence-based decision. The application of a meta-analytical approach in the medical field allows the systematic synthesis and assessment of the results across studies to draw conclusions about the main body of the research, such as a more accurate estimate of treatment effect or determining disease risk factors. Herein, we review the advantages and the basic steps of meta-analysis towards the identification of powerful cancer biomarkers.
Keywords: Meta-analysis; cancer; biomarkers

ÖZET
Sağlık uzmanları, kanıta dayalı bir karara varmak için çok sayıda, çoğu zaman birbiriyle çelişen klinik araştırmalardan elde edilen bulguları birleştirme ve tercüme etme zorluğuyla karşı karşıyadır. Meta-analitik yaklaşımların tıp alanında uygulanması, tedavi etkisinin daha doğru tahmin edilmesi veya hastalık risk faktörlerinin belirlenmesi gibi araştırmanın ana kısmı hakkında sonuçlar çıkarmak için çalışmalardaki sonuçların sistematik sentezine ve değerlendirilmesine olanak tanır. Bu çalışmada, güçlü kanser biyobelirteçlerinin tanımlanmasına yönelik meta-analizin avantajlarını ve temel adımlarını derleyeceğiz.
Anahtar kelimeler: Meta-analiz, kanser, biyobelirteçler

Introduction

The need for identifying powerful biomarkers for the accurate and timely diagnosis, prognosis and evidence-based decision making for diverse types of cancers poses a major challenge in clinical and medical research[1-5]. Given the continuous accumulation of quantitative and qualitative data from clinical trials and studies, meta-analysis has emerged as a fundamental tool in clinical practice and public health for data collection, evaluation, and interpretation, in order to obtain statistically significant and relevant information at low cost. Meta-analysis is the application of statistical methods to combine the quantitative findings from multiple scientific studies, addressing the same question, so as to increase statistical power over individual studies, and to deal with any conflict among the individual studies[6-8].

In this minireview, we provide a methodological guide for conducting meta-analyses by using transparent and reproducible ways to draw valid conclusions from the body of the research.

Defining the research question

The first step in carrying out a meta-analysis is the formulation of a clear and well-articulated research question[9]. For example, is *HOTAIR* expression associated with survival in human cancers? The researchers should provide a background of the topic, referring to the current state of knowledge, and state precisely the main goals of the meta-analysis. In the meta-analysis conducted by Toy and colleagues (2019), the authors discuss the gaps in the scientific literature and specify specify the research objectives, i.e. to perform a comprehensive and updated meta-analysis in order to investigate the prognostic value of *HOTAIR* expression in cancer [10]. In this way, a significant positive correlation between *HOTAIR* overexpression and

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poor overall survival, as well as progression/metastasis-free and recurrence/disease-free survival, was found in multiple and diverse types and subtypes of human cancers[10].

Systematic Literature Review

The systematic review of relevant studies for collecting published and unpublished information is a difficult task of meta-analysis. To maximize the number of the retrieved pertinent studies, it is recommended to search more than one of the bibliographic databases such as MEDLINE/PubMed, Scopus, Embase, The Cochrane Central Register of Controlled Trial, Web of Science, Google Scholar[11].

An extensive, usually manual, search of the scientific databases is performed by using a combination of relevant search terms. Initially, the title and abstract of the articles are scanned and the irrelevant studies are excluded from the subsequent steps of the analysis. The reference list of the review articles can be also examined to identify other articles that were omitted in the initial search. The included articles are then subjected to a selection filter based on established inclusion (English language of publication, minimal sample size etc.) and exclusion (not original research, inadequate sample size, etc.) criteria. The key variables to be extracted from the eligible studies should be defined. Broad inclusion criteria would increase heterogeneity among studies, whereas narrow inclusion criteria would limit the number of pertinent studies[8,12].

Furthermore, assessment of the quality of the included studies, could assist reviewers in determining the inclusion/exclusion criteria or the representativeness of the study sample. For example, the Jadad scale[13] is often used for assessing the quality of randomized clinical trials, Newcastle-Ottawa scale[14,15] for non-randomized studies, AXIS for cross-sectional studies[16], and QUADAS-2 for the quality assessment of diagnostic accuracy studies[17].

