Review

Seyyed Mortaza Haghgoo, Heidar Sharafi and Seyed Moayed Alavian*

# Serum cytokines, adipokines and ferritin for non-invasive assessment of liver fibrosis in chronic liver disease: a systematic review

https://doi.org/10.1515/cclm-2018-0357
Received April 8, 2018; accepted July 27, 2018; previously published online September 19, 2018

**Abstract:** Chronic liver disease (CLD) is a major health problem worldwide. Non-alcoholic fatty liver disease (NAFLD), chronic hepatitis C (CHC), chronic hepatitis B (CHB), and alcoholic liver disease (ALD) are the most common etiologies of CLD. Liver biopsy is the gold standard for assessment of liver fibrosis, however, it is an invasive method. This review attempts to evaluate the usefulness of serum adiponectin, serum leptin, serum ferritin, serum transforming growth factor-β1 (TGF-β1), and serum platelet derived growth factor-BB (PDGF-BB) as non-invasive markers in the diagnosis of liver fibrosis/cirrhosis. A systematic search in MEDLINE, Web of Science, Scopus, and local databases was performed to identify articles published in English or Persian as of November 2017. Studies conducted among CLD patients, with biopsy proven fibrosis/cirrhosis, and providing sufficient details of patients' clinicopathological characteristics were included. In the 95 studies included, there were a total of 15,548 CLD patients. More than 83% of studies were carried out in Asia and Europe. The relationship between liver fibrosis/cirrhosis and serum levels of ferritin, adiponectin, leptin, TGF-β1, and PDGF-BB was assessed in 42, 33, 27, nine, and three studies, respectively. Serum levels of the markers, particularly ferritin, could successfully predict liver fibrosis/cirrhosis, however, these data might not be clinically replicated and further studies are needed.

**Keywords:** adipokines; chronic liver disease; cirrhosis; cytokines; fibrosis; serum biomarker.

***Corresponding author: Seyed Moayed Alavian,** MD, Baqiyatallah Research Center for Gastroenterology and Liver Diseases, Baqiyatallah University of Medical Sciences, Tehran, Iran; and Middle East Liver Diseases (MELD) Center, Tehran, Iran, Phone: +98-21-88945186, Fax: +98-21-88945188, E-mail: Alavian@thc.ir
**Seyyed Mortaza Haghgoo:** Baqiyatallah Research Center for Gastroenterology and Liver Diseases, Baqiyatallah University of Medical Sciences, Tehran, Iran; and Department of Clinical Biochemistry, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
**Heidar Sharafi:** Baqiyatallah Research Center for Gastroenterology and Liver Diseases, Baqiyatallah University of Medical Sciences, Tehran, Iran; and Middle East Liver Diseases (MELD) Center, Tehran, Iran

## Background

Chronic liver disease (CLD) refers to disease of the liver which involves a process of progressive destruction and regeneration of the liver parenchyma, leading to fibrosis, cirrhosis, and hepatic insufficiency. Regardless of geographical differences, CLDs have a high prevalence worldwide [1, 2]. The most prevalent etiologies of CLD are hepatitis B virus (HBV) infection, hepatitis C virus (HCV) infection, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), and alcoholic liver disease (ALD). All these diseases can cause stages of liver fibrosis or cirrhosis [2].

NAFLD have become a serious health concern due to the rising prevalence of obesity and type 2 diabetes mellitus [3]. Currently, NAFLD and NASH are believed to be the most common cause of CLD and liver fibrosis [4]. According to epidemiological studies, it is estimated that 3%–34% of the general population have NAFLD and 2%–5% have NASH [5, 6]. It is approximated that annually three to four million new cases of HCV infection are diagnosed. Around 71 million people are chronically infected with HCV worldwide and 399,000 deaths are reported due to all HCV related causes, mostly from cirrhosis and hepatocellular carcinoma (HCC) each year [7]. HBV infection is a major health problem and it is estimated that two billion are infected throughout the world. There are nearly more than 257 million people suffering from chronic HBV infection, which resulted in 887,000 deaths in 2015, mostly from complications such as cirrhosis and HCC [7]. Alcohol consumption has been identified as a major risk factor for all liver diseases and is significantly associated

with cirrhosis. Around 47.9% of cirrhosis deaths (46.5% of women and 48.5% of men) are related to ALD [8].

Chronic damage to the hepatocytes and activation of the hepatic stellate cells (HSCs), also known as perisinusoidal cells, Ito cells, lipocytes and fat-storing cells, is believed to be the beginning of the insidious process of the liver fibrosis. Liver fibrosis is the result of repeated injury and wound-healing response of the liver, and the consequent accumulation of extracellular matrix (ECM) proteins. Most related complications occur when cirrhosis develops [2, 9]. The necessity of long-term monitoring of CLD, and also the requirement of liver biopsy as the gold standard for the diagnosis and assessment of the necroinflammatory grade and stage of fibrosis has led to some restrictions for clinicians and researchers. The invasive nature of biopsy, including possible complications such as pain and hemorrhage, technical errors, resampling limitations, the presence of some clinical conditions such as ascites and coagulation disorders, and variations between decisions made by different pathologists could justify that non-invasive, reliable, and simple sampling methods are needed for the assessment of liver fibrosis [10, 11].

Recently, several non-invasive tests have been made available for the assessment of liver fibrosis, such as aspartate transaminase to platelet ratio index, enhanced liver fibrosis, FIB-4, FibroTest, Forns index, FibroScan, and a significant hepatic fibrosis index [12–14]. Serum markers could be useful in directly and indirectly evaluating liver function and liver fibrosis [15]. A number of the serum markers which are directly associated with liver fibrogenesis and ECM degeneration, including hyaluronic acid, tissue inhibitor of metalloproteinase type 1 (TIMP-1), YKL-40, collagen subtypes, cytokines, and chemokines, are under investigation, and some have been previously tested [12, 16]. It is assumed that cytokines and adipokines play central roles in the progression from chronic liver injury to fibrosis/cirrhosis. Transforming growth factor-β1 (TGF-β1), platelet derived growth factor-BB (PDGF-BB), leptin, adiponectin and ferritin are multifunctional factors and are involved in inflammation, immune system modulation, and wound healing processes. Therefore, these molecules could affect the development and progression of liver fibrosis [17, 18]. Accordingly, we are going to focus on the five markers which may be directly or indirectly linked to the presence or extent of liver fibrosis or cirrhosis. A great number of studies have investigated the possible association of serum levels of these markers with liver fibrosis or cirrhosis, however, a systematic review or meta-analysis is needed to clarify the clinical importance of the markers. This systematic review provides an overview of the original studies carried out in the field. Moreover, this review discusses the findings and different aspects of the studies in order to better understand the clinical usefulness of the markers and present updated information to the field.

# Methods

## Literature search

Initially 13,649 records were obtained from comprehensive databases including Web of Sciences, Scopus, MEDLINE (by PubMed), and Science Direct, in addition to local databases including Scientific information database, Elmnet, Magiran, Irandoc, Barakat Knowledge Network System (IranMedex), and IslamMedex using various combination of search keywords including “liver fibrosis”, “hepatic fibrosis”, cirrhosis, leptin, adiponectin, ferritin, “platelet derived growth factor”, “transforming growth factor”, PDGF, TGF, and serum. The search was not limited by publication time and it ended at November 2017. The search was limited to English and Persian publications. The Medical subject headings database was used as the terminological search filter. The following query was formatted and used in different databases by their requirements:

(“liver fibrosis” OR “hepatic fibrosis” OR cirrhosis) AND (leptin OR adiponectin OR ferritin OR “platelet derived growth factor” OR “transforming growth factor” OR TGF OR PDGF) AND serum

The search was supplemented by manual searching of other sources including conference proceedings, reference lists, researchers in field, and related citations.

## Inclusion and exclusion criteria

All the observational clinical studies which compared serum levels of leptin, ferritin, adiponectin, PDGF-BB, and TGF-β1 by different stages of fibrosis in patients with chronic hepatitis B (CHB), chronic hepatitis C (CHC), NAFLD/NASH, and ALD were eligible for this systematic review. Studies which aimed at diagnosing fibrosis/cirrhosis in CLD using the markers were eligible as well.

In detail, studies were included in the systematic review if: (1) they were original full-text publications or abstracts containing sufficient data; (2) they included patients with biopsy-proven fibrosis/cirrhosis; (3) they made comparison between different stages of fibrosis for serum levels of leptin, adiponectin, ferritin, PDGF-BB, and

TGF-β1 or they aimed at diagnosing fibrosis/cirrhosis in CLD using these markers.

Studies were excluded from the systematic review if: (1) they included patients who had co-infection with human immunodeficiency virus (HIV), HBV, and HCV; (2) they included patients with CLDs other than NAFLD/NASH, ALD, CHC, and CHB (e.g. autoimmune hepatitis); (3) study sample size was less than 30; (4) they measured candidate markers in specimens other than serum; (5) they did not provide sufficient details about patients demographics (e.g. gender, age, etc.); (6) they included patients with unspecified cause of fibrosis/cirrhosis; (7) they were reviews, letter to editors, case reports, hypothesis, book chapter/section, editorials, animal model/cell line studies; (8) they included patients under therapeutic intervention; (9) they included patients with underlying diseases (e.g. cardiovascular diseases); (10) they included post-transplant patients.

# Results and discussion

## Literature search

Initially 13,649 records were obtained from comprehensive and local databases using various combinations of the above mentioned terms and keywords. Moreover, 158 citations were added from other aforementioned sources. To summarize the identification, screening, eligibility and final selection procedure for included articles in the systematic review, a flowchart was drawn according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines [19]. Two hundred and twelve articles were initially selected and finally, 95 articles were included in the systematic review (Figure 1).

## Characteristics of included studies

The 95 included studies were published between 1996 and 2016. Cross-sectional studies (n = 53), case-control studies (n = 34), cohort studies (n = 5), and therapeutic interventions (n = 3) were the most frequent study designs, respectively. Of the 15,548 CLD patients who were enrolled in the studies, 9,103 were male and 6,445 were female. The main characteristics of the included studies, the main demographics, clinical, and biochemical features of the patients and also the results of the statistical analyses were extracted and double checked by an investigator and then summarized in Table 1. Thirty-seven studies were conducted in Asia, 41 in Europe, 12 in America, and five in Australia. Forty-three studies were carried out among patients with CHC, 34 among patients with NAFLD/NASH, nine among patients with CHB, two among patients with ALD, and seven among groups of patients with CLD of different etiologies. Metavir, Brunt, Ishak, Kleiner, Scheuer, and Knodell were the most popular fibrosis scoring systems, which were used in 19, 19, 14, 14, 11, and eight studies, respectively. Serum levels of leptin, adiponectin, PDGF-BB, TGF-β1 and ferritin were measured using enzyme linked-immunosorbent assays (ELISAs) in 49 studies and radioimmunoassays (RIAs) in nine studies. Thirty-seven studies used spectrophotometry, chromatography, colorimetric and other commercially available methods for measurement of the markers. Details of measurement methods were not mentioned in some studies.

## Transforming growth factor-β and non-invasive assessment of liver fibrosis

TGF-β is a multifunctional growth factor and plays important roles in cell growth and differentiation, immune regulation and matrix synthesis [114]. Three different isoforms of TGF-β including TGF-β1, TGF-β2, and TGF-β3 have been identified in mammalians' tissue. TGF-β1 is found to be the predominant isoform of TGF-β. All TGF-β isoforms are synthesized and secreted in a latent form and must be activated to exert their biological effects. This latent inactive TGF-β complex consists of a TGF-β homodimer, a latency associated peptide, and a latent TGF-β-binding protein (LTBP). Processing from the latent form to the biologically active form could be induced by changes in ionic strength, changes in pH, or proteolytic enzymes. Only the biologically active form of TGF-β1 is immunoreactive and could be detected by immunoassay [115, 116].

TGF-β blocks matrix degradation by decreasing and increasing of the synthesis of proteases and the levels of protease inhibitors, respectively [117]. Additionally, TGF-β induces deposition of extracellular matrix in the site of injury and it can cause scarring and fibrosis [118]. Induction of apoptosis mediated by TGF-β in hepatocytes was found to be connected with liver cirrhosis [119]. TGF-β1 mediates HSCs activation and transformation in conjunction with other growth factors such as PDGF-BB [23].

