REVIEWS

Adv Clin Exp Med 2015, **24**, 4, 671–677
DOI: 10.17219/acem/22913

ISSN 1899–5276

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Current Possibilities to Assess the Degree of Liver Fibrosis in Patients with Haemophilia Infected with HCV – Review

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Haemophilia is an entity, wherein the HCV infection rate is greater than in the general population and ranges between 70–90%. The majority of HCV infections were acquired by hemophiliacs in the 1980s, by the use of infected cryoprecipitate or fresh frozen plasma preparations. It is therefore highly likely that many of them, more than twenty years after the infection, have developed advanced liver disease. Until recently, in order to assess its severity, it was necessary to perform a liver biopsy. Currently, we observe rapid developments of non-invasive methods that are particularly useful in patients with bleeding disorders. The most popular include elastography (Fibroscan, SWE) and the algorithms based on measurements of the relevant blood parameters (e.g. FibroTest, Fibrometer, APRI index). Ease of implementation of these studies, no need for hospitalization of the patient or specific preparation for surgery, allow for quick and minimally invasive assessment of liver disease progression and classification of the patient to the appropriate antiviral therapy (**Adv Clin Exp Med 2015, 24, 4, 671–677**).

Key words: fibrosis, haemophilia, hepatitis C, non-invasive methods, liver biopsy.

Haemophilia and HCV Infection

Haemophilia is a disease in which the incidence of HCV infection is higher than in the general population and ranges from 70–90% [1, 2]. The incidence in the Polish population is comparable to that observed in the United States and Western Europe. The study of Windyga et al. which was carried out on 172 patients born between 1935–1990 (mean age 35.3 ± 11.6), diagnosed with severe haemophilia A or B, who had received intravenous infusions of fresh frozen plasma, cryoprecipitate and blood before 1991, demonstrated that 95% of respondents had been infected with hepatitis C. More than 77% of them have not eliminated the infection and continue to actively replicate the virus. It is therefore a significantly higher incidence as compared to the general population and analogous to the above-cited statistic [3]. The percentage of HCV-infected persons in Poland is estimated at 1.5%. It can be assumed that in our country there are approximately 1,000 patients with severe haemophilia and chronic hepatitis C infection.

The majority of HCV infections was acquired by hemophiliacs in the 1980s, by the use of infected cryoprecipitate or fresh frozen plasma preparations, which did not undergo any procedures for the inactivation of infectious particles at that time. Chronic hepatitis C infection leads to progressive liver fibrosis, cirrhosis and hepatocellular carcinoma [4]. It is therefore highly likely that many patients, more than twenty years after the infection, have developed advanced liver disease.

Methods of Evaluation of Liver Fibrosis

Previously, in order to assess the severity of liver disease, it was necessary to perform liver biopsy procedure. For several years, we have seen rapid developments of non-invasive methods of evaluating fibrosis and inflammation in the liver. They are particularly useful in patients with contraindications to performing invasive procedures, including patients with bleeding disorders.

Liver Biopsy

Liver biopsy remains the "gold standard" in assessing the severity of liver damage. Histopathological examination, obtained by biopsy, assesses the degree of fibrosis and the severity of inflammation in the liver. It is also an indispensable diagnostic tool that allows to further diagnose the ongoing intrahepatic processes, including autoimmune reactions, copper or iron-storage [5]. However, it is an invasive procedure. The complication rate is estimated at 0.3–0.6%, and the mortality rate at 0.05% [6]. The most common complications of the procedure are pain and asymptomatic bleeding (subcapsular and intracapsular hematomas). Very rarely, there is bleeding into bile ducts, puncture of other organs, like lungs (to form a pneumothorax, subcutaneous edema, pleural effusion) kidney, colon or gallbladder [7].

A significant limitation of liver biopsy is the chance of puncturing of non-diagnostic sample. Its representative length should be 1.0 cm. Obtaining a shorter sample limits the correct interpretation, especially the reliable assessment of the degree of fibrosis. Obtaining a reliable biopsy sample may be difficult also because of the varying degree of fibrosis in the liver and depending on the location of the sample. Regev et al. demonstrated that non-uniformly extending liver fibrosis is a potential limitation of the liver biopsy. In this study on a group of 124 patients with chronic hepatitis C infection liver biopsies obtained laparoscopically from both the right and the left lobe of the liver were histologically assessed. Significant differences were shown in 41 patients (33.1 %) in the results of the assessment of fibrosis (at least one degree of difference in terms of fibrosis according to the Scheuer`s scale) [8]. Bedossa et al., in turn, demonstrated that a biopsy sample length of a minimum of 25 mm increases the accuracy of the assessment of fibrosis by partial eliminating the above-mentioned limitations [9]. They also showed that in up to one third of respondents histological sections may be misdiagnosed [8, 9]. Therefore, the liver biopsy, because of the above-mentioned limitations, primarily its invasiveness, is a mediocre tool in assessing the dynamics of the degree of organ damage.

