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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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)[4-9]. 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[10].

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[11,12], 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\textsuperscript{[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)\textsuperscript{[8]}. 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\textsuperscript{[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 reviews, including 11 English publications and seven Chinese articles, studied the relationship between AFP mRNA in peripheral blood after surgery and postoperative recurrence of HCC\textsuperscript{[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\textsuperscript{[22-26]}. 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\textsuperscript{[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
respectively. After this, Ijichi’s study was combined to the other six cohort studies by statistical indexer aggregation as shown in Table 4, the result showed that the presence of recurrence (7/19, 2/53) was significant ($\chi^2=48.773, P<0.001$), and the fail-safe number was 136 and 64 for $P=0.01$, respectively. The AFP mRNA positive group had a higher incidence rate of recurrence ($\chi^2=77.976, P<0.001$), 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$^{[2]}$, 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.

| 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($^{\text{O}}/{\text{O'+E}}$) (6/10, 1/5)$^{[1]}$ | 15 | Semi-quantitative |
| Minata et al | 2001 | Dichotomized by the presence of AFP mRNA | Cohort       | Case($^{\text{O}}/{\text{O'+E}}$) (11/11, 1/15) | 26 | RT-PCR           |
| Witzigmann et al | 2001 | Dichotomized by the presence of AFP mRNA | Cohort       | Case($^{\text{O}}/{\text{O'+E}}$) (5/15, 4/16) | 31 | RT-PCR           |
| Lemoine et al | 1997 | Dichotomized by the presence of AFP mRNA | Cohort       | Case($^{\text{O}}/{\text{O'+E}}$) (2/10, 2/7) | 17 | RT-PCR           |
| Okada et al | 2001 | Dichotomized by the presence of AFP mRNA | Cohort       | Case($^{\text{O}}/{\text{O'+E}}$) (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($^{\text{O}}/{\text{O'+E}}$) (17/19, 19/62) | 81 | RT-PCR           |
| Funaki et al | 1997 | Dichotomized by the presence of recurrence | Case-control | Case($^{\text{O'}}/{\text{O'+E'}}$) (11/11, 2/3)$^{[2]}$ | 14 | RT-PCR           |
| Liu et al | 1999 | Dichotomized by the presence of recurrence | Case-control | Case($^{\text{O'}}/{\text{O'+E'}}$) (7/19, 2/53) | 72 | RT-PCR           |

1The patients who received operation only were enrolled in the study. 2Prospective 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.

| 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 |
| Okada      | 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 | 4.58 | 0.000 |

**Table 1** Main characteristics of selected publications

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

Another source of bias is that the authors may report in negative ones tend to be published in native languages, while the studies that are more often published in English while the negative ones tend to be published in native languages. This selection may favor the positive 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. 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.

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

| Author | RRi | Yi | Wi | Ywi | Wi' | Ywi' |
|--------|-----|----|----|------|-----|------|
| 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   | χ²  | F   |
|---------|-----|------|-----|-----|-----|-----|-----|
| I-Ichi  | 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 |
| Okuda   | 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 | 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.
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\textsuperscript{[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\textsuperscript{[10]}. 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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