In this systematic review, nine studies which investigated the relationship between TGF-β1 and liver fibrosis/cirrhosis were eligible according to the inclusion criteria. Three studies by Kirmaz et al., Nelson et al., and Neuman et al. in patients with CHC indicated that there is positive correlation between fibrosis stages and the serum

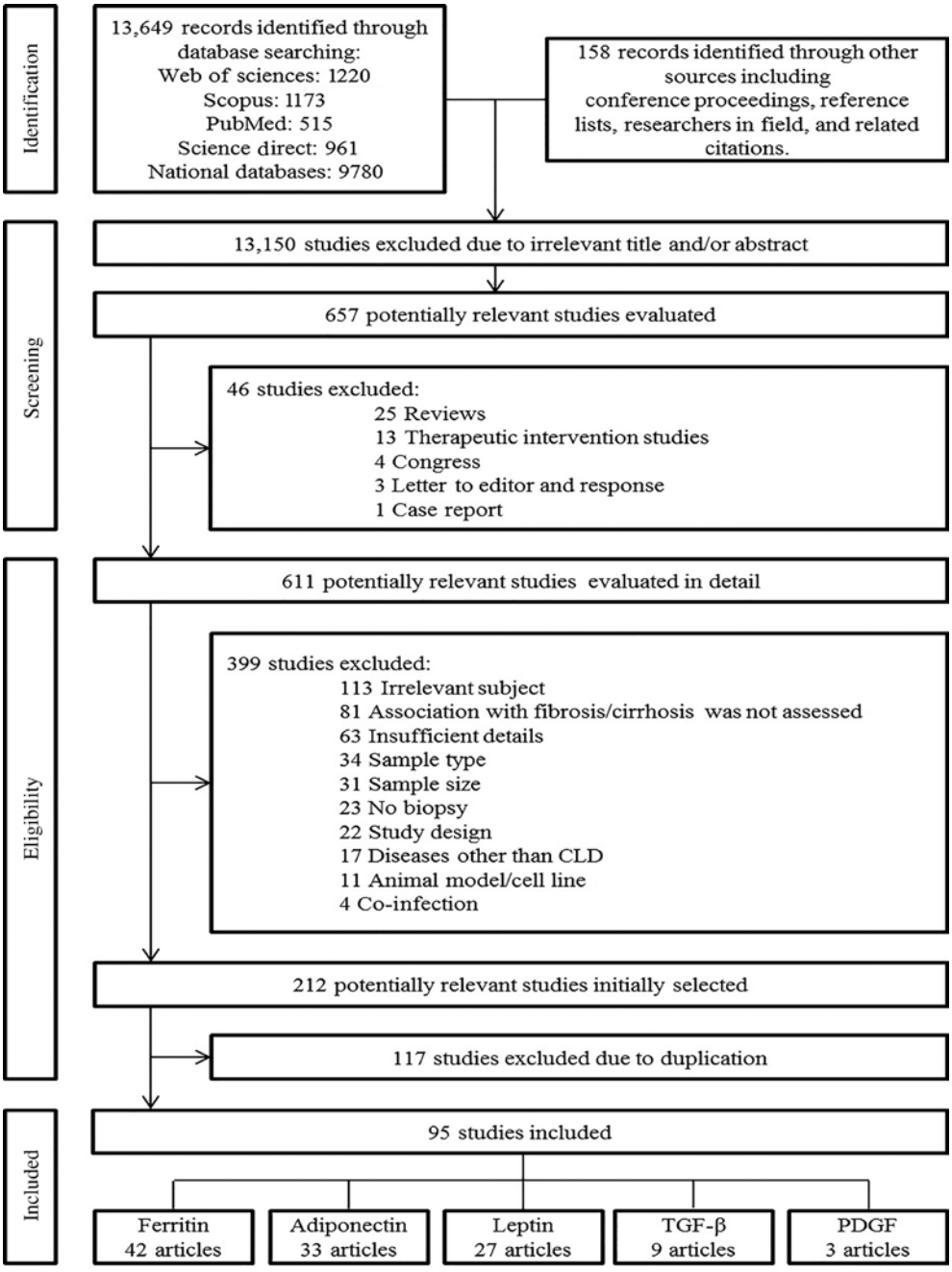


**Figure 1:** Flow diagram of article selection.
CLD, chronic liver disease; TGF-β1, transforming growth factor-β1; PDGF-BB, platelet derived growth factor-BB.

levels of total TGF-β1. However, in the case of biologically active TGF-β1 such a correlation was not observed. The study by Nelson et al. evaluated serum TGF-β1 within 12 months from liver biopsy, while the study by Neuman et al. provided sequential data on liver biopsy that were used to ascertain the serum TGF-β1 predictive value for liver fibrosis progression [20–22]. In another investigation among patients with NASH or CHC such correlation was not observed [25]. Further, in two studies serum TGF-β1 was reported as a predictor of progressive liver fibrosis [22, 26]. On the other hand, the findings of three studies conducted among patients with CHB showed that there is no correlation between the serum TGF-β1 and stage of fibrosis. No significant difference was observed when the mean concentrations of total TGF-β1 was compared by stages of fibrosis [20, 23, 24]. Palekar et al. found no significant difference of mean serum levels of TGF-β1 between simple steatosis and NASH (at different stage of

**Table 1:** Main characteristics and results of statistical analyses of included studies in the systematic review.

| Markers | Ref no. | Characteristics of included studies | | | | | | | | Results of statistical analyses | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Author | Year | Study location | Study design | Etiology | Method of measurement | Sample size | Patient gender | Fibrosis staging | Univariate analysis[a] | Multivariate analysis[b] | Correlation analysis[c] | ROC curve analysis[d] |
| TGF-β1 | [20] | Kirmaz et al. | 2004 | Turkey | Case control | CHC[e] CHB[f] | ELISA[g] | 49 | M[h]: 29 F[i]: 20 | Knodell | No significant difference between cirrhotic and CHC | – | CHC: Significant positive correlation with stages of fibrosis/cirrhosis, CHB: No significant correlation with stages of fibrosis/cirrhosis | – |
| | [21] | Nelson et al. | 1997 | USA | Case control | CHC | ELISA | 88 | M: 47 F: 41 | Knodell | – | – | Total TGF-β1[j]: Significant positive correlation with stages of fibrosis/cirrhosis, Biologically active TGF-β1: No significant correlation with stages of fibrosis/cirrhosis | – |
| | [22] | Neuman et al. | 2002 | France | Case control | CHC | ELISA | 159 | M: 107 F: 52 | Metavir Knodell | Significant difference between stages of fibrosis | – | Significant positive correlation with stages of fibrosis/cirrhosis | – |
| | [23] | Zhang et al. | 2003 | China | Case control | CHB | ELISA | 60 | M: 54 F: 6 | S1-S4 2000 | – | – | No significant correlation with stages of fibrosis/cirrhosis | – |
| | [24] | Ucar et al. | 2013 | Turkey | Cross-sectional | CHB | ELISA | 73 | M: 47 F: 26 | Metavir | Stage <2 Vs ≥2: No significant fibrosis | – | – | Could not predict advanced fibrosis (AUC[k] = 0.44) |
| | [25] | Tarantino et al. | 2008 | Italy | Cross-sectional | NAFLD[l] CHC | ELISA | 123 | M: 66 F: 57 | Ishak | – | – | No significant correlation with stages of fibrosis/cirrhosis | – |

**Table 1** (continued)

| Markers | Ref no. | Characteristics of included studies | | | | | | | | Results of statistical analyses | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Author | Year | Study location | Study design | Etiology | Method of measurement | Sample size | Patient gender | Fibrosis staging | Univariate analysis[a] | Multivariate analysis[b] | Correlation analysis[c] | ROC curve analysis[d] |
| | [26] | Kanzler et al. | 2001 | Germany | Case control | CHC | ELISA | 39 | M: 20<br>F: 19 | Knodell Chevallier | Significant association between high serum TGF-β1 and progressive fibrosis | – | – | – |
| | [27] | Palekar et al. | 2006 | USA | Cross-sectional | NAFLD | ELISA | 80 | M: 42<br>F: 38 | Brunt | Simple steatosis vs. NASH[m]: No significant difference, Non-mild vs. moderate-advanced fibrosis: Significant difference | – | – | Significantly predicts advanced fibrosis (AUC = 0.67) |
| | [28] | Zoheiry et al. | 2015 | Egypt | Case control | CHC | ELISA | 86 | M: 60<br>F: 26 | Metavir | – | – | – | Significantly predicts advanced fibrosis (AUC = 0.71), Sen[n] = 83.3%, Spe[o] = 75%, NPV[p] = 60%, PPV[q] = 90.9% |
| Total (TGF-β1) | | 9 Studies | | | | | | 757 | M: 472<br>F: 285 | | | | | |
| PDGF-BB | [18] | Zhou et al. | 2016 | China | Cross-sectional | CHB | ELISA | 465 | M: 363<br>F: 102 | Ishak | Significant difference between stages of fibrosis | Independently predicts moderate-advanced fibrosis | Significant negative correlation with stages of fibrosis/cirrhosis | Significantly predicts advanced fibrosis (AUC = 0.73) |

**Table 1** (continued)

| Markers | Ref no. | Characteristics of included studies | | | | | | | | Results of statistical analyses | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Author | Year | Study location | Study design | Etiology | Method of measurement | Sample size | Patient gender | Fibrosis staging | Univariate analysis[a] | Multivariate analysis[b] | Correlation analysis[c] | ROC curve analysis[d] |
| | [23] | Zhang et al. | 2003 | China | Case control | CHB | ELISA | 60 | M: 54<br>F: 6 | S1-S4<br>2000 | – | – | Significant positive correlation with stages of fibrosis/ cirrhosis | Significantly predicts presence of fibrosis (AUC = 0.98), Cut-off = 40.5 ng/mL[r], Sen = 90%, Spe = 95% |
| | [29] | El-Bassiouni et al. | 2012 | Egypt | Case control | CHC | ELISA | 120 | M: 80<br>F: 40 | Ishak | Significant difference between cirrhotic and CHC | – | Significant negative correlation with stages of fibrosis/ cirrhosis | – |
| Total (PDGF-BB) | | 3 Studies | – | – | – | – | – | 645 | M: 497<br>F: 148 | – | – | – | – | – |
| Leptin | [30] | Chwist et al. | 2014 | Poland | Cross-sectional | NAFLD | ELISA | 70 | M: 40<br>F: 30 | Kleiner | Non-mild vs. moderate-severe fibrosis: No significant difference | – | – | – |
| | [31] | Poorten et al. | 2013 | Belgium | Cross-sectional | NAFLD | ELISA | 119 | M: 63<br>F: 56 | Brunt | Non-mild vs. moderate-severe fibrosis: No significant difference | – | – | – |
| | [32] | Petta et al. | 2012 | Italy | Cross-sectional | NAFLD | ELISA | 142 | M: 95<br>F: 47 | Kleiner | Serum leptin was not associated with severe fibrosis | – | – | – |
| | [33] | Machado et al. | 2012 | Portugal | Cross-sectional | NAFLD | IRMA[v] | 82 | M: 13<br>F: 69 | Kleiner | No significant difference between stages of fibrosis | – | – | – |

**Table 1** (continued)

| Markers | Ref no. | Characteristics of included studies | | | | | | | | Results of statistical analyses | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Author | Year | Study location | Study design | Etiology | Method of measurement | Sample size | Patient gender | Fibrosis staging | Univariate analysis[a] | Multivariate analysis[b] | Correlation analysis[c] | ROC curve analysis[d] |
| | [34] | Koehler et al. | 2011 | USA | Cross-sectional | NAFLD | – | 160 | M: 24<br>F: 136 | Brunt | No significant difference between stages of fibrosis | – | – | – |
| | [35] | Muñoz et al. | 2009 | Mexico | Case control | NAFLD | ELISA | 52 | M: 33<br>F: 19 | Brunt | Non-mild vs. moderate-severe fibrosis: No significant difference | – | – | – |
| | [36] | Yalniz et al. | 2006 | Turkey | Case control | NAFLD | ELISA | 37 | M: 25<br>F: 12 | Brunt | No significant difference between stages of fibrosis | – | – | – |
| | [37] | Lydatakis et al. | 2006 | Greece | Cross-sectional | NAFLD | IRMA | 50 | M: 27<br>F: 23 | Matteoni | Without fibrosis vs. fibrosis: No significant difference<br>No significant difference between stages of fibrosis | – | – | – |
| | [38] | Tsochatzis et al. | 2008 | Greece | Cross-sectional | NAFLD<br>CHC<br>CHB | ELISA | 146 | M: 85<br>F: 61 | Ishak | CVH[s]: Non-mild vs. moderate-severe fibrosis: No significant difference,<br>NASH: Non-mild vs. moderate-severe fibrosis: Significant difference | – | – | – |

**Table 1** (continued)

| Markers | Ref no. | Characteristics of included studies | | | | | | | | Results of statistical analyses | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Author | Year | Study location | Study design | Etiology | Method of measurement | Sample size | Patient gender | Fibrosis staging | Univariate analysis[a] | Multivariate analysis[b] | Correlation analysis[c] | ROC curve analysis[d] |
| | [39] | Chitturi et al. | 2002 | Australia | Case control | NAFLD | Conventional automated analyzer | 47 | M: 27 F: 20 | Brunt | No significant difference between stages of fibrosis | Could not independently predict extent of liver fibrosis | – | – |
| | [40] | Hui et al. | 2004 | Australia | Case control | NAFLD | RIA | 109 | M: 68 F: 41 | Brunt | – | Could not independently predict extent of liver fibrosis | – | – |
| | [41] | Canbakan et al. | 2008 | Turkey | Cross-sectional | NAFLD | RIA | 52 | M: 28 F: 24 | Brunt | – | Could not independently predict extent of liver fibrosis | No significant correlation with stages of fibrosis/ cirrhosis | – |
| | [42] | Lemoine et al. | 2009 | France | Case control | NAFLD | ELISA | 74 | M: 38 F: 36 | Brunt | Non-mild vs. moderate-severe fibrosis: No significant difference | Could not independently predict extent of liver fibrosis | – | – |
| | [43] | Hickman et al. | 2003 | Australia | Case control | CHC | ELISA | 160 | M: 110 F: 50 | Scheuer | No significant difference between stages of fibrosis | – | – | – |
| | [44] | Manola-kopoulos et al. | 2007 | Greece | Case control | CHC CHB | ELISA | 50 | M: 36 F: 14 | Ishak | Cirrhotic vs. non-cirrhotic: Significant difference among both CHC and CHB | – | – | – |
| | [45] | Tiftikci et al. | 2009 | Turkey | Case control | CHC | ELISA | 51 | M: 22 F: 29 | Metavir | No significant difference between stages of fibrosis | – | – | – |
| | [46] | Kukla et al. | 2010 | Poland | Case control | CHC | ELISA | 40 | M: 20 F: 20 | Scheuer | No significant difference between stages of fibrosis | – | – | – |

**Table 1** (continued)

| Markers | Ref no. | Characteristics of included studies | | | | | | | | Results of statistical analyses | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Author | Year | Study location | Study design | Etiology | Method of measurement | Sample size | Patient gender | Fibrosis staging | Univariate analysis[a] | Multivariate analysis[b] | Correlation analysis[c] | ROC curve analysis[d] |
| | [47] | Wong et al. | 2010 | China | Cross-sectional | CHB | Bio-Plex Pro Human Diabetes Panel | 266 | M: 203 F: 63 | Ishak | Cirrhotic vs. non-cirrhotic: No significant difference | – | No significant correlation with stages of fibrosis/cirrhosis | – |
| | [48] | Aşçi et al. | 2012 | Turkey | Case control | CHC CHB | ELISA | 70 | M: 33 F: 37 | Ishak | CHB: Mild-moderate vs. severe fibrosis: No significant difference, CHC: Mild-moderate vs. severe fibrosis: No significant difference | – | – | – |
| | [49] | Mera et al. | 2014 | Japan | Cross-sectional | CHC | ELISA | 77 | M: 31 F: 46 | Metavir Scheuer | Non-mild vs. moderate-severe fibrosis: No significant difference | – | – | – |
| | [50] | Giannini et al. | 2000 | Italy | Case control | CHC | RIA[t] | 48 | M: 32 F: 16 | Knodell | Non-mild vs. moderate-severe fibrosis: No significant difference | – | – | – |
| | [51] | Romero-Gómez et al. | 2003 | Spain | Cross-sectional | CHC | ELISA | 131 | M: 84 F: 47 | Scheuer | – | Could not independently predict presence of liver fibrosis | Significant positive correlation with stages of fibrosis/cirrhosis | – |
| | [52] | Myers et al. | 2007 | France | Cohort | CHC | ELISA | 62 | M: 35 F: 27 | Metavir | – | Could not independently predict extent of liver fibrosis | – | Could not predict advanced fibrosis (AUC = 0.58) |