Non-Invasive Methods

Consequently, there is a growing interest in using non-invasive methods to assess the degree of fibrosis and inflammation in the liver. Easy application and no need for hospitalization of the patient or specific preparation for surgery allow for a quick and minimally invasive assessment of liver disease progression, and classifying the patient to the appropriate antiviral therapy. In addition, several studies have shown the advantages of these methods over liver biopsy in the assessment of the degree of liver damage [10, 11]. In the study of Poynard et al., the degree of compatibility between non-invasive methods such as Fibrotest and ActiTest and liver biopsy was evaluated [11]. A discrepancy was revealed in the results in 29% of patients, with 18% resulting from erroneous biopsy of the liver, and only 2.4% from the interpretation of non-invasive testing methods. Nevertheless, histopathological examination of the liver remains up to date the decisive method.

Elastography

Fibroscan

Fibroscan (TE – transient elastography; one-dimensional transient elastography) is an increasingly commonly used elastographic method, serving non-invasive measurement of the degree of stiffness of the liver, indirectly assessing the degree of fibrosis. It involves assessing the velocity of propagation of an elastic shear wave generated by the mechanical impulse from applied transducer. This rate depends on the cohesiveness of the liver, and the degree of cohesiveness increases in direct proportion to the degree of fibrosis. The velocity measurements are computer-processed and the final result is the average of 10 measurements. Known limitations of this method are: a significant degree of obesity, pregnancy, and the presence of ascites [12].

It has been shown that there is a good correspondence between this method and the stage of liver fibrosis [13, 14]. In the most recent recommendations of the European Association for the Study of the Liver elastography is mentioned as a method with a high degree of reliability in assessing the severity of chronic hepatitis C [15]. In Poland since 2011, according to the regulation of the Ministry of Health, this study can be used as an alternative to liver biopsy in the assessment of

eligibility of patients for treatment programs of chronic hepatitis B and C. Many authors believe that this is the most appropriate tool for diagnosing liver cirrhosis. In earlier stages of liver fibrosis the differences between obtained results are less pronounced [16, 17].

Shear-Wave Elastography

Shear-wave elastography (SWE) is the latest method of assessing the severity of fibrosis. Like one-dimensional transient elastography (TE), it is based on the measurement of the velocity of the induced shear wave in the imaged tissues. The essence of SWE is to generate a number of shear waves at different depths, which interfere with each other to form a cone-like shape shear wave. Due to a very high speed of acquisition the velocity of propagation of the interference wave can be measured. The software then generates a map of the velocity of propagation of the wave and calculates the relative hardness of the organ. The measurement results are expressed in kPa or m/s and also visualized by applying a color image on a conventional ultrasound image. The operator performs several measurements, with mean value calculated, on the basis of which (as in the case of Fibroscan) the degree of fibrosis is determined using Metavir scale. The advantages of this method are much independence from the investigator, its speed and overall safety of the patient. In addition, the operator is able to assess the morphology of the organ (to detect potential focal changes) and, if necessary, measurements of flow in the portal vein system can be performed. The operator can also choose the best places, where the measurements will be made (avoiding large vessels or capsula of the liver), which further increases the reliability of the study. Use of the ultrasound beam to produce a transverse wave and ability to choose the location of the measurement the stiffness of the tissue allows us also to examine patients with ascites. As in the case of the transient elastography (Fibroscan), shear wave elastography is difficult to perform in patients with significant obesity. Several studies have confirmed the high diagnostic efficacy of the SWE method and compliance of the results of the biopsy, TE and FibroTest [18, 19].

Magnetic Resonance Elastography

Magnetic resonance elastography is another radiological method allowing for the quantitative measurement of liver stiffness and the assessment of the degree of fibrosis. The velocity of propagation of the mechanical stimulus (wave) in the examined tissue is measured with the help of magnetic resonance. Based on the collected data, the software calculates the hardness of the liver and allows us to determine the degree of fibrosis. MRE option is available as an add-on software to conventional MRI scanners. An important advantage of this method is complete independence from the operator, and the ability to make measurements in the whole organ, and not a few selected points. The disadvantages include the long duration of the study, the high price of software and difficulties in availability of this method [20, 21].

