

# Progress in evaluating the status of hepatitis C infection based on the functional changes of hepatic stellate cells (Review)

WEI WANG, XUELIAN HUANG, XUZHOU FAN, JINGMEI YAN and JIANFENG LUAN

Department of Blood Transfusion Medicine, School of Medicine, Jinling Hospital,  
Nanjing University, Nanjing, Jiangsu 210002, P.R. China

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**Abstract.** Hepatitis C virus (HCV) infection is a global public health problem. Cirrhosis and hepatocellular carcinoma are the main causes of death in patients with chronic hepatitis C (CHC) infection. Liver fibrosis is an important cause of cirrhosis and end-stage liver disease after CHC infection. Along with the course of infection, liver fibrosis exhibits a progressive exacerbation. Hepatic stellate cells (HSCs) are involved in both physiological and pathological processes of the liver. During the chronic liver injury process, the activated HSCs transform into myofibroblasts, which are important cells in the development of liver fibrosis. At present, HCV infection still lacks specific markers for the accurate detection of the disease condition and progression. Therefore, the present review focused on HSCs, which are closely related to HCV-infected liver fibrosis, and analyzed the changes in the HSCs, including their surface-specific markers, cytokine production, activation, cell function and morphological structure. The present review aimed to propose novel diagnostic markers, at both the cellular and molecular level, which would be of great significance for the timely diagnosis of the disease. According to this aim, the characteristic changes of HSCs during HCV infection were reviewed in the present article.

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*Correspondence to:* Professor Jianfeng Luan, Department of Blood Transfusion Medicine, School of Medicine, Jinling Hospital, Nanjing University, 305 Zhongshan East Road, Nanjing, Jiangsu 210002, P.R. China  
Email: dbzqblood@163.com

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## 1. Introduction

Hepatitis C virus (HCV) is an infectious disease of global concern. The World Health Organization suggests that there are >71 million individuals infected with HCV worldwide and ~475,000 deaths are caused by HCV infections annually (1,2). The prevalence and incidence of HCV infections are the highest in low- and middle-income countries (3,4). The HCV can cause chronic liver disease, which is long-lasting and can progress to fibrosis, cirrhosis and hepatocellular carcinoma (HCC) if it is not treated in a timely manner (5-7). The dormant period of the HCV is long, sometimes up to 20-30 years, and the clinical symptoms are not obvious, thus they are easy to ignore once infected (8,9).

During the early course of HCV infection, the activation and proliferation of hepatic stellate cells (HSCs) is the central link in the development of liver fibrosis (10,11). When the HCV infection causes damage to the liver, cytokines and reactive oxygen species released by the tissues activate HSCs to develop into myofibroblasts (MFBs), which undergo considerable proliferation, and secrete collagen and metalloproteinase inhibitors (12-14), the secretion of which notably increases extracellular matrix deposition and decreases its degradation, respectively (15). The excessive deposition of the extracellular matrix results in the destruction of the structure of the liver, eventually leading to the development of liver fibrosis (16).

At present, several serological makers and viral antigens in the serum, such as HCV antibody (Ab), HCV core antigen (cAg) and HCV-RNA, are routinely assessed for to diagnose the status of the HCV infection and liver disease associated with the HCV (17-19). Nevertheless, given the complexity and variability in HCV infections, the levels of serological markers do not sufficiently reflect the status of HCV infection and disease progression (20,21). Therefore, it is necessary to identify additional indices that may be used to differentiate varying degrees of HCV progression. The changes in the function and status of HSCs was discovered to be closely associated with the course of HCV infection (22). Therefore, in the present review, the functional changes of important cell populations, such as HSCs, during alterations to liver immune function following the infection with HCV are described, with the aim of highlighting avenues for the identification of complementary laboratory indices that may be used for the diagnosis of HCV infection.