**Table 1** (continued)

| Markers | Ref no. | Characteristics of included studies | | | | | | | | Results of statistical analyses | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Author | Year | Study location | Study design | Etiology | Method of measurement | Sample size | Patient gender | Fibrosis staging | Univariate analysis[a] | Multivariate analysis[b] | Correlation analysis[c] | ROC curve analysis[d] |
| | [53] | Popa et al. | 2011 | Romania | Cross-sectional | CHC | ELISA | 81 | M: 22 F: 59 | Metavir | – | Could not independently predict extent of liver fibrosis | Significant positive correlation with stages of fibrosis/cirrhosis | Significantly predicts advanced fibrosis (AUC = 0.64), Sen = 76.6%, Spe = 51% |
| | [54] | Ghweil et al. | 2014 | Egypt | Therapeutic intervention | CHC | – | 40 | M: 33 F: 7 | Metavir | – | – | Significant positive correlation with stages of fibrosis/cirrhosis | – |
| | [55] | Gordon et al. | 2005 | Australia | Cross-sectional | CHC CHB | ELISA | 91 | M: 60 F: 31 | Metavir | – | – | No significant correlation with stages of fibrosis/cirrhosis | – |
| | [56] | Nicolas et al. | 2001 | Spain | Case control | ALD[s] | RIA | 100 | M: 100 | – | Compensated liver cirrhosis vs. non-cirrhotic: No significant difference | – | – | – |
| | [57] | Naveau et al. | 2006 | France | Cross-sectional | ALD | RIA | 209 | M: 161 F: 48 | – | Cirrhotic vs. non-cirrhotic: Significant difference | Independently and positively associated with presence of cirrhosis | – | – |
| Total (Leptin) | | 28 Studies | | | | | | 2485 | M: 1464 F: 1021 | | | | | |
| Adiponectin | [47] | Wong et al. | 2010 | China | Cross-sectional | CHB | Bio-Plex Pro Human Diabetes Panel | 266 | M: 203 F: 63 | Ishak | Cirrhotic vs. non-cirrhotic: No significant difference | – | No significant correlation with stages of fibrosis/cirrhosis | – |
| | [58] | Wu et al. | 2013 | China | Case control | CHB | RIA | 89 | M: 75 F: 14 | Scheuer | – | – | No significant correlation with stages of fibrosis/cirrhosis | – |

**Table 1** (continued)

| Markers | Ref no. | Characteristics of included studies | | | | | | | | Results of statistical analyses | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Author | Year | Study location | Study design | Etiology | Method of measurement | Sample size | Patient gender | Fibrosis staging | Univariate analysis[a] | Multivariate analysis[b] | Correlation analysis[c] | ROC curve analysis[d] |
| | [59] | Hui et al. | 2007 | China | Cross-sectional | CHB | ELISA | 100 | M: 67 F: 33 | Ishak | Cirrhotic vs. non-cirrhotic: Significant difference | Independently and positively predicts extent of liver fibrosis | Significant positive correlation with stages of fibrosis/cirrhosis | – |
| | [60] | Liu et al. | 2009 | Taiwan | Case control | CHB | ELISA | 160 | M: 92 F: 68 | – | High serum adiponectin associated with cirrhosis | – | – | – |
| | [61] | Liu et al. | 2005 | Taiwan | Cohort | CHC | ELISA | 95 | M: 61 F: 34 | Ishak | – | – | No significant correlation with stages of fibrosis/cirrhosis | – |
| | [62] | Durante-Mangoni et al. | 2006 | Italy | Cross-sectional | CHC | ELISA | 161 | M: 89 F: 72 | Scheuer | – | – | No significant correlation with stages of fibrosis/cirrhosis | – |
| | [63] | Grigorescu et al. | 2008 | Romania | Cross-sectional | CHC | ELISA | 152 | M: 53 F: 99 | Metavir | – | – | No significant correlation with stages of fibrosis/cirrhosis | – |
| | [64] | Meng et al. | 2009 | China | Case control | CHC | ELISA | 127 | M: 68 F: 59 | Scheuer | – | – | No significant correlation with stages of fibrosis/cirrhosis | – |
| | [65] | Ashour et al. | 2010 | Egypt | Cross-sectional | CHC | ELISA | 74 | M: 54 F: 20 | Metavir | – | – | No significant correlation with stages of fibrosis/cirrhosis | – |
| | [66] | Hung et al. | 2010 | China | Cross-sectional | CHC | ELISA | 129 | M: 61 F: 68 | Knodell | Initial vs. advanced fibrosis: No significant difference | – | – | – |
| | [67] | Derbala et al. | 2009 | Qatar | Case control | CHC | ELISA | 92 | M: 80 F: 12 | Scheuer | – | – | Significant positive correlation with stages of fibrosis/cirrhosis | – |

**Table 1** (continued)

| Markers | Ref no. | Characteristics of included studies | | | | | | | | Results of statistical analyses | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Author | Year | Study location | Study design | Etiology | Method of measurement | Sample size | Patient gender | Fibrosis staging | Univariate analysis[a] | Multivariate analysis[b] | Correlation analysis[c] | ROC curve analysis[d] |
| | [68] | Latif et al. | 2011 | Egypt | Cross-sectional | CHC | ELISA | 60 | M: 40 F: 20 | Brunt | – | – | Significant negative correlation with stages of fibrosis/cirrhosis | – |
| | [45] | Tiftikci et al. | 2009 | Turkey | Case control | CHC | Specific enzymatic Immunoassay | 51 | M: 22 F: 29 | Metavir | No significant difference between stages of fibrosis | – | – | – |
| | [49] | Mera et al. | 2014 | Japan | Cross-sectional | CHC | ELISA | 77 | M: 31 F: 46 | Metavir Scheuer | Initial vs. advanced fibrosis: No significant difference | – | – | – |
| | [69] | Kara et al. | 2007 | Turkey | Case control | CHC | ELISA | 50 | M: 22 F: 28 | Scheuer | No significant difference between stages of fibrosis | – | – | – |
| | [53] | Popa et al. | 2011 | Romania | Cross-sectional | CHC | ELISA | 81 | M: 22 F: 59 | Metavir | – | Independently predicts advanced fibrosis | Significant negative correlation with stages of fibrosis/cirrhosis | Significantly predicts advanced fibrosis (AUC = 0.72), Sen = 86.7%, Spe = 62.7% |
| | [70] | Jonsson et al. | 2005 | Australia | Cross-sectional | CHC | RIA | 194 | M: 132 F: 62 | Scheuer | Between stages of fibrosis and non- cirrhotic vs. cirrhotic: No Significant difference | Could not independently predict extent of liver fibrosis | – | – |
| | [71] | Corbetta et al. | 2011 | Italy | Case control | CHC | ELISA | 54 | M: 41 F: 13 | Ishak | – | Independently predicts moderate-advanced fibrosis | – | – |

**Table 1** (continued)

| Markers | Ref no. | Characteristics of included studies | | | | | | | | Results of statistical analyses | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Author | Year | Study location | Study design | Etiology | Method of measurement | Sample size | Patient gender | Fibrosis staging | Univariate analysis[a] | Multivariate analysis[b] | Correlation analysis[c] | ROC curve analysis[d] |
| | [72] | Shimada et al. | 2007 | Japan | Cross-sectional | NAFLD | ELISA | 100 | M: 56 F: 44 | Brunt | Significant difference between stages of fibrosis | – | – | – |
| | [27] | Palekar et al. | 2006 | USA | Cross-sectional | NAFLD | RIA | 80 | M: 42 F: 38 | Brunt | Simple steatosis vs. NASH: No significant difference, Non-mild vs. moderate-advanced fibrosis: No significant difference | – | – | – |
| | [30] | Chwist et al. | 2014 | Poland | Cross-sectional | NAFLD | ELISA | 70 | M: 40 F: 30 | Kleiner | Non-mild vs. moderate-advanced fibrosis: No significant difference | – | – | – |
| | [31] | Poorten et al. | 2013 | Belgium | Cross-sectional | NAFLD | ELISA | 119 | M: 63 F: 56 | Brunt | Non-mild vs. moderate-advanced fibrosis: No significant difference | – | – | – |
| | [32] | Petta et al. | 2012 | Italy | Cross-sectional | NAFLD | ELISA | 142 | M: 95 F: 47 | Kleiner | Non-mild vs. moderate-advanced fibrosis: No significant difference | – | – | – |
| | [35] | Muñoz et al. | 2009 | Mexico | Case control | NAFLD | ELISA | 52 | M: 33 F: 19 | Brunt | Initial vs. advanced fibrosis: No significant difference | – | – | – |

**Table 1** (continued)

| Markers | Ref no. | Characteristics of included studies | | | | | | | | Results of statistical analyses | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Author | Year | Study location | Study design | Etiology | Method of measurement | Sample size | Patient gender | Fibrosis staging | Univariate analysis[a] | Multivariate analysis[b] | Correlation analysis[c] | ROC curve analysis[d] |
| | [36] | Yalniz et al. | 2006 | Turkey | Case control | NAFLD | ELISA | 37 | M: 25 F: 12 | Brunt | No significant difference between stages of fibrosis | – | – | – |
| | [73] | Younossi et al. | 2011 | USA | Cross-sectional | NAFLD | ELISA | 79 | M: 18 F: 61 | Bondini | Fibrosis vs. without fibrosis: No significant difference, Non-mild vs. advanced fibrosis: No significant difference | – | – | – |
| | [74] | Savvidou et al. | 2009 | Greece | Cross-sectional | NAFLD | ELISA | 42 | M:17 F: 25 | Brunt | Significant difference between stages of fibrosis | Independently predicts advanced liver fibrosis | Significant negative correlation with stages of fibrosis/ cirrhosis | – |
| | [75] | Arvaniti et al. | 2008 | Greece | Cross-sectional | NAFLD | ELISA | 43 | M: 22 F: 21 | Kleiner | Non-mild vs. advanced fibrosis: No significant difference | – | No significant correlation with stages of fibrosis/ cirrhosis | – |
| | [40] | Hui et al. | 2004 | Australia | Case control | NAFLD | RIA | 109 | M: 68 F: 41 | Brunt | – | Could not independently predict extent of liver fibrosis | – | – |
| | [76] | Jarrar et al. | 2008 | USA | Case control | NAFLD | ELISA | 83 | M: 18 F: 65 | Bondini | – | Could not independently predict extent of liver fibrosis | – | – |
| | [34] | Koehler et al. | 2011 | USA | Cross-sectional | NAFLD | – | 160 | M: 24 F: 136 | Brunt | Significant difference between stages of fibrosis | Independently predicts mild-advanced liver fibrosis | – | Significantly predicts advanced fibrosis (AUC = 0.8), Sen = 63%, Spe = 94% |

**Table 1** (continued)

| Markers | Ref no. | Characteristics of included studies | | | | | | | | Results of statistical analyses | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Author | Year | Study location | Study design | Etiology | Method of measurement | Sample size | Patient gender | Fibrosis staging | Univariate analysis[a] | Multivariate analysis[b] | Correlation analysis[c] | ROC curve analysis[d] |
| | [77] | Liew et al. | 2006 | Taiwan | Cross-sectional | NAFLD CHC CHB ALD[u] | – | 160 | M: 60 F: 100 | Kleiner Brunt | – | – | Significant negative correlation with stages of fibrosis/ cirrhosis | – |
| | [38] | Tsochatzis et al. | 2008 | Greece | Cross-sectional | NAFLD CHC CHB | ELISA | 146 | M: 85 F: 61 | Ishak | Non-mild vs. moderate-severe fibrosis: No significant difference | – | – | – |
| Total (Adiponectin) | | 33 Studies | | | | | | 3434 | M: 1879 F: 1555 | | | | | |
| Ferritin | [30] | Chwist et al. | 2014 | Poland | Cross-sectional | NAFLD | Advia Centaur XP | 70 | M: 40 F: 30 | Kleiner | Non-mild vs. moderate-severe fibrosis: No significant difference | – | – | – |
| | [35] | Muñoz et al. | 2009 | Mexico | Case control | NAFLD | – | 52 | M: 33 F: 19 | Brunt | Serum ferritin changes were not associated with stages of fibrosis | – | – | – |
| | [78] | Albano et al. | 2005 | Italy | Case control | NAFLD | – | 167 | M: 101 F: 66 | Brunt | Cirrhotic vs. non-cirrhotic: No significant difference | – | – | – |
| | [79] | Bazick et al. | 2015 | USA | Cross-sectional | NAFLD | – | 346 | M: 106 F: 240 | Brunt | None-moderate vs. severe/ cirrhosis: No significant difference | – | – | – |

