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# Role of AFP mRNA expression in peripheral blood as a predictor for postsurgical recurrence of hepatocellular carcinoma: A systematic review and meta-analysis

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## Abstract

**AIM:** To identify the role of alpha-fetoprotein (AFP) mRNA expression in peripheral blood one week after surgery as a predictor for recurrence of hepatocellular carcinoma (HCC).

**METHODS:** Published studies fulfilling the selection criteria were identified by searching several databases online. After a methodology assessment using a quality scale designed by European Lung Cancer Working Party, data in each research were aggregated by means of meta-analysis.

**RESULTS:** Altogether 368 cases were included in the 9 selected studies, which fulfilled the selection criteria. The quality scores ranged from 35% to 84% with a median score of 55%. The 'design' subscore had the lowest median value (38%). By aggregating the data, a high $\chi^2$ value (77.576) was presented. The fail-safe number was 136 and 64 for $P = 0.05$ and 0.01, respectively.

**CONCLUSION:** AFP mRNA expression in peripheral blood 1 wk after surgery correlated with the recurrence of HCC and was a good predictor for tumor recurrence.



**Key words:** AFP mRNA; HCC; Meta-analysis

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## INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignant diseases in the world. Approximately 560 000 new cases of HCC are diagnosed each year, constituting 6% of all new human cancers[1]. The HCC mortality rate in China is approximately 20.4/100 000 and is the second leading cause of cancer death among Chinese males[2]. It has been putatively accepted that surgery, including curative resection and liver transplantation, is the only hope for curing this malignant disease. With the advance in surgical techniques, the surgical mortality of HCC has decreased significantly in the past decades. The 5-year survival rate after curative resection of HCC has risen from 16.0% up to 48.6%. But unfortunately, even after curative resection, approximately from 40% to 100% HCC patients will suffer from tumor recurrence[3]. So it is of great importance to find valuable prognostic markers, which could predict the recurrence and metastases of HCC, and with the help of those markers, doctors could perform proper postoperative treatment on those who are at a high risk of cancer recurrence.

It has been suggested that a small number of HCC cells could be detected in the peripheral blood of patients with HCC by reverse-transcription polymerase chain reaction (RT-PCR) targeting alpha-fetoprotein (AFP) messenger RNA (mRNA)[4,5]. Some clinical studies reported that the presence of AFP mRNA in peripheral blood was associated with blood-borne spread and poor prognosis of HCC. However, some other reports about the value of AFP mRNA as a marker for predicting the recurrence of HCC after surgery are quite different[6].

Recently some studies suggested that the meta-analysis was a scientific method to evaluate the role of biomarkers, such as p53, microvessel density, Bel-2[7-9], in predicting the recurrence and prognosis of cancer. In order to correctly evaluate the value of AFP mRNA as a predictor of HCC recurrence, we performed a systemic review of literature on the relationship between AFP mRNA expression in peripheral blood 1 wk after surgery and the recurrence of HCC, and applied meta-analysis to explore the role of AFP mRNA as a prognostic marker of HCC recurrence after surgery.

## MATERIALS AND METHODS

### Publication selection

To be eligible for this review, trials had to deal with HCC patients who received their first curative operation (including curative resection and liver transplantation) only. The data of AFP mRNA expression in peripheral blood a week after surgery should be available (the latest data was used to evaluate its relationship with tumor recurrence if AFP mRNA test was performed several times a week after operation). The survival observation should last at least 1 year. Reliable data and analysis should be presented in the article and results should be published as a full paper in medical literature in English or Chinese. Abstracts were excluded from this analysis because of insufficient data to apply the scoring system and to evaluate the methodological quality of the trial.

Articles were identified by an electronic search on Pubmed, Embase cancerlit and cnki using the keywords: HCC, liver cancer, liver tumor, alpha-fetoprotein mRNA, AFP mRNA. The bibliographies reported in all the identified studies were used for completion of the trial search. When authors reported, in several publications, on the same patient populations, only the most recent or complete study was included into the analysis, in order to avoid overlapping between cohorts. The search ended in February 2004.