## 2. Functional characteristics of HSCs

**Activation of HSCs.** As early as the 19th century, German scientist von Kuffer first discovered the presence of stellate cells in the hepatic sinus space (23). In 1996, international standardization stipulated that such stellate cells were named HSCs (24). In addition to the known functions of storing lipid droplets and participating in fibrosis, HSCs also possess several other important functions, such as their role in mediating the immune response of the liver (25-27). Previous studies have reported that HSCs were heterogeneous and plastic in the liver, and different subsets of HSC phenotypes exist, which all have various functions (28-30). In the physiological liver, HSCs exist in a quiescent non-proliferative state, termed quiescent (q)HSCs (31). qHSCs were discovered to serve a role in the storage and transport of retinoids (vitamin A compounds), and the quantity of vitamin A lipid droplets in the cytoplasm of qHSCs in the liver may account for up to 45-72% of the total content in the human body (32). Nervous system markers, such as glial fibril acidic protein and neurotensin, nerve growth factor receptor and desmin were also discovered to be expressed in qHSCs (33,34). qHSCs can also secrete extracellular matrix protein and protein substances, such as laminin, polysaccharide protein and type IV collagen, which are required for the formation of the basement membrane (35-37).

Following chronic liver damage as a result of alcoholic steatohepatitis, non-alcoholic fatty liver disease, hepatitis B or HCV infection, or cholestatic liver injury, several external stimuli and cell types converge upon HSCs to promote their activation and development into MFB cells (38-40). The other cell types involved in the activation of HSCs include liver macrophages, hepatic sinusoidal endothelial cells, natural killer (NK) cells, B cells and hepatocytes (41). These cells secrete various components, including oxidative stress products, cytokines and apoptotic bodies, amongst others, to activate the HSCs (42-44). MFBs possess a potent ability to contract and migrate through the upregulation of fibrosis markers, such as type I collagen,  $\alpha$ -smooth muscle actin (SMA), matrix metalloproteinases and tissue inhibitor of matrix metalloproteinases (45-47).  $\alpha$ -SMA is present in vascular smooth muscle cells and fibroblasts, and is a widely recognized marker of HSC activation (48,49). In addition, previous studies have identified that cytokine receptor-like factor 1, secreted phosphoprotein 1, lysyl oxidase, lysyl oxidase-like 2 and IL-17 receptor A are also recognized markers associated with the activated phenotype of qHSCs, and are upregulated following activation (50-54).

HSCs also secrete chemokines to recruit cells and regulate the local immune microenvironment (55). Current studies have reported that HSCs expressed chemokine receptors, such as C-C chemokine receptor type (CCR)5, CCR7 and C-X-C motif chemokine receptor (CXCR)3, and secreted the chemokines C-C motif chemokine (CCL)5, CCL3, CCL2, C-X-C motif chemokine ligand (CXCL)10, CXCL9 and CXCL8 (56,57).

**Immune function of HSCs.** HSCs serve an important role in regulating the immune environment in the liver (58). The liver is rich in macrophages, which are an important innate immune system response cell group physiologically (59,60). The interactions between macrophages and HSCs were discovered to serve vital roles in the development of liver

diseases; for example, a previous study revealed that HSCs induced monocytes to differentiate into specific CD14<sup>+</sup>/human leukocyte antigen-DR<sup>-</sup> phenotypes when activated HSCs were co-cultured with mature peripheral blood mononuclear cells (61). HSCs are activated via pattern recognition receptor pathways, such as Toll-like receptors (TLR)4 and TLR2, inflammatory markers produced by hepatic macrophages (TNF- $\alpha$ , NLR family pyrin domain containing 3, IL-1 $\beta$ , IL-6 and CCL5) and chemokines (CCL2, CCL8 and CX3 chemokine receptor 1) that directly influence HSC activation (62). A previous study illustrated that NK cells served an important role in inhibiting liver fibrosis by producing the antifibrotic cytokine IFN- $\gamma$  to selectively kill or age the early-activated HSCs (63). After the activation of early HSCs, vitamin A is metabolized to retinoic acid, and the expression of retinoic acid early inducible gene 1 is upregulated, which in-turn promotes the killing ability of NK cells (64). Increased retinoic acid metabolism in late-activated HSCs was discovered to inhibit the IFN- $\gamma$ /STAT1 signaling pathway, increase the secretion of TGF- $\beta$ , inhibit the activity of NK cells and reduce the antifibrotic function of NK cells (43,65). It was reported that activated HSCs functioned as antigen presenting cells, which triggered T cell proliferation (27). HSCs were also discovered to express the immunomodulatory programmed cell death receptor 1, which binds to programmed death-ligand 1 presented on the T cell surface to induce T cell apoptosis (44,66). In summary, HSCs possess a diverse and complex array of functions, serve an important role in the liver microenvironment, participate in a variety of physiological and pathological reactions and are closely associated with changes in immune function.