The key data are extracted from the primary articles and recorded in a structured form, usually in an ad hoc Excel spreadsheet. In the case key data are not available in the main text or the supplementary material of the primary research article, it is advised to contact the corresponding authors to ask for any missing data. In addition, it is recommended that the above mentioned tasks are carried out by two investigators independently and potential dispute is resolved by consensus[8,12,18].

The process of ensuring the transparency, reliability, comprehensiveness, and replicability of a systematic review is facilitated by updated reporting guidelines, established by international consortia. Examples of these guidelines for systematic reviews include QUORUM (Quality of Reporting of Meta-analyses)[19], MOOSE (Meta-analysis Of Observational Studies in Epidemiology)[20], and the most widely used PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) checklist[21]. Moreover, guidelines have been developed for reporting certain sorts of evidence and information, such as CONSORT (Consolidated Standards of Reporting Trials) guidelines for randomized clinical trials[22], STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines for various types of observational clinical studies (usually in the surgical discipline)[23], meta-analysis methods for genetic association studies[24] as well as genome-wide association studies[25].

Statistical analyses

Statistical analyses are performed on the collected data. There are several state-of-the-art, freely accessible statistical analysis software such as Stata (https://www.stata.com/) or R (https://www.r-project.org/) or standalone applications.

Effect Estimation

Selecting the appropriate effect measure depends on the types of data, i.e. dichotomous (or binary) data, continuous data, time-to-event data etc. Many epidemiological studies measure binary outcomes using defined endpoints. In this case, the overall effect can be measured by odds ratio (OR), relative risk (RR) and risk difference (RD).

The odds ratio (OR)[26,27] measures the association between two events, i.e., exposure and outcome, in case-control studies. OR is defined as the ratio of the odds of an outcome in the presence of a particular exposure

and the odds of the outcome in the absence of this exposure; OR higher that 1 indicates that the outcome (e.g. survival) is more likely to occur in the presence of a given exposure (e.g. treatment). For example, Zhu and coworkers (2017) used OR to show that PVT1 expression is significantly correlated with lymph node metastasis (OR = 2.67, 95% CI: 1.66–4.29), distant metastases (OR = 4.00, 95% CI: 1.39–11.50), advanced tumor-node-metastasis (TNM) stage (OR = 3.28, 95%CI: 2.46–4.38), and tumor size (OR = 1.47, 95% CI: 1.02–2.11)[28]. Moreover, Wang et al. (2021) found that heat shock protein 70 (HSP70) expression is robustly associated with higher tumor differentiation, (OR = 0.49, 95%CI: 0.37–0.65), intestinal gastric cancer (OR = 2.19, 95%CI : 1.59–3.01) and lymphovascular invasion (OR = 1.54, 95%CI: 1.19–2.00; invasion[29].

RR or risk ratio[27,30] is the ratio of the risk probability of an event in the presence of exposure to the risk probability of this event in the absence of the same exposure. Nassour et al. (2023) found that there is a higher RR of bladder and kidney cancer in Lynch syndrome patients[31].

Another metric, RD[32], is the difference between the risk of an event in the presence and the absence of a specific exposure. For example, in a recent study, Nakamura et al. (2024) demonstrated that the heat-shock protein HSP40 is associated with a lower probability (RD = 0.18, 95%CI: 0.03-0.33) and HSF1 (RD = -0.16, 95%CI: -0.29 to -0.04) with a higher probability of lymph node dissemination[33].

