

# Prognostic role of urokinase plasminogen activator in hepatocellular carcinoma

## A protocol for systematic review and meta analysis

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### Abstract

**Background:** Previous studies have showed that the high expression of urokinase plasminogen activator (uPA) in pathology and serology is closely related to the progression of hepatocellular carcinoma (HCC). However, there are no systematic reviews for these evidence, and the association between uPA and HCC is still not completely understood. Therefore, we will undertake a systematic review of the literature to summarize previous evidence regarding this topic, in order to clarify the prognostic significance of uPA in HCC.

**Methods and analysis:** Studies comparing the HCC patients with high and low expression of uPA on the clinicopathological features and the prognosis are eligible for this review. Outcomes include all endpoints about survival and clinicopathological features. Prospective or retrospective primary studies which published in English will be included. Four databases of Medline, EMBASE, Web of Science, and the Cochrane Library will be systematically searched from their inception to Mar 2021 to retrieve relevant studies. Reference lists of included studies will be manually reviewed and grey literatures will be identified by Google Scholar. Two reviewers will independently screen the records and extract the information and data of the included studies. The Newcastle-Ottawa Scale will be used to assess the quality of included studies. Hazard ratio and 95% confidence interval will be pooled to assess the association between uPA expression and the prognosis. Pooled odds ratio and 95% confidence interval will be used for other outcomes. Heterogeneity will be assessed using the Cochrane Q test and  $I^2$  statistic, and a subgroup analysis will be performed if necessary. Grades of Recommendation, Assessment, Development and Evaluation method will be applied to assess the certainty of evidence.

**Ethics and dissemination:** This protocol required information extracted from previously published articles. So, there is no ethical problem in this study. We plan to publish our findings in peer-reviewed journals and relevant conference proceedings.

**Systematic review registration:** This study has been registered with the International Prospective Register of Systematic Reviews database (no.CRD42020150340).

**Abbreviations:** CI = confidence interval, HCC = hepatocellular carcinoma, HR = hazard ratio, NOS = Newcastle-Ottawa Scale, uPA = urokinase plasminogen activator.

**Keywords:** systematic review, meta-analysis, urokinase plasminogen activator, hepatocellular carcinoma, protocol

PT and LG contributed equally to this work.

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Any amendments will be first submitted online via International Prospective Register of Systematic Reviews database, and a corrected protocol will be published subsequently.

This protocol is prepared according to PRISMA-P guidelines.<sup>[26]</sup> No primary data will be collected, and all required information will be extracted from previously published articles. Therefore, there is no need for ethics. We plan to publish our findings in peer-reviewed journals and relevant conference proceedings.

The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

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## 1. Introduction

Hepatocellular carcinoma (HCC), the fifth and seventh most common cancer in adult man and female with more than 700,000 new cases and over 600,000 deaths worldwide per year.<sup>[1,2]</sup> The distribution of HCC varies according to geographic location, high in Asian and sub-Saharan Africa, with incidence rates of over 20 per 100,000 individuals, and low in countries of northern Europe, Middle East, Oceania, North and South America.<sup>[3]</sup>

Earlier diagnosis is benefit for the prognostic for HCC. There are multiple methods for diagnosing, such as the alpha-fetoprotein serum values,<sup>[4]</sup> alpha-fetoprotein-L3,<sup>[4]</sup> DCP,<sup>[5]</sup> circulating miRNAs,<sup>[6]</sup> GP73, and GPC3.<sup>[7]</sup> Moreover, numerous microRNAs and genes/proteins are involved in carcinogenesis and prognosis for HCC. Urokinase plasminogen activator (uPA) is 1 of this indexes, which is proved to relate with prognosis and earlier diagnosis of HCC.<sup>[8]</sup> However, above mentioned indexes performed various specificity and sensitivity for HCC prognosis and recurrence. Thus, identifying the biological indicators of invasion and metastasis, especially with regard to the prognosis of HCC, is particularly important.