### Methodological assessment

To assess the methodology, a scoring system previously introduced by Steels *et al*[7], was used in this literature review. Nine investigators, including eight clinicians and one statistician, read each study independently and scored them according to a quality scale designed by ELCWP (European Lung Cancer Working Party)[7]. This quality scale evaluated many dimensions of the methodology, grouped in four main categories: the scientific design, the description of the methods used to identify the expression of AFP mRNA in peripheral blood, the generalizability of the results and the analysis of the study data. Each category had a maximal score of 10 points with an overall maximal theoretical score of 40 points.

The scores were compared and a consensus value for each item was reached in meetings at which at least two-thirds of the investigators needed to be present. The final scores were expressed as percentages, with higher values reflecting a better methodological quality.

### Statistical methods

For the quantitative aggregation of recurrence results, we measured the impact of AFP mRNA on recurrence of HCC from the risk ratio (RR) and *P* value between the two recurrence distributions. According to the method introduced by Riley[9], the RR and *P* values were calculated by the following steps. First, the accurate RR and *P* value were calculated depending on the exact data of RR, OR, the logrank statistic or its *P* value, the O-E statistic (difference between numbers of observed and expected events) or its variance provided in the publication. Second, if those data were not available, we used individual patient data to look for the total number of events, the number of patients at risk in each group and the number of events in each group, and calculated the RR and *P* value. Lastly, if the only exploitable data were in the form of graphical representations of survival distributions, survival rate at some specified times were extracted from them in order to reconstruct the RR estimate and *P* value, with the assumption that the rate of patients censored was constant during the follow-up study. When steps 2 or 3 were used, three independent investigators read the publication and curves to reduce the imprecision in reading variations. The individual RRs were combined into an overall RR by fixed effect model (Peto's method) assuming that the homogeneity was true. The heterogeneity was tested by $\chi^2$ test. If the homogeneity was rejected, then a random-effects model (D & L method) was applied to aggregate the data.

The trials were grouped according to their scientific design. The intra-group aggregation was performed at first, followed by the inter-group aggregation. Wilcoxon test was used to compare the distribution of the quality scores.

The statistical analysis was performed by statistical software SPSS11.0. *P* values less than 0.05 were considered statistically significant.

## RESULTS

### Studies selection and characteristics analysis

Eighteen researches, including 11 English publications and seven Chinese articles, studied the relationship between AFP mRNA in peripheral blood after surgery and postoperative recurrence of HCC[10-26]. Three English articles were excluded because identical cohorts of patients were included in other selected publications or the AFP mRNA expression was detected within a week after surgery instead of 1 wk later or because of insufficient data for meta-analysis[10,11,19]. Six Chinese articles were excluded because the survival observation lasted less than a year or because we could not determine the time when AFP mRNA was detected or because of insufficient data for performing meta-analysis[22-26]. In nine of the remaining articles published between 1997 and 2004, seven researches were cohort studies and two were case-control studies. The total number of patients was 368 ranging from 14 to 87. The main characteristics of the nine studies are shown in Table 1.

### Quality assessment

Assessed according to the scoring system, the overall quality score for the remaining nine studies ranged from 35% to 84%, with a median score of 55%. The 'design' subscore had the lowest value (median: 38%). There was no statistical quality difference between trials with significant results and non-significant results (median scores: 57% *vs* 51%, $P>0.05$).