**Table 1** (continued)

| Markers | Ref no. | Characteristics of included studies | | | | | | | | Results of statistical analyses | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Author | Year | Study location | Study design | Etiology | Method of measurement | Sample size | Patient gender | Fibrosis staging | Univariate analysis[a] | Multivariate analysis[b] | Correlation analysis[c] | ROC curve analysis[d] |
| | [80] | Parikh et al. | 2015 | India | Case control | NAFLD | – | 55 | M: 15<br>F: 40 | Brunt | Without fibrosis vs. fibrosis/ cirrhosis: Significant difference | – | – | – |
| | [72] | Shimada et al. | 2007 | Japan | Cross-sectional | NAFLD | – | 100 | M: 56<br>F: 44 | Brunt | Significant difference between stages of fibrosis | – | – | – |
| | [81] | Shimada et al. | 2002 | Japan | Cross-sectional | NAFLD | – | 81 | M: 40<br>F: 41 | Kleiner<br>Brunt | Non-mild vs. moderate-severe fibrosis: Significant difference | – | – | – |
| | [82] | Vuppalanchi et al. | 2014 | USA | Case control | NAFLD | ELISA | 105 | M: 17<br>F: 88 | Kleiner | Fibrosis vs. without fibrosis: Significant difference | – | – | – |
| | [83] | Hagström et al. | 2016 | Sweden | Cohort | NAFLD | DxI/Access-Modular E/ Cobas e602 | 222 | M: 134<br>F: 88 | Metavir | Significant difference between stages of fibrosis | – | – | – |
| | [84] | Bugianesi et al. | 2004 | Italy | Cross-sectional | NAFLD | – | 263 | M: 218<br>F: 45 | Kleiner | Serum ferritin significantly associated with mild and severe fibrosis | Independently predicts mild and severe fibrosis | – | – |
| | [85] | Bugianesi et al. | 2006 | Italy | Cross-sectional | NAFLD | – | 132 | M: 108<br>F: 24 | Kleiner | Serum ferritin significantly associated with severe fibrosis | Independently predicts severe fibrosis | – | – |

**Table 1** (continued)

| Markers | Ref no. | Characteristics of included studies | | | | | | | | Results of statistical analyses | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Author | Year | Study location | Study design | Etiology | Method of measurement | Sample size | Patient gender | Fibrosis staging | Univariate analysis[a] | Multivariate analysis[b] | Correlation analysis[c] | ROC curve analysis[d] |
| | [86] | Manousou et al. | 2011 | UK | Cross-sectional | NAFLD | – | 111 | M: 71<br>F: 40 | Kleiner | Non-mild vs. moderate-advanced fibrosis: Significant difference | Independently predicts moderate-advanced fibrosis | – | To predict the presence of fibrosis: Cut-off = 240 ng/mL Sen = 91%, Spe = 70% |
| | [87] | Kowdley et al. | 2012 | USA | Cross-sectional | NAFLD | – | 628 | M: 235<br>F: 393 | – | High serum ferritin significantly associated with severe fibrosis | Independently predicts severe fibrosis | – | – |
| | [88] | Angulo et al. | 2014 | Italy | Cohort | NAFLD | ELISA | 1014 | M: 586<br>F: 428 | Kleiner | Significant difference between stages of fibrosis | Independently predicts presence of fibrosis/severe fibrosis/ advanced fibrosis | – | The overall accuracy of serum ferritin levels to diagnose any stage or combination of stages of fibrosis was rather poor, as indicated by an AUC below 0.60 for any serum ferritin cut point analyzed. Similarly, the sensitivity of these serum ferritin cut points was between 13 and 41%, whereas the specificity was between 70 and 95%. |

**Table 1** (continued)

| Markers | Ref no. | Characteristics of included studies | | | | | | | | Results of statistical analyses | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Author | Year | Study location | Study design | Etiology | Method of measurement | Sample size | Patient gender | Fibrosis staging | Univariate analysis[a] | Multivariate analysis[b] | Correlation analysis[c] | ROC curve analysis[d] |
| | [89] | Yoneda et al. | 2014 | USA | Cross-sectional | NAFLD | – | 1201 | M: 641 F: 560 | Kleiner | Significant difference between stages of fibrosis | Independently predicts presence of fibrosis/ cirrhosis | – | The overall accuracy of serum ferritin levels to diagnose any stage or combination of stages of fibrosis was rather poor, as indicated by an AUC below 0.70 for any serum ferritin cut point analyzed. Similarly, the sensitivity of these serum ferritin cut points was between 33 and 50%, whereas the specificity was between 69 and 75%. The NPV and PPV for the cut points were between 24 and 79% and between 27 and 88%, respectively |
| | [90] | Fracanzani et al. | 2008 | Italy | Cohort | NAFLD | – | 458 | M: 370 F: 85 | Kleiner | High serum ferritin significantly associated with ≥F2 | Independently predicts ≥F2 fibrosis | – | – |

**Table 1** (continued)

| Markers | Ref no. | Characteristics of included studies | | | | | | | | Results of statistical analyses | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Author | Year | Study location | Study design | Etiology | Method of measurement | Sample size | Patient gender | Fibrosis staging | Univariate analysis[a] | Multivariate analysis[b] | Correlation analysis[c] | ROC curve analysis[d] |
| | [91] | Salomone et al. | 2013 | Italy | Cross-sectional | NAFLD | – | 285 | M: 223 F: 62 | Brunt | Non-mild vs. moderate-advanced fibrosis: Significant difference | Independently predicts moderate-advanced fibrosis | – | – |
| | [39] | Chitturi et al. | 2002 | Australia | Case control | NAFLD | Conventional automated analyzer | 47 | M: 27 F: 20 | Brunt | – | Could not independently predict extent of liver fibrosis | – | – |
| | [92] | Canbakan et al. | 2007 | Turkey | Cross-sectional | NAFLD | – | 105 | M: 54 F: 51 | Brunt | – | – | Significant positive correlation with stages of fibrosis/cirrhosis | – |
| | [93] | Boga et al. | 2015 | Turkey | Case control | NAFLD | – | 66 | M: 23 F: 43 | NIDDK NASH | – | – | Significant positive correlation with stages of fibrosis/cirrhosis | – |
| | [94] | El-Mezayen et al. | 2012 | Egypt | Cross-sectional | CHC | ELISA | 210 | M: 164 F: 45 | Metavir | Mild vs. severe fibrosis: Significant difference | – | – | Significantly predicts severe fibrosis (AUC = 0.8) |
| | [95] | Gentile et al. | 2013 | Italy | Cross-sectional | CHC | – | 249 | M: 154 F: 95 | Ishak | Cirrhotic vs. non-cirrhotic: Significant difference | – | – | – |
| | [96] | Mohammadalizadeh et al. | 2007 | Iran | Cross-sectional | CHC | Colorimetric | 60 | M: 50 F: 10 | Knodell | Initial vs. advanced fibrosis: No significant difference | – | – | – |
| | [97] | Yoshida et al. | 2010 | Japan | Case control | CHC | – | 78 | M: 40 F: 38 | – | Cirrhotic vs. non-cirrhotic: No significant difference | – | – | – |

**Table 1** (continued)

| Markers | Ref no. | Characteristics of included studies | | | | | | | | Results of statistical analyses | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Author | Year | Study location | Study design | Etiology | Method of measurement | Sample size | Patient gender | Fibrosis staging | Univariate analysis[a] | Multivariate analysis[b] | Correlation analysis[c] | ROC curve analysis[d] |
| | [54] | Ghweil et al. | 2014 | Egypt | Therapeutic intervention | CHC | – | 40 | M: 33 F: 7 | Metavir | – | – | Significant positive correlation with stages of fibrosis/ cirrhosis | – |
| | [98] | Fabris et al. | 2001 | Italy | Cross-sectional | CHC | Microparticle enzyme immunoassay | 69 | M: 42 F: 27 | Ishak | – | – | Significant positive correlation with stages of fibrosis/ cirrhosis | – |
| | [99] | Fernández-Rodriguez et al. | 2004 | Spain | Cross-sectional | CHC | – | 133 | M: 74 F: 59 | Metavir | – | Independently predicts extent of liver fibrosis among patients with history of alcohol consumption | Significant positive correlation with stages of fibrosis/ cirrhosis | – |
| | [100] | Haque et al. | 1996 | USA | Cross-sectional | CHC | Colorimetric | 72 | M: 36 F: 36 | Knodell | – | – | No significant correlation with stages of fibrosis/ cirrhosis | – |
| | [101] | Lin et al. | 2006 | Taiwan | Cross-sectional | CHC | – | 32 | M: 15 F: 17 | Metavir | Mild vs. severe fibrosis: No significant difference | – | No significant correlation with stages of fibrosis/ cirrhosis | – |
| | [102] | Guyader et al. | 2007 | France | Cross-sectional | CHC | – | 586 | M: 340 F: 246 | Metavir | Severe vs. non-severe fibrosis: No significant difference | – | Significant positive correlation with stages of fibrosis/cirrhosis | – |
| | [103] | Petta et al. | 2010 | Italy | Case control | CHC | – | 197 | M: 104 F: 93 | Scheuer | Severe vs. non-severe fibrosis: Significant difference | Independently predicts severe fibrosis | – | – |

**Table 1** (continued)

| Markers | Ref no. | Characteristics of included studies | | | | | | | | Results of statistical analyses | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Author | Year | Study location | Study design | Etiology | Method of measurement | Sample size | Patient gender | Fibrosis staging | Univariate analysis[a] | Multivariate analysis[b] | Correlation analysis[c] | ROC curve analysis[d] |
| | [104] | Lange et al. | 2012 | Switzerland | Therapeutic intervention | CHC | – | 980 | M: 638 F: 342 | Metavir | Non-mild vs. moderate-severe fibrosis: Significant difference | Independently predicts moderate-severe fibrosis | – | – |
| | [105] | Amanzada et al. | 2013 | Germany | Therapeutic intervention | CHC | Microparticle enzyme immunoassay | 191 | M: 113 F: 78 | – | None-moderate vs. severe/cirrhosis: Significant difference | Independently predicts severe fibrosis/cirrhosis | – | – |
| | [106] | Grammatikos et al. | 2015 | Germany | Cross-sectional | CHC | – | 203 | M: 89 F: 114 | Kleiner | Non-mild vs. moderate-advanced fibrosis: Significant difference | Independently predicts moderate-advanced fibrosis | – | – |
| | [107] | Metwally et al. | 2004 | USA | Cross-sectional | CHC | – | 100 | M: 59 F: 41 | Metavir | Mild vs. severe fibrosis: Significant difference | Independently predicts extent of liver fibrosis | – | – |
| | [108] | D'souza et al. | 2005 | UK | Cross-sectional | CHC | – | 339 | M: 191 F: 148 | Ishak | Significant difference between stages of fibrosis | Could not independently predict extent of liver fibrosis | – | – |
| | [109] | Sumida et al. | 2007 | Japan | Cross-sectional | CHC | – | 184 | M: 104 F: 80 | Inuyama | Significant difference between stages of fibrosis | Could not independently predict extent of liver fibrosis | – | – |
| | [110] | Won et al. | 2009 | Korea | Cross-sectional | CHC | Spectro-photometry ECL immunoassays | 105 | M: 59 F: 46 | Ishak | Stage ≤4 Vs ≥5: No significant fibrosis | Could not independently predict extent of liver fibrosis | – | – |

**Table 1** (continued)

| Markers | Ref no. | Characteristics of included studies | | | | | | | | Results of statistical analyses | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Author | Year | Study location | Study design | Etiology | Method of measurement | Sample size | Patient gender | Fibrosis staging | Univariate analysis[a] | Multivariate analysis[b] | Correlation analysis[c] | ROC curve analysis[d] |
| | [111] | Ladero et al. | 2010 | Spain | Cross-sectional | CHC | – | 429 | M: 253 F: 176 | Metavir | Non-mild vs. moderate-severe fibrosis: Significant difference | Could not independently predict extent of liver fibrosis | – | – |
| | [112] | Rajabali et al. | 2008 | Iran | Case control | CHB | Chromato-graphy Electrophoresis | 50 | M: 32 F: 18 | Knodell | – | – | Significant positive correlation with stages of fibrosis/ cirrhosis | – |
| | [113] | Chook et al. | 2011 | Malaysia | Case control | CHB | – | 40 | M: 15 F: 25 | – | Cirrhotic vs. non-cirrhotic: Significant difference | – | – | Cut-off =291μg/L[x] Sen=35%, Spe=100%, Accuracy =67.5% |
| | [25] | Tarantino et al. | 2008 | Italy | Cross-sectional | NAFLD CHC | ELISA | 123 | M: 66 F: 57 | Ishak | – | – | CHC&NASH (M): No significant correlation with stages of fibrosis/ cirrhosis, NASH (F): Significant negative correlation with stages of fibrosis/ cirrhosis | – |
| Total (Ferritin) | | 42 Studies | | | | | | 9978 | M: 5769 F: 4209 | | | | | |
| Total (All markers) | | 95 Studies | | | | | | 15548 | M: 9103 F: 6445 | | | | | |

[a]Results of independent sample t-test or Mann-Whitney or ANOVA or Kruskal-Wallis test; [b]results of multivariate regression or multivariate logistic regression analyses; [c]results of Pearson's correlation coefficient; [d]results of receiver operating characteristic curve analysis for assessment of diagnostic accuracy; [e]chronic hepatitis C; [f]chronic hepatitis B; [g]enzyme-linked immunosorbent assay; [h]male; [i]female; [j]transforming growth factor-β; [k]area under curve; [l]non-alcoholic fatty liver disease; [m]non-alcoholic steatohepatitis; [n]sensitivity; [o]specificity; [p]negative predictive value; [q]positive predictive value; [r]nano gram/mL; [s]chronic viral hepatitis; [t]radioimmunoassay; [u]alcoholic liver disease; [v]immunoradiometric assay; [w]platelet-derived growth factor-BB; [x]μg/L.

fibrosis); while serum levels of the TGF-β1 were significantly higher among non-mild fibrosis when compared to severe fibrosis [27]. Receiver operating characteristic (ROC) curve analysis in two studies by Palekar et al. and Zoheiry et al. showed that the serum TGF-β1 could significantly predict advanced liver fibrosis. However, another study in patients with CHB showed that advanced fibrosis could not be predicted by serum TGF-β1 [24, 27, 28]. More details of results and characteristics of the studies are presented in Table 1.

