The association of serum markers of fibrosis and development of liver cirrhosis in chronic hepatitis B patients: A systematic review and meta-analysis

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The association of serum markers of fibrosis and development of liver cirrhosis in chronic hepatitis B patients: A systematic review and meta-analysis

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Abstract: Liver fibrosis is reversible by immune treatments in the early stages of cirrhosis development, but not in later stages. At present, the value of the markers for indicating an increased risk of cirrhosis is controversial, and thus we executed this meta-analysis. Retrospective and longitudinal studies of chronic hepatitis B patients were retrieved from PubMed, Elsevier, Springer, Wiley, OVID and EBSCO. Mean differences with 95% confidence intervals of four fibrosis markers were calculated using Review Manager 5.1. Twenty-one studies that included 732 cases and 1,025 controls were analyzed. The pooled mean differences of the serum markers with 95% confidence interval were hyaluronic acid 229.95 (173.94–285.96) ng/mL, laminin 78.73 (45.86–111.60) ng/mL, type III precollagen 69.10 (49.55–88.65) ng/mL and type IV collagen 97.12 (62.32–131.92) ng/mL. Elevated serum hyaluronic acid, laminin, type III precollagen and type IV collagen increased the risk of liver cirrhosis development in chronic hepatitis B patients.

Subjects: Population Health; Epidemiology; Hematology; Infectious Diseases

Keywords: Hepatitis B; liver cirrhosis; fibrosis; risk factor; meta-analysis

1. Introduction
Hepatitis B virus (HBV) infection is a public health challenge. About 360 million people are chronically infected with HBV worldwide, including up to 30 million living in China (Jury, 2003; Liang, Chen, & Wang et al., 2005). The long-term consequences of HBV infection include the evolution of liver fibrosis (LF) and liver cirrhosis (LC). The 1-year cumulative incidence of LC in chronic HBV infection is estimated to be 2.1–6% (Chu & Liaw, 2006). LC is a serious condition. Five-year survival with compensated LC is 80–86%, but it is only 14–30% with decompensated LC. The 1-year cumulative incidence of decompensation has been estimated as 10% (de Jongh et al., 1992; Fattovich et al., 2002; Schuppan & Afedhol, 2008). The previous research has revealed that fibrosis is reversible in the early stages of cirrhosis development, but not in later stages (Friedman & Bansal, 2006).

In general, fibrosis can be improved by cytokine regulating and controlling immune responses, and this improvement can be performed by changing the serum cytokine levels, for instance, TGF-β, IL-6 and IL-17 (Orsatti, Hytiorglou, Thung, Ishak, & Paronetto, 1997; Wang & Chang, 2011). At present, treatments are available for changing serum cytokine levels. Moreover, Cai’s study also showed that, in CHB patients, changed of serum levels for TGF-β, IL-6, IL-10 and IL-17 could change degree of LF and risk of LC development (Cai, Wang, & Yao et al., 2018).
In a general way, serum hyaluronic acid (HA), laminin (LN), type III procollagen (PC III) and type IV collagen (C IV) are taken for revaluating the value of serum fibrotic markers in clinical practice and reflecting the presence and extent of fibrosis (Zhang, 2013; Zhang, Wu, & Du et al., 2013). As these four serological indicators can be assayed at local hospitals, studies that evaluated their use were evaluated for inclusion in this meta-analysis. Many previous studies have evaluated the effectiveness of these indicators as markers of the development of LF and LC, for instance, the studies for HA, LN, PC III and C IV (Chen, Xiao, Chen, & Tu, 2009; Chen, Ye, Yu, Huang, & Zuo, 2007; He, Zhu, & Chen et al., 2007; Huang, Xie, Yang, & Huang, 2015; Jiang, Zhang, Hu, & Wu, 2010; Li, He, & Li, 2007; Liang, Lou, & Hou, 2013; Liu, 2015; Liu, Li, & Xie, 2014; Wei, 2009; Zhang, Yang, Sheng, & Lai, 2010; Zhu, 2011), the studies for HA, PC III and C IV (Du, Han, Xu, Qin, & Chen, 2010; Kang, Shang, Li, & Yang, 2006; Lin, Gao, Huang, & Zhang, 2006; Yang, Hai, Zhang, & Wang, 2010), the studies for HA, LN and C IV (Dong, Shen, & Zhang, 2007; Zhou et al., 2006), and the studies for HA, LN and PC III (Cao, Wen, & Ye, 2008; Hong, Huang, & Ruan, 2010; Lu, Zhang, & Yang, 2015). However, conclusions of their usefulness in identifying patients at increased risk of LC are not consistent. As the influence of random errors can be reduced by conducting a meta-analysis, mean difference (MD) in the serum values and the 95% confidence interval (CI) for these four indicators were calculated to determine if they are effective as markers of the development of LC in patients with chronic HBV infection.