## 3. Role of HSCs in the process of HCV infection

Chronic HCV infection can cause liver damage and result in a range of mild to more severe diseases, such as chronic hepatitis C (CHC), fibrosis and cirrhosis (67). The activation of HSCs was identified as an important signal in the control of extracellular matrix synthesis and degradation in HCV-induced liver fibrosis (68). At the cellular level, the activatory properties on HSCs are considered to be associated with the amino domain of HCV core protein (69). Furthermore, HCV infection was discovered to stimulate the innate immune response, and changes in the functions of HSCs were also affected by other important immune cells, such as NK cells, natural killer T (NKT) cells and macrophages (70,71). At the molecular level, during HCV infection, two types of cytokines that are closely associated with the activation of HSCs were discovered to serve different roles; one type of chemokine primarily promoted fibrosis, such as CXCL9, CXCL10, CXCL11, IL-4, IL-13, IL-17 and TGF- $\beta$ , while the other type of cytokine contributed to the inflammatory response, such as IL-5, IL-20, IL-22, IFN- $\gamma$ , TNF- $\alpha$  and CCL5 (72). At the mRNA level, previous studies have illustrated that several different microRNAs (miRNAs/miRs) were abnormally expressed in HCV-induced liver fibrosis, such as miR-16, miR-21, miR-122, miR-150, miR-214 and miR-221, where they were involved in the activation of HSCs (Fig. 1) (73-75).

**Properties of immune cells in CHC infection.** HSCs can express several HCV co-receptors that interact with the HCV proteins