In order to measure the total amount of TGF-β1 (latent form + biologically active form) in serum or other biological fluids by immunoassay, the sample must be treated by an acid solution to remove the proteins attached to the latent form of TGF-β1 (e.g. LTBP). On the other hand, to measure the biologically active form, the sample must be applied to the system without acid treatment. It has been demonstrated that the concentrations of the latent form is much more than the concentrations of the biologically active form in serum or plasma. The latent form of TGF-β1 has a half-life of 100 min in plasma, and thus, each measurement may be a snapshot at that point of time [25]. Given this, changes in the total amount of TGF-β1 could not reflect the extent of liver fibrosis at a certain point of time. It is believed that simple fatty liver is not so benign as previously thought, because TGF-β1 is detectable in this condition. Therefore, increased serum TGF-β1 in fatty liver could be a consequence of excess lipid deposition and/or initial inflammation [25], nevertheless, decreased levels of TGF-β1 in NASH patients with advanced liver fibrosis has remained a controversial issue [22]. Increased serum levels of TGF-β1 in advanced stages of fibrosis might refer to the chronic background of the disease in patients with chronic viral hepatitis (CVH) [120, 121]. The significant positive correlation between serum TGF-β1 and stage of fibrosis, and also higher levels of the cytokine in patients with CHC when compared to patients with CHB may indicate different pathogenesis of the diseases. For this connection, findings of an investigation in 2004 showed an up-regulated TGF-β1 expression in response to HCV core antigen when compared with hepatitis of other etiologies [122]. It has been speculated that host genetic diversity could affect the production of TGF-β and a high producing TGF-β genotype was detected in 2000 by Powell et al. [123]. It is noteworthy that no significant difference has been observed in TGF-β levels by HCV genotypes [22]. These data support the idea of the central role of TGF-β in the progression to chronic disease. TGF-β may cause more serious damage to the liver in patients with CHC and consequently may be related to poor prognosis in these patients.

## Platelet derived growth factor-BB and non-invasive assessment of liver fibrosis

PDGF-BB is a growth factor in which its biosynthesis is stimulated by TGF-β [124]. Recently, it has been shown that PDGF-BB signaling strongly promotes HSCs activation and induces phenotypic changes, followed by collagen deposition and fibrogenesis. In the liver, PDGF-BB levels are associated with fibrosis of different etiologies [125, 126]. Moreover, it is supposed that TGF-β1 governs development of the hepatic fibrosis by inducing the mitogenic effect of PDGF-BB [127].

Out of the three studies that discussed the clinical importance of PDGF-BB in the prediction of fibrosis or cirrhosis, two studies were conducted among patients with CHB and one study was among patients with CHC. Zhou et al. reported a strong negative correlation between fibrosis stages and serum PDGF-BB, however, Zhang et al. found a positive correlation in patients with CHB [18, 23]. In accordance with Zhou et al., the study among patients with CHC reported that serum PDGF-BB negatively correlated with fibrosis stages and decreased PDGF-BB was associated with cirrhosis [29]. More details of results and characteristics of the studies are presented in Table 1.

It appears that serum levels of PDGF-BB tend to be decreased during progression of liver fibrosis and in cirrhosis it reaches its lowest level. The extrahepatic concentration of PDGF-BB is believed to be related to the platelet count, and therefore, decreased serum levels of PDGF-BB could be justified by the decreasing platelet count during progression of liver fibrosis in patients with CHB and CHC [126, 128, 129]. The positive correlation reported by Zhang et al. might be due to the small sample size compared to the others, differences in the response of immune system to the injury or extent of the liver damage in those patients.

## Leptin and non-invasive assessment of liver fibrosis

Leptin, an important adipokine, plays a central role in the regulation of lipid accumulation, inflammation and immune system functions by paracrine or endocrine mechanisms [17].

In addition to the role of leptin in the regulation of food intake and energy expenditure, its important role in liver fibrogenesis has also been demonstrated. Briefly, leptin participates in modulating the response to the injury by increasing the expression of type I procollagen and potentiates the effect of TGF-β through binding to

its receptor on HSCs, up-regulation of TIMP-1, activating MAP kinase and PI3K/Akt signaling pathways, amplification of inflammatory responses mediated by NF-κB in HSCs, and stimulation of angiogenesis through up-regulation of the vascular endothelial growth factor in HSCs [130–133].

Of 28 studies which investigated the possible association of serum leptin with liver fibrosis/cirrhosis, 13 were carried out in patients with CVH, 12 in patients with NAFLD, two in patients with ALD, and one in patients with NAFLD or CVH. Eight studies conducted in patients with NAFLD used univariate analysis and found no significant association; however, a study in Greece reported a significant association after stratifying results by gender [30–38]. Multivariate analysis revealed that serum leptin could not predict any stage of liver fibrosis or cirrhosis in NAFLD [39–42]. Out of the 12 studies among patients with CVH, seven studies found no significant association between serum leptin and liver fibrosis/cirrhosis [43–50]. Results of multivariate analysis in patients with CVH showed that serum leptin could not predict stage of liver fibrosis as well [51–53]. Despite the fact that three studies found a positive correlation between serum leptin and liver fibrosis/cirrhosis [51, 53, 54], two studies did not find such a correlation [47, 55]. A study in Greece showed that the etiology of CLD may affect the serum leptin levels [38]. Results of the ROC curve analysis showed poor diagnostic accuracy of the serum leptin [52, 53]. More details of results and characteristics of the studies are presented in Table 1.

Two studies carried out in patients with ALD had opposite results. The study by Nicolas et al. among men reported no significant association [56], however, the other study by Naveau et al. among both genders found a significant association using both univariate and multivariate analyses [57]. This controversy may be attributed to the study populations. Indeed, the first study by Nicolas et al. was conducted among alcoholics with compensated cirrhosis. Moreover, the larger sample size and inclusion of female patients in the second study might have affected the findings.

It appears that serum levels of leptin may not be significantly affected by the origin of the study population or the etiology of the CLD. However, the serum levels of leptin seem to be affected by patients' age and gender and percentage of body fat. Therefore, the effects of these factors should be adjusted in future studies. Additionally, the categorization of quantitative variables could affect the results of statistical analyses and must be considered.

## Adiponectin and non-invasive assessment of liver fibrosis

Besides the role of adiponectin in carbohydrates and lipid metabolism, its hepatoprotective and anti-inflammatory actions are also well established [134]. Modulation of the activated phenotype of HSCs is found to be the main mechanism by which adiponectin mediates its effects in liver wound healing procedures [135]. Adiponectin suppresses the proliferation and migration of HSCs stimulated with PDGF-BB. Also, adiponectin attenuates the effect of TGF-β1 on the expression of fibrogenic genes such as the connective tissue growth factor [136]. Hence, a mutual regulation of leptin and adiponectin appears with activated phenotype of the HSCs [137].

Thirty-three studies discussed the possible relationship between serum adiponectin and liver fibrosis/cirrhosis. Four studies were conducted among patients with CHB and 14 among patients with CHC. Thirteen studies were carried out in patients with NAFLD and two studies in patients with CLD of different etiologies. Two studies among patients with CHB found no correlation between serum adiponectin and the stage of liver fibrosis [47, 58]. The remaining two studies by Hui et al. and Liu et al. reported a significant association between the increased serum adiponectin and advanced liver fibrosis/cirrhosis using multivariate and univariate analyses, respectively [59, 60].

However, six studies among patients with CHC showed no significant correlation between serum adiponectin and fibrosis stage [61–66] while two studies reported a significant positive correlation [53, 67]. Interestingly, findings of a study in Egypt showed a significant negative correlation between serum adiponectin and liver fibrosis [68]. No significant relationship was seen when univariate analysis was used to assess the association of serum adiponectin with liver fibrosis/cirrhosis [45, 49, 69]. Results of the multivariate and ROC curve analyses used in three studies [53, 70, 71] are summarized in Table 1.

Of the studies in patients with NAFLD, eight studies assessed the relationship using univariate analysis. Except for a study by Shimida et al. in Japan [72], the others could not find any significant relationship between serum adiponectin and liver fibrosis [27, 30–32, 35, 36, 73]. Two studies assessed the correlation between serum adiponectin and liver fibrosis. In the study by Savvidou et al., the adiponectin concentrations were normally distributed after a logarithmic transformation and then, the correlation was assessed and a significant negative correlation was observed [74]. In contrast, another study reported no

significant correlation [75]. It is interesting to note that multivariate analysis showed no significant results [40, 76], although an investigation in the US reported the usefulness of decreased serum adiponectin in the diagnosis of advanced fibrosis [34].

Two studies enrolled patients with CLD of different etiologies. A study by Liew et al. which included patients with CHC, patients with CHB, and patients with NAFLD showed a significant negative correlation between serum adiponectin and stage of liver fibrosis [77]. However, the second study reported a significant association between the increased serum adiponectin and advanced liver fibrosis [38]. The latter study obtained the results after conducting statistical analysis among men and women separately to control the effect of the gender of patients. They stated that the etiology of CLDs and gender could affect the serum concentrations of adiponectin. More detailed results and characteristics of the studies are presented in Table 1.

It seems that in contrast to serum leptin, serum levels of adiponectin could be affected by the etiology of CLD and that decreased and increased levels of this adipokine could predict advanced liver fibrosis in NAFLD and CVH, respectively. Regarding the inverse correlation between serum adiponectin and BMI, percentage of body fat, serum triglycerides and fasting insulin, the result could be affected by these factors in patients suffering from metabolic syndromes. Thus, the results should be interpreted with caution. In addition, categorization of the quantitative variables included in the analyses could affect the results and must be considered.

## Ferritin and non-invasive assessment of liver fibrosis

In addition to the role of ferritin in iron metabolism, it could also affect the inflammatory response, angiogenesis, and suppression of cell mediated immunity [138]. Ferritin is speculated to be a pro-inflammatory signaling molecule in HSCs. It activates PI3K and MAP kinase pathways and finally activates NF-κB which results in the up-regulation of the expression of pro-inflammatory cytokines such as interleukine-1-β and inducible nitric oxide synthase [139]. Interestingly, the binding of ferritin to its receptor on activated HSCs is believed to be responsible for enhanced production of collagen and liver fibrosis [138]. As a clinical tool, serum ferritin is applied for several diagnostic panels and recently has been used for the prediction of cirrhosis [138].

Forty-two studies assessed the possible link between liver fibrosis/cirrhosis and serum ferritin. Twenty studies were carried out among patients with NAFLD, 19 among patients with CHC, two among patients with CHB, and one among patients with NAFLD or CHC. Nine studies in patients with NAFLD used univariate analysis to assess the association. Five studies found no association [30, 35, 78–80] and four studies reported a significant association [72, 81–83]. The majority of studies that used multivariate analysis reported increased levels of the serum ferritin associated with advanced liver fibrosis [84–91], although, a study by Chitturi et al. found no association [39]. The correlation analysis showed a significant positive correlation between the serum ferritin and liver fibrosis [92, 93]. Interestingly, in two big studies by Angulo et al. and Yoneda et al., results of ROC curve analysis showed that serum ferritin could not diagnose any stage or combined stages of liver fibrosis [88, 89]. However, a study in 2011 which excluded cirrhotics from the analyses claimed that the increased serum ferritin could successfully diagnose the presence of liver fibrosis [86]. It seems that these opposite results are affected by the sample size or variables included in the analyses.

Of the studies among patients with CHC, four studies used univariate analysis and two studies found a significant relationship between increased serum ferritin and advanced liver fibrosis/cirrhosis [94, 95]. However, two studies observed no association [96, 97]. There were controversies in the correlation analysis results [54, 98–102] and they are summarized in Table 1. Additionally, there were controversies in the results of multivariate analyses and most of them reported that increased serum ferritin can predict advanced liver fibrosis in CHC [99, 103–107], although, some studies did not find such an association [108–111]. Results of the ROC curve analysis revealed poor accuracy of serum ferritin for the diagnosis of advanced fibrosis in CHC [94]. Interestingly, the study by Fernandez et al. showed that increased serum ferritin among CHC patients with a history of alcohol consumption could predict faster progression of liver fibrosis [99]. Obviously, alcohol and iron have toxic effects on the liver and it was demonstrated that alcohol consumption can cause disruption to the iron metabolism and be reflected in iron indexes such as elevated serum iron, ferritin, and transferrin saturation [140–143]. Therefore, this finding could be due to increasing oxidative stress and cell damage related to alcohol and iron toxicity together. Moreover, the elevated serum ferritin may be caused by chronic inflammation and then cause faster progression of fibrosis [138, 144, 145].

Of the two studies among patients with CHB, the study by Rajabali et al. indicated a significant positive correlation between liver fibrosis and serum ferritin [112]. The other study by Chook et al. found no difference between

**Table 2:** Involved cell types, signaling pathways, and possible changes of the serum markers during liver fibrosis in patients with CHC, CHB, NAFLD/NASH, and ALD, confounding factors may affect serum levels of the markers, and relevance of the markers to liver fibrosis.

| Markers | Involved cell types | Involved signaling pathways | Effect(s) | Possible changes of the serum markers | | | | Confounding factors[e] | Relevance to the liver fibrosis[f] |
|---|---|---|---|---|---|---|---|---|---|
| | | | | CHC | CHB | NAFLD/NASH | ALD | | |
| TGF-β1[a] | HSCs[c], endothelial cell, hepatocytes | Smad, JAK/STAT, MAP Kinase, PI3K/Akt, NF-κB | Proliferation, fibrogenesis, EMT[d], collagen production, apoptosis | ↑ | ↑ | ↓ | – | Variations between individual's immune system, genetic diversity | + |
| PDGF-BB[b] | HSCs, vascular smooth muscle cell | JAK/STAT, MAP kinase, PI3K/Akt, NF-κB, p38, Rho, PLCγ | proliferation, fibrogenesis, angiogenesis, collagen production | ↓ | ↓ | – | – | Variations between individual's immune system | + |
| Leptin | HSCs, endothelial cell, macrophages | JAK/STAT, MAP kinase, PI3K/Akt, NF-κB | proliferation, fibrogenesis, inflammation, angiogenesis, collagen production | ↑ | ↑ | ↑ | ↑ | Demographic factors, variations between individual's immune system | ++ |
| Adiponectin | HSCs, vascular smooth muscle, macrophages | JAK/STAT, MAP kinase, PI3K/Akt, NF-κB | Anti-fibrogenesis, Anti-inflammation, Angiogenesis | ↑ | ↑ | ↓ | – | Demographic factors, variations between individual's immune system, etiology of the disease | ++ |
| Ferritin | HSCs | MAP kinase, PI3K/Akt, NF-κB | Fibrogenesis, Angiogenesis, Inflammation, Collagen production | ↑ | ↑ | ↑ | – | Variations between individual's immune system, genetic disorders | +++ |

CHC, chronic hepatitis C; CHB, chronic hepatitis B; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; ALD, alcoholic liver disease. [a]Transforming growth factor-β. [b]Platelet-derived growth factor-BB. [c]Hepatic stellate cells. [d]Epithelia-to-mesenchymal transition. [e]Categorizing of variables included in the study could also be considered as a factor which affects the statistical test results and conclusion. [f]It is a ranking based on the number of the studies for each marker, sample size of the studies and also statistical analyses used for assessment of the relationships. This ranking might be applicable in future clinical studies. The highest rank was considered as five pluses (+++++).

cirrhotics and non-cirrhotics in terms of the mean serum ferritin [113]. An investigation among patients suffering from CHC or NAFLD assessed the correlation between liver fibrosis and serum ferritin. In female patients with NAFLD, a significant negative correlation was observed, however, such a correlation was not observed in other groups [25]. More detailed results and characteristics of the studies are presented in Table 1.