2. Materials and methods

2.1. Search strategy

Articles were retrieved from the Chinese Medical Journal Database, PubMed, Elsevier, Springer, Wiley, OVID and EBSCO by searching the titles and abstracts of articles published between 2006 and 2016 for the key words “fibrosis”, “LC” and “hepatitis B”. The analysis was conducted following the PRISMA meta-analysis guidelines (Key documents, 2017).

2.2. Data extraction

Two independent reviewers assessed the retrieved articles using a standard data extraction form designed by the study investigators, being total number of cases, mean and standard deviation in case group, and total number, mean and standard deviation in control group. The unit of measurement for the studied variables is "ng/mL" or can be converted to "ng/mL". The normal critical value of four serum fibrosis markers for healthy people are HA<84 ng/mL, LN<133 ng/mL, PC III<120 ng/mL and C IV<84 ng/mL.

Discrepancies in reviewer decisions on which articles should be included were resolved by discussion. The standards for final evaluation should be following inclusion and exclusion criteria: retrospective or longitudinal studies of consecutive patient series published in English or Chinese were eligible for inclusion. Studies were excluded if they reported outcomes of patients with hepatitis viruses other than HBV as the etiological agent, or did not report usable values of serum markers of fibrosis. Duplicate published reports of the same study were excluded.

2.3. Sensitivity analysis

We omitted the studies with wide interval of 95%CI for MD values in subgroup analysis, and pooled and gained the pooled MDCI with 95%CI for LN, PC III, C IV and HA, and compared the pooled MDCI with the pooled MD, respectively. And then, we omitted the studies with maximum value of weight in subgroup analysis, and pooled and gained the pooled MDweight with 95%CI for LN, PC III, C IV and HA, and compared the pooled MDweight with the pooled MD, respectively.

2.4. Statistical analysis

MD and 95% CI were the primary outcomes of efficacy. Fixed- or random-effect models were used to perform the meta-analysis. Q and I (Liang et al., 2005) statistics were used to assess study heterogeneity. If P was > 0.1, then a fixed effects model was used, and if it was ≤ 0.1 a random-effects model was used. Statistical analysis was performed using Review Manager 5.0 software.
(Cochrane Collaboration, http://www.cc-ims.net/RevMan/relnotes.htm). The MDs were not pooled if there were fewer than five evaluable differences in the values of a serum fibrosis marker.

3. Results

3.1. Literature search
A flow chart of the study selection process is shown in Figure 1. A total of 21 study reports were included in the meta-analysis.

3.2. Study characteristics
The 21 studies included 732 cases and 1,025 controls. The MD and their 95% CI for serum fibrosis markers are shown in Figures 2 and 3. The study characteristics, region, study type, numbers of case and control participants, serum fibrosis markers, sample size, male/female ratio and mean participant age (years) are shown in Table 1.

3.3. Effectiveness of serum fibrosis markers for predicting the development of LC
The four serum fibrosis markers that were evaluated included HA (21 studies, 1,757 research objects), LN (18 studies, 1,489 research objects), PC III (18 studies, 1,499 research objects) and C IV (17 studies, 1,397 research objects). The pooled MDs and 95% CI were HA 229.95 (173.94–285.96) ng/mL, LN 78.73 (45.86–111.60) ng/mL, PC III 69.10 (49.55–88.65) ng/mL, and C IV 97.12 (62.32–131.92) ng/mL.
The study heterogeneity evaluation found significant variation of the study-specific MDs for all four serum markers (all \( p < 0.10 \)). Consequently, the MDs of the four markers were pooled with random-effect models. The pooled MD results for serum HA and LN and the development of LC in HBV patients are shown in Figure 2, and the results for serum PC III and C IV are shown in Figure 3.

### 3.4. Publication bias

A funnel plot of the articles included in the meta-analysis (Figure 4) is symmetrical, with the axis of symmetry (MD = 0) to the right of center.

### 3.5. Sensitivity analysis

Considering the reliability of pooled MD for LN, PC III, C IV and HA, we omitted the studies with wide interval of 95%CI for MD values, the pooled MD\(_{C1}\) with 95%CI was as follows: the pooled MD\(_{C1}\) with 95%CI was 69.67 [37.38, 101.96] for LN after omitting Liu’s studies (Liu, 2015), and the pooled MD\(_{C1}\) with 95%CI was 64.84 [45.16, 84.52] for PC III after omitted Zhang’s studies (Zhang et al., 2010), and the pooled MD\(_{C1}\) with 95%CI was 97.20 [62.11, 132.30] for C IV after omitted Jiang’s studies (Jiang et al., 2010), and the pooled MD\(_{C1}\) with 95%CI was 228.69 [172.38, 285.00] for HA after omitted Jiang’s studies (Jiang et al., 2010). These pooled MD\(_{C1}\) with 95%CI were very close to respective pooled MD with 95%CI. The subgroup

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**Figure 2. The pooled MD results for serum HA and LN.**

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characteristics of serum markers of fibrosis associated with LC in CHB patients after omitting the studies with wide interval of 95%CI for MD values in subgroup analysis were shown in Table 2.