Hazard ratio (HR)[34] metric is usually applied for time-to-event data. HR measures the hazard rate of an event (e.g. survival rate) in an exposed group (e.g. treated) compared to the hazard rate of the same event in an unexposed group (e.g. untreated). HR is most often used in survival studies since it represents the instantaneous risk at different time points of the entire study period, unlike OD, RR and RD, which are cumulative over the length of the study. In case the HR is not reported in the article, it can be estimated from the survival curves (i.e. Kaplan-Meier curves) with the Cox proportional hazards model[35]. For example, in a comprehensive meta-analysis by Toy and colleagues (2019), the HR in cancer patients with high *HOTAIR* expression was estimated to be greater than 1, indicating that the overall survival rate of the patients over-expressing *HOTAIR* is lower compared to those with low *HOTAIR* expression[10]. Fang et al. (2020) found a significant positive correlation between elevated *lncSNHG15* expression and poor overall (HR = 2.07, 95%CI, 1.48-2.88) and disease-free (HR = 2.32, 95%CI: 1.53-3.53;) survival[36]. In a recent study, de Moraes and collaborators (2024) showed that the progression-free survival rate is higher in the breast cancer patients treated only with CDK inhibitors (CDKi) compared to those treated with CDKi and PPI (HR = 2.0901, 95%CI: 1.410-2.9498)[37].

Forest Plot

The results of the meta-analyses are typically presented using forest plots[38] (Figure 1), a graphical display of the estimated effect sizes for each study with the corresponding 95% confidence interval (95%CI), as well as the pooled or overall effect, which is the weighted average of the individual estimates.

Selection of the best fit statistical model

Most meta-analyses are based on two statistical models, fixed- or random-effect model[39], to calculate the overall effect. The fixed-effects model assumes that studies share a single common true effect size, and the overall effect is an estimate of the common effect size. The random-effects model assumes that true effects vary among studies and the overall effect is the weighted average of the effects reported in the individual studies.

Heterogeneity

The studies included in a meta-analysis have inherent considerable differences due to the overall design, methodology, data processing and analysis etc. Heterogeneity represents the degree of disagreement among studies in a meta-analysis, which is essential to be detected and measured in order to determine whether the heterogeneity is acceptable and, hence, appropriate to combine these studies in the meta-analysis or not. Several heterogeneity metrics are applied to assess heterogeneity[40,41].

Cochran's Q test[42] is a non-parametric (chi-square) statistical test used to examine whether all studies have the same effect. The Q test calculates the sum of the weighted squared differences between the effects of

the individual studies and the overall effect. The null hypothesis is rejected if the Q test p-value is less than 0.05, indicating the presence of heterogeneity. Another robust metric, the Higgins I^2 statistic[43] estimates the percentage of observed total variation across studies that is attributed to real heterogeneity rather than random chance. I^2 is calculated with the formula $(Q-df)/Q\times100\%$, where 'Q' is the Cochran test and 'df' is the degrees of freedom. I^2 values range between 0% (indicating lack of heterogeneity) and 100% (indicating high level of heterogeneity). Generally, if there is high heterogeneity ($I^2 \geq 50\%$), the random-effects model is applied; alternatively, if the heterogeneity is low ($I^2 < 50\%$), the fixed-effects model is used[10].

Subgroup analysis[44] is a method often used to assess heterogeneity. The studies are divided into groups based on certain features and characteristics (e.g. data extraction method, ethnicity, income). Separate meta-analyses are conducted for each subgroup in order to detect any statistically significant differences among the subgroups.