uPA is a serine protease of 55 kDa molecular mass, mainly composed of a growth factor-like domain, a kringle domain and a serine protease domain.<sup>[9]</sup> In the tumor progression, uPA binds to its cell surface receptor uPAR, which then catalyzes the further transformation of pro-metalloproteinases into metalloproteinases.<sup>[10]</sup> These activated metalloproteinases then promote the degradation of the extracellular matrix,<sup>[11]</sup> and contribute to tumor cell invasion and metastasis at secondary tumor sites. During previous research, some case have showed that the high expression of uPA in pathology and serology is closely related to the poor prognosis of HCC,<sup>[12,13]</sup> although the majority of published reports are of small or medium size. Obviously, heterogeneity in study design, data collection, documentation and presentation limit the accessibility of these data. So far, there is no a reliable evaluation system on overall survival and survival rate between uPA expression and HCC. However, there are no systematic reviews for these evidence, and the association between uPA and HCC is still not completely understood. Therefore, we will undertake a systematic review of the literature to summarize previous evidence regarding this topic, in order to clarify the prognostic significance of uPA in HCC.

## 2. Objectives

To determine the relationship between uPA expression and clinicopathological features in HCC patients, and to analyze the prognostic value of uPA in HCC.

## 3. Methods and analysis

### 3.1. Registration

This protocol was registered with International Prospective Register of Systematic Reviews database (CRD42020150340)

### 3.2. Participants

Patients with HCC will be considered for this review. The diagnosis is histologically confirmed. The patients received success hepatectomies, and tissue samples were preserved for the test of uPA expression. The stage and histological degree of tumours are determined according to the Barcelona Clinic Liver

Cancer staging system<sup>[14]</sup> and Edmondson's grade,<sup>[15]</sup> respectively.

### 3.3. Exposure

The expression level of uPA in tissue is the exposure variable of this review. We will include studies detecting uPA expression in HCC tissues by the immunohistochemistry analysis. The expression level of uPA is defined as high and low expression based on the immunohistochemical staining. Studies should provide data comparing the HCC patient with high and low expression of uPA on the clinicopathological features and the prognosis.

### 3.4. Outcomes

Outcomes that indicate the associations of uPA expression with prognosis or clinicopathological features will be included for this review. Any outcomes about prognosis are eligible as long as sufficient information are provided to estimate the hazard ratio (HR). The clinicopathological features will include tumor differentiation, lymph node metastasis, tumor size, and TNM stage, and so on.

### 3.5. Studies

Prospective or retrospective published studies which evaluate the association between the uPA expression and HCC are eligible for this review. Studies will be excluded if they are letters, case reports, reviews, and animal studies. Non-English studies will also be excluded.

### 3.6. Information sources

Medline, EMBASE, Web of Science, and the Cochrane Library will be systematically searched from their inception to Mar 2021.

### 3.7. Search strategy

A combination of subject terms with free-text terms are used during the searches of 4 databases. The following search words is adopted for each database: (((tumour\* or carcinom\* or tumor\* or malign\* or neoplasm\* or cancer\*) and (hepat\* or liver)) or HCC) and (uPA or Urokinase\* plasminogen or Urokinase-Type Plasminogen Activator). Other relevant articles which are not recorded in databases will be also manually reviewed from the reference lists of eligible studies. In addition, we will also search in Google Scholar to identify any grey literatures.

## 4. Study records

### 4.1. Data management

All study records are processed through EndNote X9, which can identify and remove duplicates. All extracted data are stored in a Microsoft Excel spreadsheet.

### 4.2. Study selection and data collection process

Two reviewers will independently assess the titles and abstracts of records identified by the literature searches. The full-text articles of any potentially relevant studies will be obtained. The same reviewers then will assess the full text of these potentially relevant

studies for inclusion based on our inclusion criteria. Any disagreements will be solved by face-to-face discussion, with arbitration by a third researcher, when necessary. The data and information of the finally included study will be independently extracted by 2 reviewers using pre-piloted electronic data extraction forms. When differences in extracted data arise, 2 reviewers will discuss to reach consensus, or a third researcher will be consulted if necessary.