### Meta-analysis

As shown in Table 2, the nine studies enrolled in this study could be grouped into seven cohort studies and two case-control studies. Six of the seven cohort studies provided detailed individual patient data, and RR and *P* value were calculated according to the total number of events, the number of patients at risk in each group and the number of events in each group. As only exact *P* value was available

**Table 1** Main characteristics of selected publications

| Author | Yr | Cut-off | Study design | Data used for meta-analysis | *n* | Method |
|---|---|---|---|---|---|---|
| Wong *et al* | 2001 | The upper limit of normal value | Cohort | Case($^{D/n}/_{D'/n'}$) (6/10, 1/5)[1] | 15 | Semi-quantitative RT-PCR |
| Minata *et al* | 2001 | Dichotomized by the presence of AFP mRNA | Cohort | Case($^{D/n}/_{D'/n'}$) (11/11, 1/15) | 26 | RT-PCR |
| Witzigmann *et al* | 2001 | Dichotomized by the presence of AFP mRNA | Cohort | Case($^{D/n}/_{D'/n'}$) (5/15, 4/16) | 31 | RT-PCR |
| Lemoine *et al* | 1997 | Dichotomized by the presence of AFP mRNA | Cohort | Case($^{D/n}/_{D'/n'}$) (2/10, 2/7) | 17 | RT-PCR |
| Okuda *et al* | 2001 | Dichotomized by the presence of AFP mRNA | Cohort | Case($^{D/n}/_{D'/n'}$) (6/12, 2/13) | 25 | RT-PCR |
| Ijichi *et al* | 2002 | Dichotomized by the presence of AFP mRNA | Cohort | *P* value (0.014) | 87 | RT-PCR |
| I-Shyan *et al* | 2004 | Dichotomized by the presence of AFP mRNA | Cohort | Case($^{D/n}/_{D'/n'}$) (17/19, 19/62) | 81 | RT-PCR |
| Funaki *et al* | 1997 | Dichotomized by the presence of recurrence | Case-control | Case($^{D/n}/_{D'/n'}$) (11/11, 2/3)[2] | 14 | RT-PCR |
| Liu *et al* | 1999 | Dichotomized by the presence of recurrence | Case-control | Case($^{D/n}/_{D'/n'}$) (7/19, 2/53) | 72 | RT-PCR |

[1]The patients who received operation only were enrolled in the study. [2]Prospective survival observation was also performed on eight cases of HCC who were operated on during this study, but as AFP mRNA was tested 2–3 d after operation, these eight cases were excluded.

**Table 2** Aggregation of six cohort studies by Pote's fix-effect model

| Author | O | E | O–E | V(O–E) | (O–E)/V | Z | *P* |
|---|---|---|---|---|---|---|---|
| Wong | 6 | 4.67 | 1.33 | 1.60 | 1.11 | 1.05 | 0.146 |
| Minata | 11 | 5.10 | 5.90 | 1.66 | 21.10 | 4.58 | 0.000 |
| Witzigmann | 5 | 4.35 | 0.65 | 2.68 | 0.16 | 0.39 | 0.347 |
| Lemoine | 2 | 2.35 | -0.35 | 1.69 | 0.07 | -0.27 | 0.607 |
| Okuda | 6 | 3.84 | 2.16 | 2.07 | 2.26 | 1.50 | 0.067 |
| I-Shyan | 17 | 8.44 | 8.56 | 3.75 | 19.50 | 4.42 | 0.000 |
| Aggregation | | | 18.24 | 13.44 | 43.95 | 11.68 | |

in Ijichi's study[16], we combined the six studies initially and then Ijichi's data was added into the aggregation by statistical index combination.

The six cohort studies were analyzed with a fix-effect model, RR was 3.884 (95%CI: 2.276-6.629). However, the test of heterogeneity was significant ($P = 0.002$). Thus we calculated the RR using a random-effects model, the adjusted RR was 9.102 (95%CI: 1.677-49.405) and *P* value was 0.011. The fail-safe number was 44 and 19, for $P = 0.05$ and $P = 0.01$, respectively. After this, Ijichi's *P* value was combined to the former six cohort studies by statistical indexer aggregation. The result showed that $\chi^2$ was 48.773 and $P<0.001$ after combination. The fail-safe number was 64 and 29 for $P = 0.05$ and $P = 0.01$, respectively (Table 3).