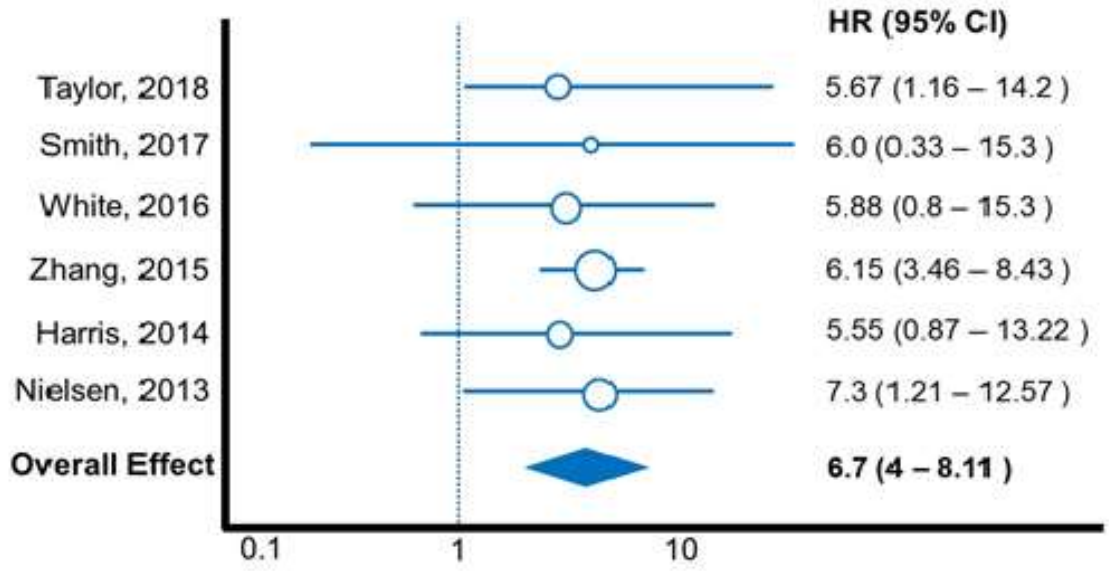

Figure 1. Example forest plot of hazard ratios. On the left column, the individual studies (indicated by the first author's name and the date) are shown in chronological order. The measure of the effect for each of these studies is indicated by circles, incorporating 95%CI (represented by whiskers). The marker's size is proportional to the study's weight in the meta-analysis; larger sample sizes are given more weight. The overall effect is represented by a diamond and the width of the diamond reflects the 95%CI of the estimate.

Sensitivity analysis

Sensitivity analysis is performed to verify the consistency of outcomes (Figure 2), and it is conducted by consecutively omitting one study, repeating the meta-analysis, and examining the effect of the excluded study on the overall effect. In case an individual study has an impact on the overall effect size this study most likely accounts for the between-study variability. For instance, in a meta-analytical study by Bonovas and colleagues (2008), where the association of statins with the risk for pancreatic cancer was investigated, a particular study was found to contribute mostly to the between study variation; when this study was excluded from the subsequent analysis, the heterogeneity was markedly reduced[45].

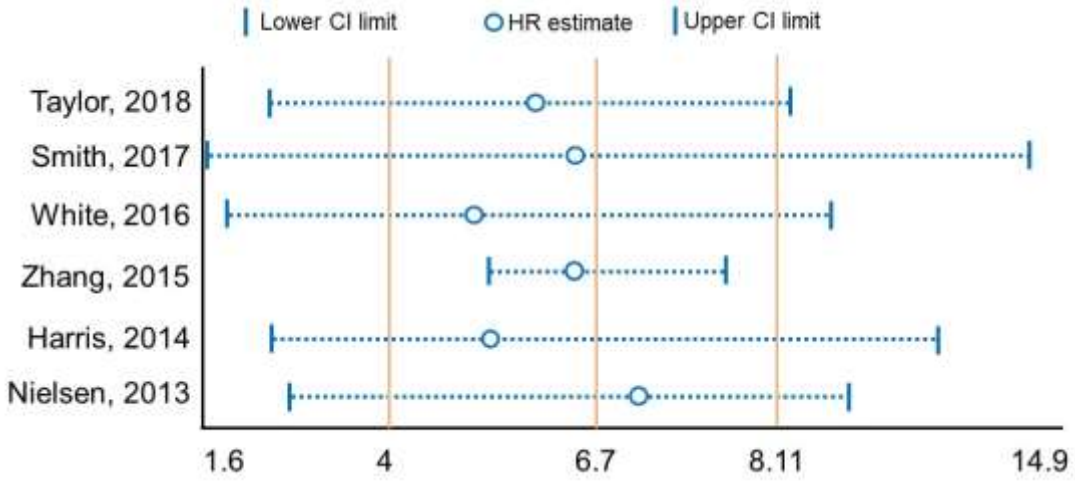

Figure 2. Example sensitivity analysis. There is no alteration in the results due to the inclusion of any individual study; the pooled HR and 95%CI remain the same.