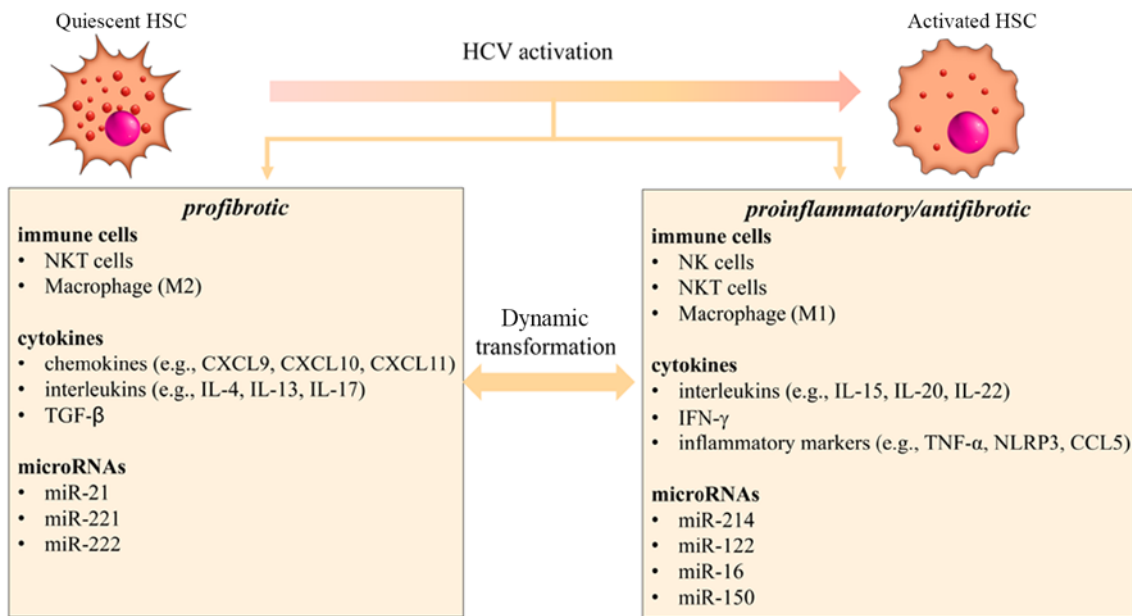


Figure 1. Indicators associated with HSCs in patients with CHC infection. In HCV-related liver fibrosis, the activation of HSCs is a dynamic process. On the one hand, NK cells generally help clear senescent-activated HSCs, including via directly killing of activated HSCs and by producing IFN- $\gamma$ . M1 macrophages promote antifibrotic differentiation of HSCs and are associated with proinflammatory response, which involves proinflammatory and antifibrotic cytokines, such as IL-5, IL-20, IL-22, IFN- $\gamma$ , TNF- $\alpha$ , NLRP3 and CCL5. Antifibrotic miRNAs include miR-214, miR-16, miR-122 and miR-150. On the other hand, NKT cells and M2 macrophages exert profibrotic effects by producing CXCL9, CXCL10, CXCL11, IL-4, IL-13, IL-17 and TGF- $\beta$ . Profibrogenic miRNAs include miR-21, miR-221 and miR-222. HSC, hepatic stellate cell; HCV, hepatitis C virus; CHC, chronic hepatitis C; NK, natural killer; NKT, natural killer T; CXCL, C-X-C motif chemokine ligand; miR, microRNA; CCL5, C-C motif chemokine ligand 5; NLRP3, NLR family pyrin domain containing 3; M1, classically activated; M2, alternatively activated.

to promote liver fibrosis (76). For example, the binding of the HCV E2 protein and CD81 on the surface of HSCs may result in an increase in the fibrogenic effects occur to HSCs (77,78). In addition, the expression of HCV core and NS3-NS5 proteins was suggested to promote HSC proliferation and induce the secretion of proinflammatory cytokines in HSCs, such as IL-8 and monocyte-chemotactic protein-1 (79,80).

Exosomes are small extracellular vesicles that are secreted by the majority of cells through the endocytic pathway; however, there is no direct contact between the different cells. Exosomes carry different biomolecules and are therefore an important vehicle for intracellular and intercellular communication (81). Notably, previous studies have confirmed the existence of exosome-mediated communications between HCV-infected hepatocytes and HSCs (82,83). Exosomes secreted from HCV-infected hepatocytes (HCV-exo) were discovered to possess the potential to activate HSCs (84). A high expression of miR-19a in exosomes was observed from HCV-exo, which in turn enhances fibrosis marker genes and activates the STAT3-mediated TGF- $\beta$  signaling pathway (85). In a previous study on the activation and function of NK cells in the pathogenesis of HCV infection, hepatocytes were reported to produce a host of cytokines, including IFN- $\alpha/\beta$ , which activated NK cells and enhanced NK cell-mediated cell cytotoxicity (86). NK cells generally display antifibrotic properties, including the inhibition of liver fibrosis by selectively expressing death receptor ligands for the receptors on activated HSCs and by producing the antifibrotic cytokine IFN- $\gamma$  (87). In addition, NKT cells were revealed to perform similar antifibrotic functions as NK cells by killing HSCs and producing IFN- $\gamma$  (88). However, NKT cells also produce

profibrotic cytokines to promote liver fibrogenesis; for example, the secretion of IL-4 and IL-13 from NKT cells were significantly increased in patients with HCV with cirrhosis (89). Macrophages are divided into two phenotypically and functionally distinct subsets, classically and alternatively (M2) activated macrophages (90). M2 macrophages are considered to possess anti-inflammatory and profibrotic effects by producing profibrotic cytokines (CCL3, CCL5, TGF- $\beta$  and TNF- $\alpha$ ) (91). Clinically, it has also been reported that the expression levels of CCL5 are upregulated in the serum and liver of patients with HCV (56).