FibroTest

FibroTest is another method, an alternative to the liver biopsy, which can be used in patients with chronic hepatitis C. The method involves determining serum alfa 2-macroglobulin, haptoglobin, apoliprotein A-1, total bilirubin, GGTP, and then calculating (patent protected) the indicator of fibrosis. In addition, the determination of GTP allows us to perform an ActiTest, evaluating necroinflammatory activity in chronic viral hepatitis. The limitations of this method concern disorders in which there is a decrease or increase of the value of these parameters. This might include: severe hemolysis, acute hepatitis, massive hepatic necrosis, acute inflammation associated with viral or bacterial infection, extrahepatic cholestasis and hypercholesterolemia with high concentrations of HDL. The value of the method is determined by the value of AUROC (area under the receiver operating characteristic), which also allows us to assess its sensitivity and specificity. In the case of FibroTest AUROC = 0.84 is achieved as compared to the results obtained by liver biopsy [22, 23].

Other Non-Invasive Methods

Other non-invasive methods applied in chronic hepatitis C include determination of serum fibrosis markers or more complex algorithms taking into account the values of the selected markers. The former are divided into two groups: direct and indirect ones. Direct markers of fibrosis include: (1) the concentration of hyaluronic acid [24], (2) laminin [25], (3) some of the collagen proteins (such as PNP III, type IV collagen), [26] (4) collagenase or their inhibitors (e.g. MMP, TIMP-1) [27, 28]. The latter include, among others, (1) the number of platelets (2) prothrombin activity, (3) the GOT/GPT ratio.

There are also algorithms based on the calculation of the relevant parameters of blood. These include: (1) APRI index, taking into account the activity of GOT and platelet count, (2) FIB-4, which also takes into account the age of the patient. There are also more complex algorithms, for instance Fibrometer, which takes into account

the values of parameters such as platelet count, prothrombin time, GOT, alpha 2-macroglobulin concentration, hyaluronic acid, urea, and age. Another algorithm of this type is the Hepascore model. It is based on the calculation of parameters such as serum bilirubin, GGTP, alpha 2-macroglobulin, hyaluronic acid, the age and sex of the patient [29, 30]. Table 1 shows the most popular models.

Table 1. Selected non-invasive models to assess liver fibrosis

FibroTest	alfa 2-macroglobulin, GGTP, apolipoprotein A-1, haptoglobin, total bilirubin, age, sex
APRI	GOT, PLT
Hepascore	bilirubin, GGTP, hyaluronic acid, sex, age
Fibrometer	PLT, prothrombin time, GOT, alfa 2-macroglobulin, hyaluronic acid, urea, age
Forns Index	age, PLT, cholesterol, GGTP
ELF (Enhanced Liver Fibrosis score)	age, hyaluronic acid, MMP-3, TIMP-1
FibroSpect	alfa 2-macroglobulin, hyaluronic acid, TIMP-1
MP3	MMP-3, TIMP-1
FIB-4	ALT, AST, PLT, age

AUROC values for the above methods in advanced liver fibrosis are 0.72–0.78, and are slightly higher in cirrhosis, AUROC between 0.77–0.86.

Limitations of these methods stem from a lack of specific markers for liver fibrosis. Either an increase or decrease of the mentioned parameters for reasons independent of liver fibrosis, can significantly affect the test result. An example is the increased bilirubin level in patients with Gilbert's syndrome, lower concentration of haptoglobin in patients with hemolysis, or increased concentration of hyaluronic acid after a meal or in patients with chronic inflammatory processes such as rheumatoid arthritis. Studies have shown that hypercholesterolemia can lead to a false result of Forns index. It is based on the calculated parameters such as platelet count, age, GGTP and cholesterol level. In contrast, normal GOT activity may prevent the proper assessment of fibrosis by APRI index. Hence, it is difficult to assess the overall consistency of results of liver fibrosis, which is obtained by different methods.

A Comparative Study of Methods to Assess Liver Fibrosis

The study of Caster et al. found the compatibility of the results obtained by two methods, i.e. using FibroTest and TE (Fibroscan), ranging from 70 to 79% [31]. It was also shown that if the results of both methods are consistent, then a liver biopsy confirms the previously established diagnosis in 84% of cases for F ≥ 2, in 95% for F ≥ 3, and in 94% for F = 4, respectively (based on a 5-point METAVIR scale, where F = 0 indicates no fibrosis, F = 4 is liver cirrhosis). It was found thus that the combination of both non-invasive methods assesses hepatic fibrosis more accurately than in the case when each of them is used separately. [37].

Similar results were obtained in a study by Mayor et al., in which histopathological examination of the liver was not performed, and non-invasive methods i.e. TE and Fibrotest were used to assess the degree of liver damage. The study demonstrated a gradual increase in the rate of compliance index in ever more advanced stages of fibrosis. When fibrosis was assessed for F ≥ 2, for F ≥ 3 and for F = 4, this index was 62%, 69% and 85%, respectively [32].