We omitted the studies with maximum value of weight in subgroup analysis, the pooled MD weight with 95%CI was 78.57 [52.24, 104.89] for LN after omitted four studies (Chen et al., 2009; He et al., 2007; Huang et al., 2015; Kang et al., 2006), and the pooled MD weight with 95%CI was 86.62 [43.56, 129.67] for PC III after omitted three studies (Chen et al., 2009, 2007; Du et al., 2010), and the pooled MD weight with 95%CI was 99.37 [73.52, 125.22] for C IV after omitted two studies (Chen et al., 2009; Du et al., 2010), and the pooled MD weight with 95%CI was 272.02 [166.59, 377.45] for HA after omitted seven studies (Chen et al., 2007; Dong et al., 2007; Hong et al., 2010; Huang et al., 2015; Jiang et al., 2010; Liang et al., 2013; Liu et al., 2014). These pooled MD weight with 95%CI were close to respective pooled MD with 95%CI. The subgroup characteristics of serum markers of fibrosis associated with LC in CHB patients after omitting the studies with maximum value of weight in subgroup analysis are shown in Table 2.

4. Discussion
The meta-analysis found that elevated serum HA, LN, PC III and C IV indicated an increased risk of developing LC. The result is consistent with the findings of a previous study by Liu et al. (Liu, 2015), who reported increases group by group for serum HA, LN, PC III and C IV in healthy controls group, mild hepatitis group, moderate hepatitis group, and LC groups. All four markers were significantly higher in LC than in the other groups (Liu, 2015). Therefore, in patients with chronic HBV infection,
| Number of reference | Study region         | Study type          | Participants category(case/control) | Serum fibrosis markers ‡ | Sample size (n) | Male/female | Age (years)                  |
|---------------------|----------------------|---------------------|-------------------------------------|--------------------------|----------------|-------------|-----------------------------|
| 13                  | Gansu, lanzhou       | Longitudinal study  | LC with chronic HBV infection/CHB   | HA, LN, PC III, C IV    | 25, 28         | 36/17       | 42 ± 12,                   |
| 14                  | Zhejiang, Hangzhou   | Retrospective study | LC with chronic HBV infection/CHB   | HA, LN, PC III, C IV    | 43, 29         | 33/10, 23/6 | 48.0 ± 6.9, 44.5 ± 7.8     |
| 15                  | Xinjiang, Wulumuqi    | Longitudinal study  | LC with chronic HBV infection/CHB   | HA, LN, PC III, C IV    | 90, 60         | 63/27, 38/22| 47.3 ± 9.2, 30.9 ± 9.5     |
| 16                  | Zhejiang, Linhai      | Longitudinal study  | LC with chronic HBV infection/CHB   | HA, LN, PC III, C IV    | 56, 65         | 50/6, 50/15 | 30-77, 18-68               |
| 17                  | Jiangxi, Nanchang     | Longitudinal study  | LC with chronic HBV infection/CHB   | HA, LN, PC III, C IV    | 61, 115        | 53/8, 102/13| 17-62                      |
| 18                  | Shandong, Weifang     | Longitudinal study  | LC with chronic HBV infection/CHB   | HA, LN, PC III, C IV    | 134, 143       | 187/90      | 48.21 ± 15.23              |
| 19                  | Guangdong, Longchuan  | Longitudinal study  | LC with chronic HBV infection/CHB   | HA, LN, PC III, C IV    | 17, 48         | 38/27       | 48.3 ± 6.8                 |
| 20                  | China, Shanghai       | Longitudinal study  | LC with chronic HBV infection/CHB   | HA, LN, PC III, C IV    | 30, 40         | 18/12, 23/17| 55 ± 15.12, 34 ± 12.22     |
| 21                  | China, Guangzhou      | Longitudinal study  | LC with chronic HBV infection/CHB   | HA, LN, PC III, C IV    | 42, 112        | 106/48      | 21-68                      |
| 22                  | Fujian, Nanping       | Longitudinal study  | LC with chronic HBV infection/CHB   | HA, LN, PC III, C IV    | 17, 54         | 51/20       | 45 ± 12                    |
| 23                  | Hubei, Xianjing       | Longitudinal study  | LC with chronic HBV infection/CHB   | HA, LN, PC III, C IV    | 38, 144        | 102/80      | 26.4 ± 7.2                 |
| 24                  | Ningxia, Yinchuan     | Longitudinal study  | LC with chronic HBV infection/CHB   | HA, LN, PC III, C IV    | 24, 49         | 55/18       | 36.96 ± 12.28              |
| 25                  | Fujian, Fuzhou        | Longitudinal study  | LC with chronic HBV infection/CHB   | HA, PC III, C IV        | 19, 35         | 57/17       | 45.1 ± 11.2                |
| 26                  | Jilin, Changchun      | Longitudinal study  | LC with chronic HBV infection/CHB   | HA, PC III, C IV        | 25, 93         | 22/3, 78/15| 51 ± 10, 39 ± 11           |