It seems that the origin of study population, etiology of CLD, and patients' demographics may not significantly affect serum ferritin. Nevertheless, the effect of genetic disorders associated with hyperferritinemia or other common clinical conditions such as iron deficiency anemia and also variations between individuals' immune system should be adjusted. Additionally, the differences between results of the studies may arise from the categorization of variables included in the analyses. For instance, some studies compared serum ferritin by stages of fibrosis, some compared between cirrhotics and non-cirrhotics, some compared between non-mild fibrosis and advanced fibrosis, and some excluded special groups of patients from the analyses. In the same way, serum ferritin could also be classified as a continuous quantitative variable or a categorical variable.

## Cytokines, adipokines and liver fibrosis

Generally, it is believed that to progress from chronic liver injury to fibrosis and cirrhosis, cytokines, adipokines, growth factors, and other biological mediators play pivotal roles. TGF-β1, PDGF-BB, leptin and ferritin have inflammatory and fibrogenic effects on different cell types, particularly HSCs, during liver fibrogenesis [146]. TGF-β1, PDGF-BB, leptin, and ferritin induce the production and accumulation of ECM which play a crucial role in the liver fibrogenesis [146]. The biosynthesis and mitogenic effects of PDGF-BB are induced by TGF-β1 [124, 127]. Leptin potentiates the effect of TGF-β1 on HSCs; however, adiponectin appears as an anti-fibrogenic and anti-inflammatory adipokine and attenuates the effects of TGF-β1 and PDGF-BB in fibrogenesis [131, 134, 136]. Adiponectin, leptin, ferritin, TGF-β1, and PDGF-BB involve three major signaling pathways including MAP kinase, PI3K/Akt, and NF-κB in different cell types involved in fibrogenesis, and further exploration could be useful in discovering drugable targets to prevent progression of the liver fibrosis. Further, angiogenesis and collagen production are two critical events which influence the extent and severity of the disease and might be applicable as targets for treatment [146]. A list of candidate proteins, involved cell types and signaling pathways, possible changes in the serum markers in fibrosis/cirrhosis and their relevance to the disease, and factors that might affect the results of biomarkers examination are summarized in Table 2.

## Measurement methods of serum markers

The ELISA and the RIA are two commonly used immunoassay approaches in clinical and research laboratories. In the current review, these two approaches were the most frequently used assays for measurement of the candidate serum biomarkers.

Immunoassays, either for clinical or research uses, for both the qualitative and quantitative measurement of small molecules and larger peptides and proteins are available. Most of the cytokines and adipokines could be measured by extremely sensitive immunoassays which can detect a very small amount of analyte in the biological fluid (e.g. 0.1 pg/mL) [147]. RIA has a high sensitivity and a low background effect because of its detection system (i.e. γ counter). However, it has some disadvantages such as the cost of equipment and reagents, short shelf-life of radiolabeled compounds, and the problems related to the disposal of radioactive waste. In the ELISA method, the lower sensitivity and background effect may cause erroneous results. A part of these problems could be attributed to its detection system. Visible spectrophotometry, used in ELISA readers, has some advantages such as cost-effectiveness, ease of use, and revealing contaminants, although, this method could be affected by temperature, pH, impurities, and contaminants which lead to inaccurate results. It should be noted that most of the problems with ELISA can be handled by an experienced technician [147, 148]. Of the other rarely used immunoassays in the included studies, we can refer to the microparticle enzyme immunoassay (MEIA) and chemiluminescence immunoassay (CLIA) which have very high sensitivity and specificity and are also faster compared to ELISAs and RIAs. MEIA and CLIA do not have the disadvantages of the RIA and ELISA, but they are expensive methods [149]. It seems that selection of a detection method for the measurements could be mainly depending on the nature and concentration of the analyte, optimum operating condition, and cost-effectiveness of the method.

## Strengths and limitations

Our study has some limitations. We only included articles published in English and Persian languages and it

may have resulted in a language bias. However, it is supposed that restricting the systematic review to English language articles has a minor effect on the conclusion [150]. We did not aim to do meta-analysis owing to the fact that very few studies reported sensitivity, specificity, negative and positive predictive values, and diagnostic accuracy for the aforementioned markers among patients with CLD of different etiologies. Moreover, variations in the study populations and methods used to measure the serum biomarkers and also variations in definition and staging of the liver fibrosis and cirrhosis dissuaded us from pooling the studies and doing a meta-analysis. Our study has some strength. We applied rigorous exclusion criteria to minimize the effects of confounding factors. Additionally, we reviewed all the studies about potential predicting serum markers of fibrosis or cirrhosis in the patients with CLD of different etiologies that have never been applied to a routine test panel and it could be applicable in clinical decision making.

## Conclusions

This systematic review showed that serum levels of adiponectin, leptin, ferritin, TGF-β1, and PDGF-BB could be affected by patients' gender, age, genetic diversity, and underlying hereditary or non-hereditary disorders and must be considered in future studies. Investigating the correlation between histological stage, liver tissue expression and serum levels of markers such as TGF-β1 and PDGF-BB could be helpful in the validation of the markers for the prediction of liver fibrosis or cirrhosis. It is interesting to note that the involved signaling pathways as well as events such as angiogenesis and collagen production could be useful in discovering drugable targets to prevent the progression of the liver fibrosis. Accordingly, analyzing the receptors of the proteins on the target cells is strongly recommended.

We conclude that serum levels of the markers, particularly ferritin, could successfully predict liver fibrosis/cirrhosis, however, these data might not be clinically replicated and further studies are needed.

**Acknowledgments:** The authors thank Emran Askari, MD (Mashhad University of Medical Sciences) for his critical comment on study design and his contribution to the literature search.

**Author contributions:** All the authors have accepted responsibility for the entire content of the submitted manuscript and approved submission.

**Research funding:** This systematic review was funded by Baqiyatallah Research Center for Gastroenterology and Liver Diseases, Baqiyatallah University of Medical Sciences (Funder Id: 10.13039/501100005849, grant No. 95-11-001168).

**Employment or leadership:** None declared.

**Honorarium:** None declared.

**Competing interests:** The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

## References

1. Manos MM, Leyden WA, Murphy RC, Terrault NA, Bell BP. Limitations of conventionally derived chronic liver disease mortality rates: results of a comprehensive assessment. Hepatology 2008;47:1150–7.
2. Rockey DC, Friedman SL. Hepatic fibrosis and cirrhosis. In: Boyer TD, Manns MP. Arun J. Sanyal, editors. Philadelphia, PA: Saunders, an imprint of Elsevier Inc., 2012.
3. Yoon K-H, Lee J-H, Kim J-W, Cho JH, Choi Y-H, Ko S-H, et al. Epidemic obesity and type 2 diabetes in Asia. The Lancet 2006;368:1681–8.
4. Brunt EM. Nonalcoholic steatohepatitis. Semin Liver Dis 2004;24:3–20.
5. Vernon G, Baranova A, Younossi Z. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. Aliment Pharmacol Ther 2011;34:274–85.
6. Farrell GC, Wong VW, Chitturi S. NAFLD in Asia – as common and important as in the West. Nat Rev Gastroenterol Hepatol 2013;10:307–18.
7. Global Hepatitis Report 2017. Geneva: World Health Organization. Licence: CC BY-NC-SA 3.0 IGO, 2017.
8. Rehm J, Samokhvalov AV, Shield KD. Global burden of alcoholic liver diseases. J Hepatol 2013;59:160–8.
9. Friedman SL. Liver fibrosis–from bench to bedside. J Hepatol 2003;38:38–53.
10. Regev A, Berho M, Jeffers LJ, Milikowski C, Molina EG, Pyrsopoulos NT, et al. Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. Am J Gastroenterol 2002;97:2614–8.
11. Castera L, Pinzani M. Biopsy and non-invasive methods for the diagnosis of liver fibrosis: does it take two to tango? Gut 2010;59:861–6.
12. Chou R, Wasson N. Blood tests to diagnose fibrosis or cirrhosis in patients with chronic hepatitis C virus infection a systematic review. Ann Intern Med 2013;158:807–20.
13. Arslan FD, Karakoyun I, Tatar B, Pala EE, Yıldırım M, Ulasoglu C, et al. SHFI: a novel noninvasive predictive model for significant fibrosis in patients with chronic hepatitis B. Hepat Mon 2018;18:e63310.
14. Korkmaz P, Demirturk N, Batırel A, Yardimci AC, Cagir U, Nemli SA, et al. Noninvasive models to predict liver fibrosis in patients with chronic hepatitis B: a study from Turkey. Hepat Mon 2017;17:e60266.

15. Hagan M, Asrani SK, Talwalkar J. Non-invasive assessment of liver fibrosis and prognosis. Expert Rev Gastroenterol Hepatol 2015;9:1251–60.
16. Lurie Y, Webb M, Cytter-Kuint R, Shteingart S, Lederkremer GZ. Non-invasive diagnosis of liver fibrosis and cirrhosis. World J Gastroenterol 2015;21:11567–83.
17. Fantuzzi G. Adipose tissue, adipokines, and inflammation. J Allergy Clin Immunol 2005;115:911–9.
18. Zhou J, Deng Y, Yan L, Zhao H, Wang G, Group CH-RFAR. Serum platelet-derived growth factor BB levels: a potential biomarker for the assessment of liver fibrosis in patients with chronic hepatitis B. Int J Infect Dis 2016;49:94–9.
19. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Int J Surg 2010;8:336–41.
20. Kirmaz C, Terzioglu E, Topalak O, Bayrak P, Yilmaz O, Ersoz G, et al. Serum tumor growth factor-β1 levels in patients with cirrhosis, chronic hepatitis B and chronic hepatitis C. Eur Cytokine Netw 2004;15:112–6.
21. Nelson D, Gonzalez-Peralta R, Qian K, Xu Y, Marousis C, Davis G, et al. Transforming growth factor-ß1 in chronic hepatitis C. J Viral Hepat 1997;4:29–35.
22. Neuman M, Benhamou J, Malkiewicz I, Ibrahim A, Valla D, Martinot-Peignoux M, et al. Kinetics of serum cytokines reflect changes in the severity of chronic hepatitis C presenting minimal fibrosis. J Viral Hepat 2002;9:134–40.
23. Zhang B-B, Cai W-M, Weng H-L, Hu Z-R, Lu J, Zheng M, et al. Diagnostic value of platelet derived growth factor-BB, transforming growth factor-β1, matrix metalloproteinase-1, and tissue inhibitor of matrix metalloproteinase-1 in serum and peripheral blood mononuclear cells for hepatic fibrosis. World J Gastroenterol 2003;9:2490–6.
24. Ucar F, Sezer S, Ginis Z, Ozturk G, Albayrak A, Basar O, et al. APRI, the FIB-4 score, and Forn's index have noninvasive diagnostic value for liver fibrosis in patients with chronic hepatitis B. Eur J Gastroenterol Hepatol 2013;25:1076–81.
25. Tarantino G, Conca P, Riccio A, Tarantino M, Di Minno MN, Chianese D, et al. Enhanced serum concentrations of transforming growth factor-beta1 in simple fatty liver: is it really benign? J Transl Med 2008;6:1–8.
26. Kanzler S, Baumann M, Schirmacher P, Dries V, Bayer E, Gerken G, et al. Prediction of progressive liver fibrosis in hepatitis C infection by serum and tissue levels of transforming growth factor-β. J Viral Hepat 2001;8:430–7.
27. Palekar NA, Naus R, Larson SP, Ward J, Harrison SA. Clinical model for distinguishing nonalcoholic steatohepatitis from simple steatosis in patients with nonalcoholic fatty liver disease. Liver Int 2006;26:151–6.
28. Zoheiry MM, Hasan SA, El-Ahwany E, Nagy FM, Taleb HA, Nosseir M, et al. Serum markers of epithelial mesenchymal transition as predictors of HCV-induced liver fibrosis, cirrhosis and hepatocellular carcinoma. Electron Physician 2015;7:1626–37.
29. El-Bassiouni N, Nosseir M, Madkour M, Zoheiry M, Bekheit I, Ibrahim R, et al. Role of fibrogenic markers in chronic hepatitis C and associated hepatocellular carcinoma. Mol Biol Rep 2012;39:6843–50.
30. Chwist A, Hartleb M, Lekstan A, Kukla M, Gutkowski K, Kajor M. A composite model including visfatin, tissue polypeptide-specific antigen, hyaluronic acid, and hematological variables for the diagnosis of moderate-to-severe fibrosis in nonalcoholic fatty liver disease: a preliminary study. Pol Arch Med Wewn 2014;124:704–12.
31. Poorten D, Samer CF, Ramezani-Moghadam M, Coulter S, Kacevska M, Schrijnders D, et al. Hepatic fat loss in advanced nonalcoholic steatohepatitis: are alterations in serum adiponectin the cause? Hepatology 2013;57:2180–8.
32. Petta S, Amato M, Di Marco V, Cammà C, Pizzolanti G, Barcellona MR, et al. Visceral adiposity index is associated with significant fibrosis in patients with non-alcoholic fatty liver disease. Aliment Pharmacol Ther 2012;35:238–47.
33. Machado MV, Coutinho J, Carepa F, Costa A, Proença H, Cortez-Pinto H. How adiponectin, leptin, and ghrelin orchestrate together and correlate with the severity of nonalcoholic fatty liver disease. Eur J Gastroenterol Hepatol 2012;24:1166–72.
34. Koehler E, Swain J, Sanderson S, Krishnan A, Watt K, Charlton M. Growth hormone, dehydroepiandrosterone and adiponectin levels in non-alcoholic steatohepatitis: an endocrine signature for advanced fibrosis in obese patients. Liver Int 2012;32:279–86.
35. Muñoz LE, Cordero P, Torres L, Sauceda AY, Flores JP, Segura JJ. Adipokines in a group of Mexican patients with nonalcoholic steatohepatitis. Ann Hepatol 2009;8:123–8.
36. Yalniz M, Bahcecioglu IH, Ataseven H, Ustundag B, Ilhan F, Poyrazoglu OK, et al. Serum adipokine and ghrelin levels in non-alcoholic steatohepatitis. Mediators Inflamm 2006;2006:1–5.
37. Lydatakis H, Hager IP, Kostadelou E, Mpousmpoulas S, Pappas S, Diamantis I. Non-invasive markers to predict the liver fibrosis in non-alcoholic fatty liver disease. Liver Int 2006;26:864–71.
38. Tsochatzis E, Papatheodoridis GV, Hadziyannis E, Georgiou A, Kafiri G, Tiniakos DG, et al. Serum adipokine levels in chronic liver diseases: association of resistin levels with fibrosis severity. Scand J Gastroenterol 2008;43:1128–36.
39. Chitturi S, Farrell G, Frost L, Kriketos A, Lin R, Liddle C, et al. Serum leptin in NASH correlates with hepatic steatosis but not fibrosis: a manifestation of lipotoxicity? Hepatology 2002;36:403–9.
40. Hui JM, Hodge A, Farrell GC, Kench JG, Kriketos A, George J. Beyond insulin resistance in NASH: TNF-α or adiponectin? Hepatology 2004;40:46–54.
41. Canbakan B, Tahan V, Balci H, Hatemi I, Erer B, Ozbay G, et al. Leptin in nonalcoholic fatty liver disease. Ann Hepatol 2008;7:249–54.
42. Lemoine M, Ratziu V, Kim M, Maachi M, Wendum D, Paye F, et al. Serum adipokine levels predictive of liver injury in non-alcoholic fatty liver disease. Liver Int 2009;29:1431–8.
43. Hickman IJ, Powell EE, Prins JB, Clouston AD, Ash S, Purdie DM, et al. In overweight patients with chronic hepatitis C, circulating insulin is associated with hepatic fibrosis: implications for therapy. J Hepatol 2003;39:1042–8.
44. Manolakopoulos S, Bethanis S, Liapi C, Stripeli F, Sklavos P, Margeli A, et al. An assessment of serum leptin levels in patients with chronic viral hepatitis: a prospective study. BMC Gastroenterol 2007;7:1–8.
45. Tiftikci A, Atug O, Yilmaz Y, Eren F, Ozdemir FT, Yapali S, et al. Serum levels of adipokines in patients with chronic HCV infection: relationship with steatosis and fibrosis. Arch Med Res 2009;40:294–8.
46. Kukla M, Zwirska-Korczala K, Gabriel A, Waluga M, Warakomska I, Szczygiel B, et al. Chemerin, vaspin and insulin resistance in chronic hepatitis C. J Viral Hepat 2010;17:661–7.