The study of Ferraioli et al. rated the accuracy of results obtained by both elastographic methods i.e. FibroScan (TE) and SWE elastography, compared to results obtained by liver biopsy. This was the first study comparing the two elastographic methods with liver biopsy performed on the same day. The study was performed on a group of 121 patients with chronic hepatitis C infection. Patients co-infected with HIV, with overt cirrhosis and with ascites were excluded from the study. It has been shown that SWE is more accurate in assessing of significant fibrosis i.e. for F ≥ 2 as compared to TE study, although there was no significant difference in the accuracy of assessment of tissue stiffness for advanced fibrosis for F ≥ 3. Moreover, the SWE correctly assessed the severity of fibrosis in 83.1% of patients, and TE in 66.7% of patients, while the discrepancy in results between TE and liver biopsy was probably a result of an incorrect result of histological examination [33].

Poynard et al. compared the results obtained by both elastographic methods, i.e. TE and SWE, and FibroTest. The study was conducted in 422 patients. It was shown that the most appropriate method was FibroTest, and the value of the 2 elastographic methods was comparable ("applicability" – 97.9% for the FT vs about 90% for the TE and SWE). In the case of liver cirrhosis results obtained

by all methods were comparable, while in patients with ascites, SWE method was more useful than TE [34]. Further, it has been demonstrated that it is useful to combine different non-invasive methods in order to increase the accuracy of the result. This gives the opportunity to withdraw from performing a liver biopsy in situations where there is a risk of significant complications.

In the observations made by Boulier et al. of a group of 235 patients with chronic hepatitis C, hepatic fibrosis was evaluated by noninvasive tests such as FT, APRI, Forns index and by histological examination of liver biopsy sample. All tests were performed on the same day. It was shown that the overall consistency of the results using all 4 methods was obtained only in 29% of patients, while using only non-invasive methods – in 35% of patients. The use of all three methods on the other hand made it possible to establish correct non-invasive assessment of fibrosis in 191 patients (81.3%), reducing the need for a biopsy to 44 patients (18.7%).

Similar observations were obtained in the SAFE (Sequential Algorithm for Fibrosis Evaluation) study. APRI index and FibroTest were used to determine the degree of liver fibrosis. An increase in precision of the test of up to 90% was achieved, thus reducing the need for liver biopsy by 47% of the patients [35].

Another way to strengthen the reliability of the measurement of liver fibrosis by non-invasive methods is the use of practical algorithms. In the available publications, there are primarily 2 of them discussed – Halfon algorithm and IQR/LSM ratio [11]. The former has been shown to have a high predictive value as to the accuracy of the determinations by FibroTest. According to this algorithm, if the result of the assessment of fibrosis obtained by FibroTest is in conformity with the result of APRI or Forns index result, it can be relied on. The latter algorithm, proposed by Lucidarme et al, was developed after analyzing factors responsible for the inconsistency of the results obtained from liver biopsy and elastography [36]. It is an indicator allowing for improved reliability of results obtained by FibroScan. Using these 2 different algorithms, Mayor et al. have created another one, which they claim allows reliable assessment of the anatomic condition of the organ in almost 87% of subjects with severe fibrosis [38].

Assessment of Liver Fibrosis in Patients with Haemophilia Infected with HCV

Although liver biopsy is not absolutely contraindicated in patients with hemophilia, and studies did not show an increased number of complications in this group of patients, this procedure is much less frequently performed in patients with bleeding disorders [37]. It involves additional costs of hospitalization, resulting from the need of transfusion of the respective coagulation factors. In view of the fact that non-invasive methods are being used more and more often, individuals with bleeding disorders have a better chance of diagnosis, without having to undergo a liver biopsy.

In the study of Mayor et al., FibroTest was used to evaluate hepatic fibrosis in 132 patients with haemophilia and positive anti-HCV antibodies. It has been shown that in patients infected with HCV with underlying bleeding disorder advanced fibrosis at the level of F3 was present in 13% of patients, and liver cirrhosis in 17% [38]. Similar results were obtained by Posthouwer et al., who conducted a study in 124 patients with chronic hepatitis C (average duration of infection was 34 years, range 14–40 years). They used an elastographic method, finding cirrhosis in 17% of patients, and advanced fibrosis in 18% of patients [39]. This was much more than suggested by the results of laboratory tests and ultrasound. Based on these latter studies, the presence of liver cirrhosis was estimated in only 7% of respondents.

Summary

Opportunities of assessing liver fibrosis by non-invasive methods are growing. Therefore, it seems sensible to use them primarily in patients with bleeding disorders. While currently there are no strict guidelines as to the choice of method in haemophiliac patients, the validity of combining different non-invasive methods and the use of practical algorithms is being emphasized in order to overcome their shortcomings. None of the studies used individually can be perceived as the gold standard.

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Conflict of interest: None declared

Received: 1.02.2014
Revised: 4.03.2014
Accepted: 18.06.2014