Publication bias

Publication bias[46] is a major aspect of concern in meta-analyses, since there is less probability of studies with non statistically significant results being published than significant findings. Thus, assessing the presence and potential effects of publication bias is critical for ensuring validity and reliability of the outcome[9,47]. There are several methods to deal with publication bias in meta-analyses[48].

A quasi-statistical approach, the funnel plot [49], is a scatterplot which allows the visual inspection of the presence of publication bias. In the funnel plot, the standard errors of the effect estimates of the individual studies are plotted on the horizontal axis versus the standard error of the estimated effect on the vertical axis. A symmetrical or asymmetrical inverted funnel plot indicates the absence or presence of publication bias, respectively (Figure 3). In a funnel plot, small studies have a tendency to be more widely scattered at the bottom of the funnel plot, whilst larger studies typically have narrower spread, since they are more precise and are closer to the true effect size.

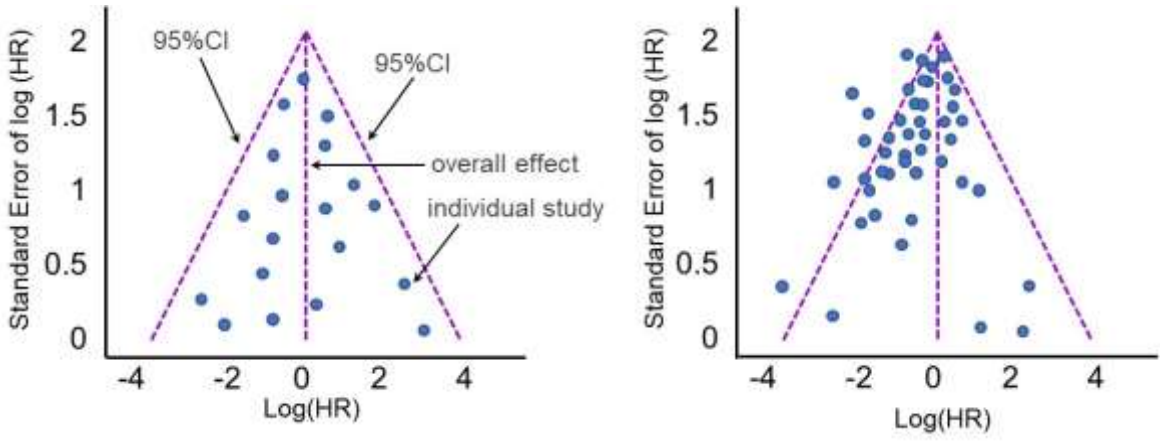

Figure 3. Example funnel plots. Left: symmetrical funnel plot. Right: asymmetrical funnel plot.

The Begg-Mazumdar adjusted rank correlation test can be also employed to identify any significant correlation between the effect estimates and their variances; this test is a statistical analogue of the funnel plot[50]

Egger's test[49] is also used to perform a linear regression of the standardized effect estimates on their standard errors; a p-value less than 0.05 indicates statistically significant publication bias.

Conclusion

Herein, we describe the core methodology and statistical techniques most commonly used to conduct meta-analyses for the discovery of potential cancer biomarkers. These biomarkers can be diagnostic molecular markers, prognostic predictors, disease monitoring biomarkers or predictors of response to therapy. Meta-analysis enables the investigators to synthesize the outcomes of diverse studies accurately and systematically, deal with controversies arising from conflict among studies and meaningfully interpret the available biological or epidemiological data, towards addressing the needs of the patients and the oncology healthcare systems.

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Correspondence Address / Yazışma Adresi
Athanasia Pavlopoulou
Izmir International Biomedicine and Genome Institute
Genomics and Molecular Biotechnology Department
Dokuz Eylül University
Izmir, Türkiye
e-mail: athanasia.pavlopoulou@deu.edu.tr

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