47. Wong VW, Wong GL, Yu J, Choi PC, Chan AW, Chan H-Y, et al. Interaction of adipokines and hepatitis B virus on histological liver injury in the Chinese. Am J Gastroenterol 2010;105:132–8.
48. Aşçi Z, Demirtürk N, Demirdal T, Acartürk G, Aktepe OC, Doğan N, et al. Kronik Viral Hepatitli Hastalarda Serum Fibrozis Belirleyicilerinin (Endoglin, Leptin ve İmmünoglobulinler) Karaciğer Histopatolojisi ile Uyumu. Turkiye Klinikleri J Med Sci 2012;32:1569–75.
49. Mera K, Uto H, Mawatari S, Ido A, Yoshimine Y, Nosaki T, et al. Serum levels of apoptosis inhibitor of macrophage are associated with hepatic fibrosis in patients with chronic hepatitis C. BMC Gastroenterol 2014;14:1–10.
50. Giannini E, Ceppa P, Botta F, Mastracci L, Romagnoli P, Comino I, et al. Leptin has no role in determining severity of steatosis and fibrosis in patients with chronic hepatitis C. Am J Gastroenterol 2000;95:3211–7.
51. Romero-Gómez M, Castellano-Megias VM, Grande L, Irles JA, Cruz M, Nogales MC, et al. Serum leptin levels correlate with hepatic steatosis in chronic hepatitis C. Am J Gastroenterol 2003;98:1135–41.
52. Myers RP, Messous D, Poynard T, Imbert-Bismut F. Association between leptin, metabolic factors and liver histology in patients with chronic hepatitis C. Can J Gastroenterol Hepatol 2007;21:289–94.
53. Popa S, Pîrcălăboiu L, Mota M, Mota E, Dinu R, Rogoz S, et al. The impact of adipocytokines on liver histology in chronic hepatitis C. Diabetes Stoffwech H 2011;20:229–34.
54. Ghweil AA, Meguid MM, Bazeed SE, Ahmed SS, Arafa U. Serum leptin, ferritin and uric acid as predictors of fibrosis and sustained virological response in chronic hepatitis C patients. Life Sci J 2014;11:129–39.
55. Gordon A, McLean CA, Pedersen JS, Bailey MJ, Roberts SK. Hepatic steatosis in chronic hepatitis B and C: predictors, distribution and effect on fibrosis. J Hepatol 2005;43:38–44.
56. Nicolas J, Fernández-Solà J, Fatjo F, Casamitjana R, Bataller R, Sacanella E, et al. Increased circulating leptin levels in chronic alcoholism. Alcohol Clin Exp Res 2001;25:83–8.
57. Naveau S, Perlemuter G, Chaillet M, Raynard B, Balian A, Beuzen F, et al. Serum leptin in patients with alcoholic liver disease. Alcohol Clin Exp Res 2006;30:1422–8.
58. Wu D, Li H, Xiang G, Zhang L, Li L, Cao Y, et al. Adiponectin and its receptors in chronic hepatitis B patients with steatosis in China. Hepat Mon 2013;13:1–10.
59. Hui C-K, Zhang H-Y, Lee NP, Chan W, Yueng Y-H, Leung K-W, et al. Serum adiponectin is increased in advancing liver fibrosis and declines with reduction in fibrosis in chronic hepatitis B. J Hepatol 2007;47:191–202.
60. Liu C-J, Chen P-J, Lai M-Y, Liu C-H, Chen C-L, Kao J-H, et al. High serum adiponectin correlates with advanced liver disease in patients with chronic hepatitis B virus infection. Hepatol Int 2009;3:364–70.
61. Liu C-J, Chen P-J, Jeng Y-M, Huang W-L, Yang W-S, Lai M-Y, et al. Serum adiponectin correlates with viral characteristics but not histologic features in patients with chronic hepatitis C. J Hepatol 2005;43:235–42.
62. Durante-Mangoni E, Zampino R, Marrone A, TRIPODI MF, Rinaldi L, Restivo L, et al. Hepatic steatosis and insulin resistance are associated with serum imbalance of adiponectin/tumour necrosis factor-α in chronic hepatitis C patients. Aliment Pharmacol Ther 2006;24:1349–57.
63. Grigorescu M, Radu C, Crisan D, Grigorescu MD, Serban A, Neculoiu D, et al. Metabolic syndrome, insulin resistance and adiponectin level in patients with chronic hepatitis C. J Gastrointestin Liver Dis 2008;17:147–54.
64. Meng Q-H, Duan Z-H, Lin Z-H, Yu H-W, Li J, Liu Y. Serum level of adiponectin correlated with gender and genotype in patients with chronic hepatitis C. Dig Dis Sci 2009;54:1120–7.
65. Ashour E, Samy N, Sayed M, Imam A. The relationship between serum adiponectin and steatosis in patients with chronic hepatitis C genotype-4. Clin Lab 2010;56:103–10.
66. Hung C-H, Wang J-H, Hu T-H, Chen C-H, Chang K-C, Yen Y-H, et al. Insulin resistance is associated with hepatocellular carcinoma in chronic hepatitis C infection. World J Gastroenterol 2010;16:2265–71.
67. Derbala M, Rizk N, Al-Kaabi S, Amer A, Shebl F, Al Marri A, et al. Adiponectin changes in HCV-Genotype 4: relation to liver histology and response to treatment. J Viral Hepat 2009;16:689–96.
68. Latif HA, Assal HS, Mahmoud M, Rasheed WI. Role of serum adiponectin level in the development of liver cirrhosis in patients with hepatitis C virus. Clin Exp Med 2011;11:123–29.
69. Kara B, Gunesacar R, Doran F, Kara IO, Akkiz H. Correlation of serum adiponectin levels and hepatic steatosis in hepatitis C virus genotype 1 infection. Adv Ther 2007;24:972–82.
70. Jonsson JR, Moschen AR, Hickman IJ, Richardson MM, Kaser S, Clouston AD, et al. Adiponectin and its receptors in patients with chronic hepatitis C. J Hepatol 2005;43:929–36.
71. Corbetta S, Redaelli A, Pozzi M, Bovo G, Ratti L, Redaelli E, et al. Fibrosis is associated with adiponectin resistance in chronic hepatitis C virus infection. Eur J Clin Invest 2011;41:898–905.
72. Shimada M, Kawahara H, Ozaki K, Fukura M, Yano H, Tsuchishima M, et al. Usefulness of a combined evaluation of the serum adiponectin level, HOMA-IR, and serum type IV collagen 7S level to predict the early stage of nonalcoholic steatohepatitis. Am J Gastroenterol 2007;102:1931–8.
73. Younossi ZM, Page S, Rafiq N, Birerdinc A, Stepanova M, Hossain N, et al. A biomarker panel for non-alcoholic steatohepatitis (NASH) and NASH-related fibrosis. Obes Surg 2011;21:431–9.
74. Savvidou S, Hytiroglou P, Orfanou-Koumerkeridou H, Panderis A, Frantzoulis P, Goulis J. Low serum adiponectin levels are predictive of advanced hepatic fibrosis in patients with NAFLD. J Clin Gastroenterol 2009;43:765–72.
75. Arvaniti V, Thomopoulos K, Tsamandas A, Makri M, Psyrogiannis A, Vafiadis G, et al. Serum adiponectin levels in different types of non alcoholic liver disease. Correlation with steatosis, necroinflammation and fibrosis. Acta Gastroenterol Belg 2008;71:355–60.
76. Jarrar M, Baranova A, Collantes R, Ranard B, Stepanova M, Bennett C, et al. Adipokines and cytokines in non-alcoholic fatty liver disease. Aliment Pharmacol Ther 2008;27:412–21.
77. Liew P-L, Lee W-J, Lee Y-C, Wang H-H, Wang W, Lin Y-C. Hepatic histopathology of morbid obesity: concurrence of other forms of chronic liver disease. Obes Surg 2006;16:1584–93.
78. Albano E, Mottaran E, Vidali M, Reale E, Saksena S, Occhino G, et al. Immune response towards lipid peroxidation products as a predictor of progression of non-alcoholic fatty liver disease to advanced fibrosis. Gut 2005;54:987–93.
79. Bazick J, Donithan M, Neuschwander-Tetri BA, Kleiner D, Brunt EM, Wilson L, et al. Clinical model for NASH and advanced fibrosis in adult patients with diabetes and NAFLD: guidelines for referral in NAFLD. Diabetes Care 2015;38:1347–55.