Two case-control studies were enrolled in the meta-analysis. One was Funaki's study in which AFP mRNA tests were positive in all the patients suffering from HCC recurrence[15]. As this was an extreme case, we combined the two case-control studies into one case-control study. When it was aggregated with the other seven cohort studies by statistic index aggregation as shown in Table 4, the result was significant ($P<0.001$). The AFP mRNA positive group had a higher incidence rate of recurrence ($\chi^2=77.576$, $P<0.001$), and the fail-safe number was 136 and 64 for $P = 0.05$ and $P = 0.01$, respectively.

## DISCUSSION

HCC ranks fifth in frequency worldwide among all malignancies. Most patients with HCC die quickly because of the rapid progression of cancer, and the mortality rate of HCC is almost equal to the morbidity rate[1]. Hepatic resection or transplantation is the only potential curative treatment for HCC patients. With the development of surgical technique, the surgical mortality of HCC has decreased significantly[27], but even after curative resection, about 60-100% patients suffered from cancer recurrence ultimately[3]. The recurrence has become the most important factor that limits the long-term survival of patients with HCC. So there is an urgent need for proper markers to predict the postoperative recurrence, and guide us to take adequate treatment for those who are at a high risk of HCC recurrence.

**Table 3** Aggregation of six cohort studies by D & L random-effects model

| Author | RRi P1′/P0′ | Yi Ln (RRi) | Wi 1/Vi | Ywi Wi × yi/V | Wi′ 1/(D+1/Wi) | Ywi′ Wi′ × yi/W′ |
|---|---|---|---|---|---|---|
| Wong | 6.000 | 1.792 | 3.650 | 0.455 | 0.207 | 0.369 |
| Minata | 1 694.000 | 7.435 | 1.162 | 0.278 | 0.284 | 0.902 |
| Witzigmann | 1.500 | 0.406 | 3.667 | 0.048 | 0.341 | 0.059 |
| Lemoine | 0.625 | –0.470 | 2.300 | –0.035 | 0.323 | –0.065 |
| Okuda | 5.500 | 1.705 | 3.591 | 0.197 | 0.340 | 0.248 |
| I-Shyan | 19.237 | 2.957 | 1.865 | 0.340 | 0.219 | 0.481 |
| Aggregation | | 13.8236 | 16.2345 | 1.677 | 1.343 | 2.208 |

**Table 4** Conversion of statistical index of the nine selected studies

| Authors | *P* | *Z* | *t* | *D* | *r* | $\chi^2$ | *F* |
|---|---|---|---|---|---|---|---|
| Ijichi | 0.014 | 2.197 | 2.236 | 0.485 | 0.236 | 4.828 | 5.000 |
| Wong | 0.146 | 1.054 | 1.098 | 0.609 | 0.291 | 1.110 | 1.215 |
| Minata | 0.000 | 4.580 | 5.357 | 2.143 | 0.731 | 20.976 | 29.248 |
| Witzigmann | 0.347 | 0.394 | 0.398 | 0.148 | 0.074 | 0.155 | 0.159 |
| Lemoine | 0.393 | –0.272 | –0.277 | –0.143 | –0.071 | 0.074 | 0.077 |
| Okudal | 0.067 | 1.499 | 1.554 | 0.648 | 0.308 | 2.248 | 2.423 |
| I-Shyan | 0.000 | 4.420 | 4.460 | 1.004 | 0.449 | 19.536 | 19.927 |
| Liu and Funki[1] | 0.000 | 5.354 | 5.338 | 1.165 | 0.503 | 28.665 | 28.546 |

[1]Liu and Funki's study were combined into a single study.