*Effect of chemokines and ILs in CHC infection.* Chemokines are divided into four groups: CC, CXC, CX3C and C families (92). HSCs primarily express receptors, such as CXCR4 and CXCR3, and can secrete chemokines, such as CXCL10, CXCL9 and CXCL8, which participate in the induction process of liver fibrosis by recruiting inflammatory immune cells and inhibiting the secretion of collagen I by HSCs, all of which exhibit immunomodulatory functions in the liver (93-95). Following HCV infection in the liver, CXCR4 expression levels were revealed to be upregulated, which subsequently activated HSCs (96). CXCR4 was found to bind to its ligand CXCL12 to stimulate the activation of HSCs, increasing the secretion of CXCL12 and leading to further proliferation and activation (97). Following the initial HCV infection, TLR3 and retinoic acid-inducible gene I were identified to activate CXCR3 ligands, such as CXCL10, in infected hepatocytes, thereby increasing their activity and stimulating the production of IFN- $\gamma$  (98). During acute HCV infection, the presence of CXCR3-related ligands, such as CXCL9, CXCL10, CXCL11

Table I. Comparison of different methods for detecting HCV.

Diagnostic marker	Technology	Advantages	Disadvantages	(Refs.)
HCV antibodies, HCV core antigen	ELISA, chemiluminescence immunoassays, gold immunochromatographic assay, recombinant immunoblot assay	Sensitive and specific.	Easily susceptible to interference and produces false positive and false negative results.	(140-143)
HCV-RNA	Reverse transcription-quantitative PCR, transcription mediated amplification	Higher specificity and often used as a confirmatory experiment.	Inaccurate quantification due to numerous sources of variation.	(17)
HCV genetic	PCR-sequence specific primers, line probe assay, PCR-restriction fragment length polymorphism	Helps to determine the establishment of different treatment methods.	Troublesome operation and expensive equipment and reagents.	(2,144,146)
Fibrosis	APRI, FIB-4 score	Improve prediction of advanced fibrosis and cirrhosis in patients with chronic hepatitis C infection.	Low sensitivity of APRI and FIB-4 in gauging improvements in liver fibrosis following therapy.	(23,154)
Other indicators	hematoxylin and eosin staining, immunohistochemical staining, abdominal ultrasound examination, CT, magnetic resonance imaging	More intuitive observation.	Invasive and only provides an auxiliary diagnosis.	(150,153)

HCV, hepatitis C virus; APRI, aspartate aminotransferase to platelet ratio index; FIB-4, fibrosis-4.

and other chemokines, increases rapidly within a few weeks of viral infection (99). For instance, Zeremski *et al* (100) demonstrated that CXCL9-11 induction began at 38-53 days and peaked at 72-83 days after viral infection. In the process of HCV infection, the upregulation of CXCL12, CXCL9, CXCL10, CXCL11 and other chemokines significantly accelerates the process of fibrosis, which gradually advances to cirrhosis and potentially even liver cancer, both of which are considerably harder to treat (101-103). Therefore, studies have proposed that chemokines, such as CXCL9, CXCL10 and CXCL11, related to CXCR3 in the peripheral blood of patients with CHC infection may be used as markers of fibrosis, and changes in their secretion may be useful to evaluate the degree of fibrosis for more targeted and effective treatment options (104-106).