**4.3. Data items**

The following data are extracted: author name, publication year, study location, study design, number of patients, follow-up period, outcome measures, baseline information of patients, clinicopathology (such as tumour differentiation, lymph node metastasis, tumour size, and TNM stage), HR, and 95% confidence interval (CI) related to uPA expression. Any items associated with a risk of bias will be also summarized.

**4.4. Risk of bias in individual studies**

The Newcastle-Ottawa Scale (NOS)<sup>[16]</sup> will be applied to assess the quality of each included study. Scores of the NOS are split

into 3 aspects: object selection, inter-group comparability, and outcome measurement. The NOS scores range from 0 to 9, and it is generally considered that an article with a score >6 is of high quality.

**4.5. Data synthesis**

All statistical tests will be performed using the software STATA. HR and 95% confidence interval (CI) will be calculated for each study to assess the association between uPA expression and the prognosis. The HR and 95% CI of each study will be first directly extracted from the original full text. For studies where HR and 95% CI are not provided, data will be obtained from the survival curves using Engauge Digitizer, and the method from Tierney et al<sup>[17]</sup> will be used to calculate the HR and 95% CI. The HR and 95% CI of individual study will be finally combined using the STATA software. Pooled HR > 1 indicates poor prognosis for the group of high uPA expression. The level of statistical significance is set at the 5%. The association between uPA expression and clinicopathological features is assessed using the pooled odds ratio and 95% CI. We will conduct qualitative statistics if a meta-analysis is not appropriate due to small number of studies or concerns regarding substantial variability. Statistical heterogene-