By now, many clinical, tumor biological, and molecular biological markers have been used to predict HCC recurrence, but it is a frequently encountered situation that different conclusions were drawn from different researches concerning the value of the same predictor. Meta-analysis offers us a method to solve this problem. By performing a systemic review on relevant studies and quantitative analysis of the results, we could take advantage of different opinions and draw an objective conclusion to the value of those biomarkers as predictors for HCC recurrence. This method has been successfully applied to evaluate the prognostic value of some biomarkers such as p53, microvessel density in several cancers[7,8]. To our knowledge, this is the first time that meta-analysis was used to evaluate the value of biomarkers for predicting HCC recurrence.

As the cancer cells in the circulation are an important source for HCC metastasis and recurrence, the biomarkers that indicate the existence of malignant cells in the circulation may be a useful predictor for HCC recurrence. In 1994, Matsumura reported that single HCC cell in circulation could be detected by means of RT-PCR, targeting AFP mRNA[4]. From then on, many researchers studied the value of AFP mRNA as a predictor for HCC recurrence, but the results are rather controversial. This may be due to the blood-borne dispersion of both tumor cells and normal liver cells during surgical manipulation and the mis-transcription of AFP mRNA by peripheral mononuclear cells[28,29], which may damage the specialty and prognostic value of AFP mRNA. In this study, we performed a systemic review and meta-analysis on the researches concerning the relation between serum AFP mRNA a week after operation and recurrence of HCC. We focused on patients treated for the first time by relative curative operation only, we could not thus extrapolate our results to re-resection patients. It demonstrated that AFP mRNA is a valuable predictor for HCC recurrence. This observation is potentially important for both prognostic reasons and HCC treatment guidance.

In order to ensure the generalizability and credibility of the result of meta-analysis, we excluded researches that could not fulfill the selection criteria. To have sufficient time to exhibit the impact of AFP mRNA on HCC recurrence, the survival observation is required to be more than 1 year. We used a methodology score system that adapted to the field of biological prognostic factors to perform the meta-analysis. The absence of statistically significant difference in quality score between researches with statically significant results and non-significant results allowed us to perform a quantitative aggregation of the individual trial results.

This study does not however prevent all potential biases. The review was restricted to English and Chinese articles because other language publications could not be accessed by the investigators. This selection may favor the positive studies that are more often published in English while the negative ones tend to be published in native languages[31]. Another source of bias is that the authors may report in several publications on the same patient populations. We have excluded articles where that seemed to be the case, and sent emails to some of the authors in order to have more information on patients' cohort, but unfortunately had no response. In several studies, the data used for meta-analysis was based on the detailed information of individual patients, this approach might cause errors due to imprecision in the reading, although three independent investigators read the article to reduce the reading variation.

Only fully published studies were selected for review and meta-analysis. This selection neglected the unpublished

data and abstracts and thus might be less accurate[30]. However, as the meta-analysis based on all performed studies on the topic, published or not, requires individual data to be updated by the investigators is much more time-consuming, even sometimes impossible; on the other hand, Meert and Auperin's studies showed that the same results of meta-analysis on the role of prophylactic cerebral irradiation in small-cell lung cancer were obtained respectively either based on published or individual data[31,32], we considered this approach might not have much effect on the accuracy of the result of meta-analysis in this study.

The variations in recurrence among the studies could be explained by the heterogeneity in the time AFP mRNA was tested, in addition to variations in patients' population and science design. It has been reported that the surgical manipulation would cause the blood-borne dispersion of both tumor cells and normal liver cells[13,28]. However, normal liver cells will diminish from the circulation due to the mechanical destruction or filtering out of the circulation when passing through the capillary network. At least 1-2 wk are needed for these normal liver cells in the circulation to become undetectable after surgery by RT-PCR targeting AFP mRNA[19]. As a result, although only trials that studied the AFP mRNA expression in peripheral blood a week after operation were selected, the impact of remaining normal liver cells in the circulation on the AFP mRNA detection could still be quite different as the difference in the time interval for testing AFP mRNA ranged from a week to several months.

In conclusion, the presence of AFP mRNA in peripheral blood a week after curative surgery is a valuable predictor for HCC recurrence.

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