IL-15 and its high-affinity receptor IL-15 receptor (R) $\alpha$  are widely expressed in immune cells and liver cells (107,108). NK cells, NKT cells and CD8<sup>+</sup> T cells generate IL-15 to maintain system homeostasis and promote liver regeneration (109). A previous study confirmed that IL-15 and IFN- $\gamma$  exhibited protective effects against HCV via the ERK signaling pathway *in vitro* (110). Jiao *et al* (108) reported that increased fibrosis was observed in IL-15R $\alpha$  knockout (KO) mice. Furthermore, the study demonstrated that collagen production was increased in HSCs isolated from IL-15R $\alpha$ KO mice. Therefore, IL-15 and IL-15R $\alpha$  may serve a protective role in the development

of liver fibrosis by regulating the expression levels of fibrotic molecules and collagen in HSCs and maintaining the balance of NK cells *in vivo*.

IL-17 was discovered to be important in the development of liver fibrosis in mice (111). It was reported that IL-17 was strongly associated with an improved prognosis in patients with CHC (112). Thus, IL-17 may be a therapeutic target for the treatment of fibrosis. IL-17 regulates fibrosis through two separate mechanisms. First, IL-17 stimulates macrophages to express the inflammatory cytokines IL-6, IL-1 $\beta$  and TNF- $\alpha$ , as well as the major fibrogenic cytokine, TGF- $\beta$ 1 (113). Additionally, IL-17 directly stimulates HSCs to express type I collagen and promotes their activation, and MFBs were discovered to be formed through the STAT3 signaling pathway during the fibrosis of HSCs (114-116).

IL-20, a proinflammatory cytokine in the IL-10 cytokine family, reportedly activates qHSCs and upregulates TGF- $\beta$  expression levels (117,118). In a mouse model of cell injury induced by CCL4, the use of antibodies to neutralize IL-20 or IL-20 receptors inhibited HSC activation and liver fibrosis, and downregulated TGF- $\beta$  production (119).

IL-22 is also a member of the IL-10 cytokine family that activates the STAT3 signaling pathway in hepatocytes, which has been illustrated to promote the development of HCC (120). IL-22 simultaneously expresses IL-10 receptor 2 and IL-22

receptor 1, both of which were identified to induce the senescence of HSCs, thereby improving liver fibrosis (121). Previous studies have revealed that the increase in IL-22 in the liver of mice reduced the expression of fibrosis-associated genes, and accelerated the recovery of the liver damage caused by fibrosis by increasing the number of senescent HSCs and decreasing the expression levels of  $\alpha$ -SMA (122-124).

**Role of miRNAs in CHC infection.** miRNAs are small non-coding RNAs of ~22 nucleotides in length that regulate post-transcriptional gene expression by altering mRNA degradation (125). The abnormal expression of different miRNAs, such as miR-122, miR-126, miR-129, miR-199a and miR-155, in HCV-induced liver fibrosis and HCC has been previously reported (126). Activated HSCs were discovered to express a low number of miRNAs ( $n=259$ ), of which 47 were downregulated and 212 were upregulated upon activation (127). Clinical data also revealed that miR-21 expression levels were associated with the viral load, fibrosis and serum liver transaminase levels (128). It was also identified that miR-221/222 expression levels were upregulated in the human liver, and the upregulation was dependent on the progression of fibrosis (129). In addition, the increased expression levels of miR-221/222 have also been confirmed in a mouse model of liver fibrosis (130). By contrast, antifibrotic miRNAs include miR-19 (85), miR-214 (131), miR-16 (132), miR-122 (133) and miR-150 (134), amongst others. For example, connective tissue growth factor 2 (CCN2) was discovered to drive fibrogenesis in HSCs (135), while in the fibrotic or steatotic liver, the upregulation of CCN2 was associated with the mutual down-regulation of miR-214 (131). The expression levels of miR-122 were also discovered to be negatively correlated with fibrosis, liver transaminase levels and patient age in another study (134).