(Continued)
| Number of reference | Study region         | Study type        | Participants category(case/control) | Serum fibrosis markers ‡ | Sample size (n) | Male/female | Age (years)  |
|--------------------|----------------------|-------------------|-------------------------------------|--------------------------|----------------|-------------|-------------|
| 27                 | Shandong, Jinan      | Longitudinal study | LC with chronic HBV infection/CHB   | HA, PC III, C IV         | 20, 55         | 14/6, 41/14 | 45.8 ± 15.0, 39.8 ± 13.4 |
| 28                 | Guangdong, Yangjiang | Longitudinal study | LC with chronic HBV infection/CHB   | HA, PC III, C IV         | 31, 91         | 83/39       | 15-61       |
| 29                 | Zhejiang, Huzhou     | Longitudinal study | LC with chronic HBV infection/CHB   | HA, LN, C IV             | 41, 75         | 66/50       | 39.8 ± 12.5 |
| 30                 | China, Beijing       | Longitudinal study | LC with chronic HBV infection/CHB   | HA, LN, C IV             | 49, 108        | 37/12, 79/29| 42.1 ± 16.2, 41.7 ± 18.5 |
| 31                 | Hubei, Shiyan        | Longitudinal study | LC with chronic HBV infection/CHB   | HA, LN, PC III           | 42, 63         | 27/15, 33/30| 39 ± 3, 36 ± 5 |
| 32                 | Henan, Zhengzhou     | Longitudinal study | LC with chronic HBV infection/CHB   | HA, LN, PC III           | 38, 71         | 61/48       | 18-69       |
| 33                 | Guangdong, Huizhou   | Longitudinal study | LC with chronic HBV infection/CHB   | HA, LN, PC III           | 50,100         | 37/13, 70/30| 43.1 ± 17.2, 42.7 ± 19.5 |
once serum value of any marker of HA, LN, PC III and C IV are higher than cut off value of normal critical value of corresponding marker in healthy people, being HA>84 ng/mL, LN>133 ng/mL, PC III>120 ng/mL and C IV>84 ng/mL, these high-risk patients should be closely monitored to prevent the development of LC.

Fibrosis and inflammation of liver tissue are both involved in the pathogenesis of LC, and the findings of this meta-analysis are consistent with Du et al. (2010), who found that the serum levels of HA, LN, PC III and C IV were positively correlated with both fibrosis stage and liver inflammation grade. The demonstration by Zhou et al. that showed serum HA, LN, PC III and C IV were positively correlated with hepatic histopathological stage (Zhou, Huang, & Huang, 2013), adds to the evidence that elevations of serum levels might accompany disease progression, and that early treatment is indicated for chronic HBV infection with elevated serum HA, LN, PC III and C IV.

This meta-analysis also found by Sensitivity Analysis that, the studies with wide interval of 95% CI for MD values and the studies with maximum value of weight in subgroup analysis were omitted, the numerical size of pooled MD for LN, PC III, C IV and HA were similar to the numerical
size of pooled MD before omitted in subgroup analysis respectively, and these showed that the pooled MD for LN, PC III, C IV and HA were reliable and stable.

The study limitations included the eligibility of only articles published in English or Chinese, and the heterogeneity of the included studies. Even though the MDs of the four markers were pooled using a random-effects method, study heterogeneity may have influenced the findings.

5. Conclusions
Elevated serum HA, LN, PC III and C IV in patients with chronic HBV infection increased the risk of developing LC. High-risk patients should be closely monitored and receive early treatment to prevent the development of LC.

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Authors' contributions
Study design: C.H., H.L., B.Z., Y.C., L.L. Statistical analysis and interpretation: H.L., K.L., Y.C. manuscript preparation: H.L., C.Z., Zhe W., Zhi W., J.Y., G.C., L.L. Critical review of manuscript: H.L., B.Z., C.H., Y.C. All authors read and approved the final manuscript.

List of abbreviations
- HA: hyaluronic acid
- LC: liver cirrhosis
- LN: laminin
- LF: liver fibrosis
- PC III: type III procollagen
- C IV: type IV collagen
- CI: confidence intervals
- CHB: chronic hepatitis B
- HBV: hepatitis B virus
- MD: mean difference
- CMJD: Chinese Medical Journal Database

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Clinical Focus

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