80. Parikh P, Patel J, Ingle M, Sawant P. Serum ferritin levels predict histological severity in patients with nonalcoholic fatty liver disease in India. Ind J Gastroenterol 2015;34:200–8.
81. Shimada M, Hashimoto E, Kaneda H, Noguchi S, Hayashi N. Nonalcoholic steatohepatitis: risk factors for liver fibrosis. Hepatol Res 2002;24:429–38.
82. Vuppalanchi R, Troutt JS, Konrad RJ, Ghabril M, Saxena R, Bell LN, et al. Serum hepcidin levels are associated with obesity but not liver disease. Obesity 2014;22:836–41.
83. Hagström H, Nasr P, Bottai M, Ekstedt M, Kechagias S, Hultcrantz R, et al. Elevated serum ferritin is associated with increased mortality in non-alcoholic fatty liver disease after 16 years of follow-up. Liver Int 2016;36:1688–95.
84. Bugianesi E, Manzini P, D'Antico S, Vanni E, Longo F, Leone N, et al. Relative contribution of iron burden, HFE mutations, and insulin resistance to fibrosis in nonalcoholic fatty liver. Hepatology 2004;39:179–87.
85. Bugianesi E, Marchesini G, Gentilcore E, Cua IH, Vanni E, Rizzetto M, et al. Fibrosis in genotype 3 chronic hepatitis C and nonalcoholic fatty liver disease: role of insulin resistance and hepatic steatosis. Hepatology 2006;44:1648–55.
86. Manousou P, Kalambokis G, Grillo F, Watkins J, Xirouchakis E, Pleguezuelo M, et al. Serum ferritin is a discriminant marker for both fibrosis and inflammation in histologically proven nonalcoholic fatty liver disease patients. Liver Int 2011;31:730–9.
87. Kowdley KV, Belt P, Wilson LA, Yeh MM, Neuschwander-Tetri BA, Chalasani N, et al. Serum ferritin is an independent predictor of histologic severity and advanced fibrosis in patients with nonalcoholic fatty liver disease. Hepatology 2012;55:77–85.
88. Angulo P, George J, Day CP, Vanni E, Russell L, Anna C, et al. Serum ferritin levels lack diagnostic accuracy for liver fibrosis in patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 2014;12:1163–9.
89. Yoneda M, Thomas E, Sumida Y, Imajo K, Eguchi Y, Hyogo H, et al. Clinical usage of serum ferritin to assess liver fibrosis in patients with non-alcoholic fatty liver disease: proceed with caution. Hepatol Res 2014;44:E499–502.
90. Fracanzani AL, Valenti L, Bugianesi E, Andreoletti M, Colli A, Vanni E, et al. Risk of severe liver disease in nonalcoholic fatty liver disease with normal aminotransferase levels: a role for insulin resistance and diabetes. Hepatology 2008;48:792–8.
91. Salomone F, Li Volti G, Rosso C, Grosso G, Bugianesi E. Unconjugated bilirubin, a potent endogenous antioxidant, is decreased in patients with non-alcoholic steatohepatitis and advanced fibrosis. J Gastroenterol Hepatol 2013;28:1202–8.
92. Canbakan B, Senturk H, Tahan V, Hatemi I, Balci H, Toptas T, et al. Clinical, biochemical and histological correlations in a group of non-drinker subjects with non-alcoholic fatty liver disease. Acta Gastroenterol Belg 2007;70:277–84.
93. Boga S, Alkim H, Alkim C, Koksal AR, Bayram M, Yilmaz Ozguven M, et al. The relationship of serum hemojuvelin and hepcidin levels with iron overload in nonalcoholic fatty liver disease. J Gastrointest Liver Dis 2015;24:293–300.
94. El-Mezayen HA, Toson E-SA, Shiha GE. Role of hyaluronic acid, its degrading enzymes, degradation products, and ferritin in the assessment of fibrosis stage in Egyptian patients with chronic hepatitis C. Eur J Gastroenterol Hepatol 2013;25:69–76.
95. Gentile I, Coppola N, Pasquale G, Liuzzi R, D'Armiento M, Di Lorenzo ME, et al. A simple noninvasive score based on routine parameters can predict liver cirrhosis in patients with chronic hepatitis C. Hepat Mon 2013;13:1–8.
96. Mohammadalizadeh A, Fallahian F, Alavian M, Rahimi F, Hedayati M, Eini E, et al. Serum iron, hepatic iron deposition, and inflammatory. Pejouhesh dar Pezeshki (Research in Medicine) 2007;31:141–5.
97. Yoshida Y, Imai Y, Sawai Y, Saito Y, Cao J, Fukuda K, et al. Hydroxyoctadecadienoic acid as a potential biomarker for oxidative stress in patients with chronic hepatitis C. J Gastroenterol Hepatol 2010;25:107–15.
98. Fabris C, Toniutto P, Scott CA, Falleti E, Avellini C, Del Forno M, et al. Serum iron indices as a measure of iron deposits in chronic hepatitis C. Clinica Chimica Acta 2001;304:49–55.
99. Fernández-Rodriguez CM, Gutiérrez ML, Serrano PL, Lledó JL, Santander C, Fernández TP, et al. Factors influencing the rate of fibrosis progression in chronic hepatitis C. Dig Dis Sci 2004;49:1971–6.
100. Haque S, Chandra B, Gerber M, Lok A. Iron overload in patients with chronic hepatitis C: a clinicopathologic study. Human Pathol 1996;27:1277–81.
101. Lin T-J, Liao L-Y, Lin S-Y, Lin C-L, Chang T-A. Influence of iron on the severity of hepatic fibrosis in patients with chronic hepatitis C. World J Gastroenterol 2006;12:4897.
102. Guyader D, Thirouard A-S, Erdtmann L, Rakba N, Jacquelinet S, Danielou H, et al. Liver iron is a surrogate marker of severe fibrosis in chronic hepatitis C. J Hepatol 2007; 46:587–95.
103. Petta S, Camma C, Scazzone C, Tripodo C, Di Marco V, Bono A, et al. Low vitamin D serum level is related to severe fibrosis and low responsiveness to interferon-based therapy in genotype 1 chronic hepatitis C. Hepatology 2010;51:1158–67.
104. Lange CM, Kutalik Z, Morikawa K, Bibert S, Cerny A, Dollenmaier G, et al. Serum ferritin levels are associated with a distinct phenotype of chronic hepatitis C poorly responding to pegylated interferon-alpha and ribavirin therapy. Hepatology 2012;55:1038–47.
105. Amanzada A, Goralczyk AD, Moriconi F, van Thiel DH, Ramadori G, Mihm S. Vitamin D status and serum ferritin concentration in chronic hepatitis C virus type 1 infection. J Med Virol 2013;85:1534–41.
106. Grammatikos G, Ferreiros N, Bon D, Schwalm S, Dietz J, Berkowski C, et al. Variations in serum sphingolipid levels associate with liver fibrosis progression and poor treatment outcome in hepatitis C virus but not hepatitis B virus infection. Hepatology 2015;61:812–22.
107. Metwally MA, Zein CO, Zein NN. Clinical significance of hepatic iron deposition and serum iron values in patients with chronic hepatitis C infection. Am J Gastroenterol 2004;99:286–91.
108. D'souza R, Feakins R, Mears L, Sabin C, Foster G. Relationship between serum ferritin, hepatic iron staining, diabetes mellitus and fibrosis progression in patients with chronic hepatitis C. Aliment Pharmacol Ther 2005;21:519–24.
109. Sumida Y, Kanemasa K, Fukumoto K, Yoshida N, Sakai K. Hepatic iron accumulation may be associated with insulin resistance in patients with chronic hepatitis C. Hepatol Res 2007;37:932–40.
110. Won J-E, Jeong S-H, Chung JI, Lee JH, Hwang SH, Kim J-W, et al. Hepatic iron, serum ferritin, HFE mutation, and hepatic fibrosis in chronic hepatitis C. Intervirology 2009;52:239–46.
111. Ladero JM, Delkader J, Ortega L, Fernández C, Devesa MJ, López-Alonso G, et al. Non-invasive evaluation of the fibrosis stage in chronic hepatitis C: a comparative analysis of nine scoring methods. Scan J Gastroenterol 2010;45:51–9.

112. Rajabali A, Qujeq D, Kashifard M. Determination of serum IRON, TIBC and ferrtin of patients with hepatitis B and fibrosis of the liver, a case-control study. J Ardabil Univ Med Sci 2008;8:45–8.
113. Chook JB, Ngeow YF, Yap SF, Tan TC, Mohamed R. Combined use of wild-type HBV precore and high serum iron marker as a potential tool for the prediction of cirrhosis in chronic Hepatitis B infection. J Med Virol 2011;83:594–601.
114. Massague J. The transforming growth factor-beta family. Annu Rev Cell Bio 1990;6:597–641.
115. Annes JP, Munger JS, Rifkin DB. Making sense of latent TGFβ activation. J Cell Sci 2003;116:217–24.
116. Massagué J. TGF-β signal transduction. Annu Rev Biochem 1998;67:753–91.
117. Edwards DR, Murphy G, Reynolds JJ, Whitham SE, Docherty AJ, Angel P, et al. Transforming growth factor beta modulates the expression of collagenase and metalloproteinase inhibitor. EMBO J 1987;6:1899–904.
118. Border WA, Ruoslahti E. Transforming growth factor-beta in disease: the dark side of tissue repair. J Clin Invest 1992; 90:1–7.
119. Oberhammer FA, Pavelka M, Sharma S, Tiefenbacher R, Purchio AF, Bursch W, et al. Induction of apoptosis in cultured hepatocytes and in regressing liver by transforming growth factor beta 1. Proc Natl Acad Sci 1992;89:5408–12.
120. Letterio JJ, Roberts AB. Regulation of immune responses by TGF-β. Annu Rev Immunol 1998;16:137–61.
121. Kanto T, Takehara T, Katayama K, Ito A, Mochizuki K, Kuzushita N, et al. Neutralization of transforming growth factor β1 augments hepatitis C virus-specific cytotoxic T lymphocyte induction in vitro. J Clin Immunol 1997;17:462–71.
122. Taniguchi H, Kato N, Otsuka M, Goto T, Yoshida H, Shiratori Y, et al. Hepatitis C virus core protein upregulates transforming growth factor-β1 transcription. J Med Virol 2004;72:52–9.
123. Powell EE, Edwards-Smith CJ, Hay JL, Clouston AD, Crawford DH, Shorthouse C, et al. Host genetic factors influence disease progression in chronic hepatitis C. Hepatology 2000;31:828–33.
124. Ross R. Platelet-derived growth factor. The Lancet 1989;333:1179–82.
125. Ikura Y, Morimoto H, Ogami M, Jomura H, Ikeoka N, Sakurai M. Expression of platelet-derived growth factor and its receptor in livers of patients with chronic liver disease. J Gastroenterol 1997;32:496–501.
126. Yoshida S, Ikenaga N, Liu SB, Peng Z-W, Chung J, Sverdlov DY, et al. Extrahepatic platelet-derived growth factor-β, delivered by platelets, promotes activation of hepatic stellate cells and biliary fibrosis in mice. Gastroenterology 2014;147:1378–92.
127. Carr BI, Hayashi I, Branum EL, Moses HL. Inhibition of DNA synthesis in rat hepatocytes by platelet-derived type β transforming growth factor. Cancer Res 1986;46:2330–4.
128. Hart CE, Bailey M, Curtis DA, Osborn S, Raines E, Ross R, et al. Purification of PDGF-AB and PDGF-BB from human platelet extracts and identification of all three PDGF dimers in human platelets. Biochemistry 1990;29:166–72.
129. Kajihara M, Okazaki Y, Kato S, Ishii H, Kawakami Y, Ikeda Y, et al. Evaluation of platelet kinetics in patients with liver cirrhosis: similarity to idiopathic thrombocytopenic purpura. J Gastroenterol Hepatol 2007;22:112–8.
130. Saxena NK, Ikeda K, Rockey DC, Friedman SL, Anania FA. Leptin in hepatic fibrosis: evidence for increased collagen production in stellate cells and lean littermates of ob/ob mice. Hepatology 2002;35:762–71.
131. Aleffi S, Petrai I, Bertolani C, Parola M, Colombatto S, Novo E, et al. Upregulation of proinflammatory and proangiogenic cytokines by leptin in human hepatic stellate cells. Hepatology 2005;42:1339–48.
132. Cao Q, Mak KM, Ren C, Lieber CS. Leptin stimulates tissue inhibitor of metalloproteinase-1 in human hepatic stellate cells respective roles of the JAK/STAT and JAK-Mediated H2O2-Dependent MAPK pathways. J Biol Chem 2004;279:4292–304.
133. Saxena NK, Titus MA, Ding X, Floyd J, Srinivasan S, Sitaraman SV, et al. Leptin as a novel profibrogenic cytokine in hepatic stellate cells: mitogenesis and inhibition of apoptosis mediated by extracellular regulated kinase (Erk) and Akt phosphorylation. FASEB J 2004;18:1612–4.
134. Tsochatzis E, Papatheodoridis GV, Archimandritis AJ. The evolving role of leptin and adiponectin in chronic liver diseases. Am J Gastroenterol 2006;101:2629–40.
135. Caligiuri A, Bertolani C, Guerra CT, Aleffi S, Galastri S, Trappoliere M, et al. Adenosine monophosphate–activated protein kinase modulates the activated phenotype of hepatic stellate cells. Hepatology 2008;47:668–76.
136. Kamada Y, Tamura S, Kiso S, Matsumoto H, Saji Y, Yoshida Y, et al. Enhanced carbon tetrachloride-induced liver fibrosis in mice lacking adiponectin. Gastroenterology 2003;125: 1796–807.
137. Ding X, Saxena NK, Lin S, Xu A, Srinivasan S, Anania FA. The roles of leptin and adiponectin: a novel paradigm in adipocytokine regulation of liver fibrosis and stellate cell biology. Am J Pathol 2005;166:1655–69.
138. Wang W, Knovich MA, Coffman LG, Torti FM, Torti SV. Serum ferritin: past, present and future. Biochim Biophys Acta 2010;1800:760–9.
139. Ruddell RG, Hoang-Le D, Barwood JM, Rutherford PS, Piva TJ, Watters DJ, et al. Ferritin functions as a proinflammatory cytokine via iron-independent protein kinase C zeta/nuclear factor kappaB–regulated signaling in rat hepatic stellate cells. Hepatology 2009;49:887–900.
140. Friedman IM, Kraemer HC, Mendoza FS, Hammer LD. Elevated serum iron concentration in adolescent alcohol users. Am J Dis Child 1988;142:156–9.
141. Lindenbaum J, Lieber CS. Hematologic effects of alcohol in man in the absence of nutritional deficiency. N Engl J Med 1969;281:333–8.
142. Ford C, Wells F, Rogers J. Assessment of iron status in association with excess alcohol consumption. Ann Clin Biochem 1995;32:527–31.
143. Fletcher L, Halliday J, Powell L. Interrelationships of alcohol and iron in liver disease with particular reference to the iron-binding proteins, ferritin and transferrin. J Gastroenterol Hepatol 1999;14:202–14.
144. Ramm GA, Britton RS, O'Neill R, Bacon BR. Identification and characterization of a receptor for tissue ferritin on activated rat lipocytes. J Clin Invest 1994;94:9–15.
145. Tran TN, Eubanks SK, Schaffer KJ, Zhou CY, Linder MC. Secretion of ferritin by rat hepatoma cells and its regulation by inflammatory cytokines and iron. Blood 1997;90:4979–86.
146. Cong M, Iwaisako K, Jiang C, Kisseleva T. Cell signals influencing hepatic fibrosis. Int J Hepatol 2012;2012:158547.

147. Robertson D, Williams GH. Clinical and translational science: principles of human research. Academic Press is an imprint of Elsevier, 2009.
148. Sittampalam GS, Coussens NP, Brimacombe K, Arkin M, Auld D, Austin C, et al. Assay guidance manual. Bethesda (MD): Eli Lilly & Company and the National Center for Advancing Translational Sciences, 2004.
149. Koivunen ME, Krogsrud RL. Principles of immunochemical techniques used in clinical laboratories. Lab Med 2015;37:490–7.
150. Morrison A, Polisena J, Husereau D, Moulton K, Clark M, Fiander M, et al. The effect of English-language restriction on systematic review-based meta-analyses: a systematic review of empirical studies. Int J Technol Assess Health Care 2012;28:138–44.