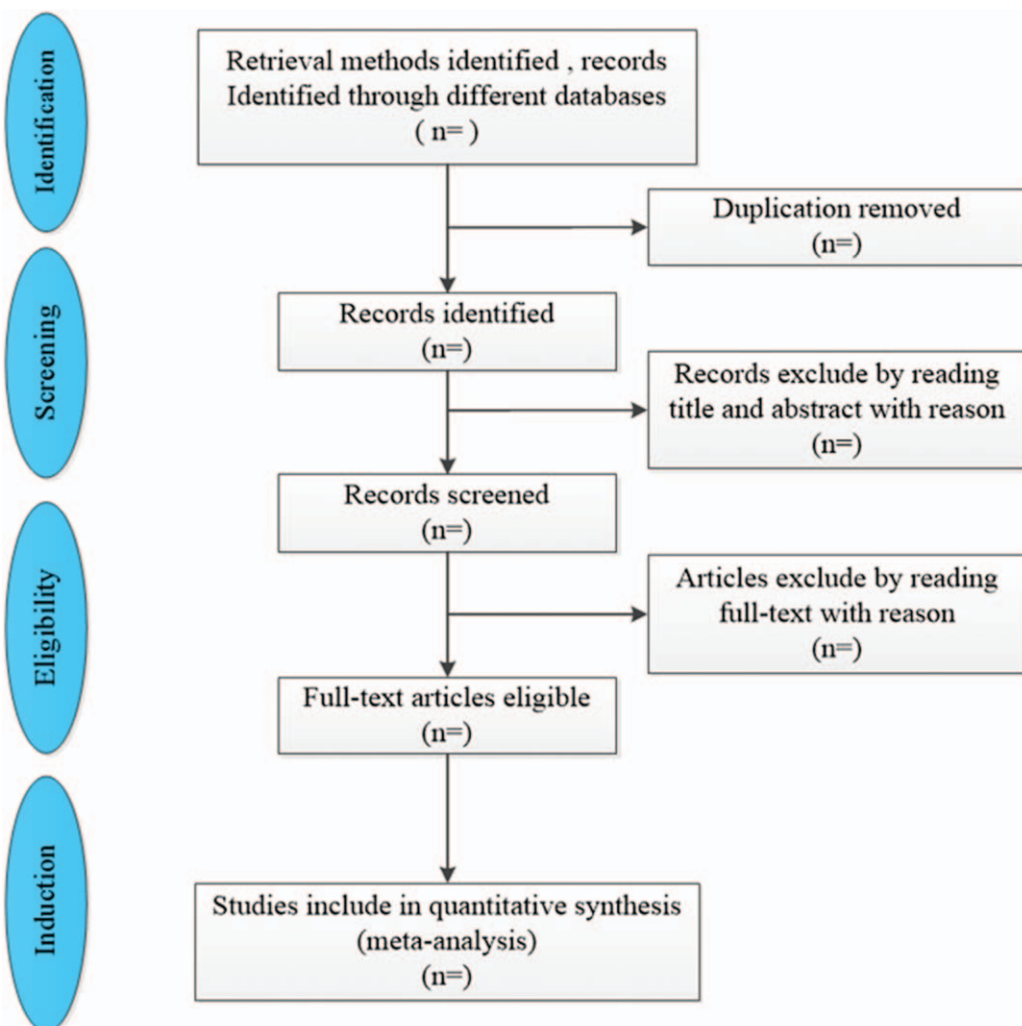


Figure 1. Flowchart: selection of studies for inclusion in meta-analysis.

ity will be assessed using the Cochrane  $Q$  test and  $I^2$  statistic. A fixed effect model will be applied when the effects are assumed to be homogenous ( $P > .05$ ,  $I^2 \leq 50\%$ ); otherwise, the random effect model will be adopted ( $P < .05$ ,  $I^2 \geq 50\%$ ). To explore the source of heterogeneity, subgroup analysis will be performed through the HR obtain method, sample size, country, and study design. Sensitivity analysis will also be conducted to assess the stability of the results.

#### 4.6. Meta-biases

If there were 10 or more studies included in each meta-analysis, we will assess the publication bias graphically by a funnel plot and statistically by the Egger test.<sup>[18]</sup>  $P < .05$  is considered to indicate publication bias.

#### 4.7. Confidence in cumulative evidence

We will assess the certainty of evidence obtained from this systematic review using the Grades of Recommendation, Assessment, Development, and Evaluation method.<sup>[19]</sup>

### 5. Discussion

uPA has inestimable potential for HCC prognostic marker. In the previous study, numerous of literatures have demonstrated that fibrinolytic system or uPA were positive or negative relation with prognosis for various cancer types.<sup>[20–22]</sup> For instance, uPA/PAI-1 as a molecular biomarker was recommend by the American Society of Clinical Oncology and the Arbeitsgemeinschaft Gynäkologische Onkologie (German Gynecological Oncology Group) guidelines to avoid unnecessary CT/X for moderate risk patients with primary breast cancer.<sup>[23]</sup> At present, several studies have reported the association between uPA and HCC.<sup>[24,25]</sup> However, small sample of individual study and uncertain quality of evidence limited the clinical application of these results.

Therefore, there is an urgent requirement to make a systematic review of studies on the prognostic significance of uPA for HCC. This article is a protocol of our systematic review, which presented the detailed description of review implement. The results of our review will be reported strictly following the PRISMA criteria. By integrating the data from previous articles, this review will objectively reveal the relationship between the expression of uPA and clinicopathological features of patients with HCC, and clarify the prognostic value of uPA for HCC.

The results of this upcoming study will help us understand the role of uPA for HCC, and it will provide a reference for developing a new strategy of prognosis assessment in HCC patients. It will also indicate the areas, where further research is necessary, before clinical application of uPA as a prognostic marker can be considered.

#### 5.1. Reporting of the review

The findings will be published as per PRISMA guidelines. A flow chart will be employed to outline the search producer (Fig. 1). Text description will be used to review the qualitative data of the included studies. Outputs of meta-analyses will be depicted in a forest plot and survival curve. Publication bias will be represented in the inverted funnel plot. The search strategy and quality appraisal tool will be provided in the supplement.

### Author contributions

PXT, YZ, and HC conceived the idea and plan for the study. PXT and LG wrote the manuscript of this protocol. LG, YZ and HYL designed the search strategy and planned the data extraction. BFW and XML made contributions in conceiving this research project. All authors revised and approved the final version of the manuscript.

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**Writing – review & editing:** Haiyuan Li, Bofang Wang, Xuemei Li, Ying Zhang, Hao Chen.

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