#### 4. Indicators for the detection of CHC infection

**Serological test.** At present, the primary markers used to detect the presence of the HCV in the laboratory include HCV-Ab, HCV-cAg, HCV-RNA, and the presence of an HCV genotype and subtype (136,137). For the initial diagnosis, the most commonly used methods for detection are ELISAs and chemiluminescence immunoassays (CLIAs) for analyzing the presence of HCV-Ab in the blood, as they are relatively easy to perform and provide results quickly (138,139). However, the HCV-Ab test often provides false-positive results in patients with a chronic infectious disease (140-142). For example, it was reported that in 477 individuals with an anti-HCV response analyzed using a recombinant immunoblot assay (RIBA), 105 (22%) were confirmed as false positives (143). Thus, if a sample is reactive in the primary screening test, further tests are required to confirm this result. The additional tests usually used are RIBAs or nucleic acid amplification assays (NATs) (2,144). NATs are more specific than ELISAs or CLIAs and have a higher detection accuracy, but a shorter time window for detection (145). In addition, NATs require specific laboratory equipment and trained personnel; thus, it is difficult to perform this assay in conventional laboratories (146).

**Diagnosis of fibrosis.** Liver biopsies to measure liver fibrosis have been almost completely replaced by noninvasive methods, including the detection of biochemical markers,

such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), as well as scoring systems, such as the AST to platelet ratio index (APRI) score and fibrosis (FIB)-4 score (147,148). The scoring systems of APRI and FIB 4 are generally cheap and simple to use for the evaluation of liver fibrosis; however, FIB-4 and APRI have been shown to have a considerably higher rate of false-negatives or false-positives in the detection of both fibrosis and cirrhosis (20). Thus, the results should be further confirmed using more accurate tests.

**Other diagnostic methods.** Aberrant lymphocyte proliferation is a primary characteristic of CHC, with evidence of focal and bridging necrosis and lobular degeneration in the portal area (149). These lesions can be observed by pathological examination, such as hematoxylin and eosin staining and immunohistochemical staining (150). However, the pathogenesis and mechanism underlying the formation of liver lesions in the process of CHC infection has not been fully determined; therefore, it is difficult to accurately diagnose HCV infection through pathological methods (151). Other diagnostic methods, such as image-based examinations, including abdominal ultrasound examination, CT, magnetic resonance (MR) imaging or MR scans, can be used for the partial screening of liver diseases (152); however, these methods are not without their own problems. For example, regarding specificity, it is difficult to determine whether the presence of lesions was caused by the HCV infection or not and it is hard to accurately detect the course of HCV (153). A breakdown of the markers, methods and their advantages and disadvantages are described in Table I.

#### 5. Conclusions

Despite numerous studies investigating the role of HSCs in different models of liver fibrosis caused by various types of disease, there remains a lack of research into the changes in the characteristics and functions of HSCs following HCV infection. At present, routine laboratory serological examinations, pathological examinations and other methods are used for the diagnosis of HCV infection; however, each technique has its limitations. For example, considering that HCV infection is a dynamic process, the serum indicators are unstable and patients with varying degrees of HCV infection and courses have differing serum marker levels. Thus, a new test is required to supplement or replace preliminary screening, particularly in patients who are diagnosed as positive, to assess the extent and status of HCV infection. HSCs are a vital immune cell population activated during HCV infection. Following an HCV infection, the expression of specific molecular markers and chemokines or the secretion of cytokines associated with HSCs are synchronously altered. At present, to the best of our knowledge, there remains a lack of studies investigating the alterations to HSC-related indicators. If the expression of one or several of the markers are discovered to be consistently altered during the course of HCV infection, they may serve as a suitable marker to assess the stage of HCV infection and they may also highlight novel avenues for understanding and eventually treating an HCV infection.

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## Authors' contributions

WW reviewed the functional characteristics of the HSCs and the role of HSCs in the process of HCV infection; XZF wrote the introduction and the conclusion. XLH and XZF corrected the grammar of the manuscript; JFL reviewed the indicators for the detection of CHC infection section; and JMY edited the figure and table in the manuscript. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

Not applicable.

## Patient consent for publication

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## Competing interests

The authors declare that they have no competing interests.

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