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Hyaluronic acid in the evaluation of liver fibrosis in patients with hepatitis C on haemodialysis

ABSTRACT

Background: This study evaluated the role of HA as a marker of liver fibrosis in patients with hepatitis C on haemodialysis. **Methods:** This is a cross-sectional study in which 52 patients were divided into two groups: Group 1: patients with hepatitis C and end-stage renal disease (ESRD) undergoing haemodialysis (n = 23); and Group 2: patients with hepatitis C without ESRD (n = 29). Plasma levels of HA were associated with histological data of the samples obtained by liver biopsy and classified by METAVIR group scoring system. **Results:** Higher plasma levels were significantly correlated to significant liver fibrosis (METAVIR ≥ F2). In Group 1, the HA cutoff to discriminate significant fibrosis was 984.8 ng/mL, with accuracy, sensitivity and specificity of 80.8%, 83.0%, and 70.0%, respectively. In Group 2, the HA cutoff was 222.3 ng/mL, with accuracy, sensitivity and specificity of 74.5%, 70.0%, and 94.0%, respectively. **Conclusion:** HA was an accurate noninvasive marker in predicting significant fibrosis in patients with hepatitis C on haemodialysis.

Keywords: liver fibrosis, hyaluronic acid, end-stage renal disease, haemodialysis, hepatitis C.

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INTRODUCTION

Chronic hepatitis C virus (HCV) infection is common in patients on haemodialysis (HD) and has been associated with chronic liver disease in this setting. The prevalence of HCV infection in HD units ranges, according to geographical area, from 9% to 80% worldwide.[1] In Brazil, several dialysis centers[2-4] reported rates from 11% to 52%. The transfusion-associated transmissions have been reduced due to the implementation of blood donor screening for hepatitis C in the early 1990s and the use of recombinant erythropoietin. Currently, nosocomial transmission is the main cause of HCV infection in this population.[5,6]

The mortality rate of HCV infected HD patients is higher than non-infected subjects.[7,8] Candidates for renal transplantation have to be treated for hepatitis C before surgery, since HCV infection has a negative impact on graft and patient survival.[9] The degree of fibrosis evaluation is paramount for treatment decision.

Liver biopsy remains the gold standard procedure for diagnosing liver fibrosis. However, it is an invasive method associated with sampling error, interobserver variability and potential complications.[10] In HD patients, the rate of complications is elevated due to higher risk of bleeding secondary to haemostatic disorders.[11,12] In a recent study, severe complications of liver biopsies were demonstrated in 13.2% of HD patients.[13] Therefore, there is a need to assess the utility of noninvasive markers of fibrosis in this population.

Hyaluronic acid (HA) has been used to predict liver fibrosis in patients with chronic hepatitis C.[14-17] APRI (aspartate-aminotransferase to platelet ratio index) is another promising liver fibrosis marker in hepatitis C.[18-20] In a recent study, APRI identified significant liver fibrosis (METAVIR ≥ F2) with accuracy of 80.1% in patients with hepatitis C on haemodialysis.[21]

However, the value of HA in the diagnosis of liver fibrosis in this population with HCV infection has yet to be defined. The aim of this study was to evaluate the role of HA as a noninvasive marker of fibrosis in patients with chronic hepatitis C on haemodialysis.

PATIENTS AND METHODS

This is a cross-sectional study of patients attending an Infectious Diseases Outpatient Clinic and Haemodialysis Unit at the Hospital of the Universidade Federal de Minas Gerais-HC-UFMG (Belo Horizonte, Brazil).

Patients with chronic hepatitis C and end-stage renal disease (ESRD) on haemodialysis are referred to our outpatient clinic to be evaluated and treated for hepatitis C. Our current practice is to treat hepatitis C according to the degree of fibrosis found in liver fragments obtained by needle liver biopsy. From May 2000 to October 2007, 69 patients with chronic hepatitis C and ESRD on HD who underwent liver biopsy in a previous study[13] were registered under our care. Among those subjects, all individuals who had stored plasma sample (used in HCV-RNA detection) and liver fragments collected within one year by liver biopsy were considered for enrollment. These patients were selected for group 1 (hepatitis C and ESRD on HD). The other 67 consecutive patients with chronic hepatitis C without ESRD who had undergone liver biopsy for treatment evaluation and collection of plasma sample were also considered for enrollment (group 2).

The study was approved by the Ethics Committee of Universidade Federal de Minas Gerais (Belo Horizonte, MG, Brazil). A written informed consent was obtained from each participant prior to commencement of the study.

Criteria for selection of subjects

The inclusion criteria for subjects of groups 1 and 2 were availability of liver fragments obtained by liver biopsy and a plasma sample collected within one year; age between 18 and 70 years old; presence of chronic HCV infection (defined as a reactive anti-HCV antibodies for more than six months and a positive HCV-RNA by PCR); absence of other chronic liver diseases such as hepatitis B (defined as negative reaction to HBV surface antigen and HBV core antibody), auto-immune hepatitis (negative reaction to antinuclear, anti-smooth muscle, anti-mitochondrial and anti-liver-kidney microsomal antibodies), schistosomiasis mansoni (no previous history and negative stool examination), negative reaction to anti-HIV-1/2 (EIA); no previous history of regular use of hepatotoxic drugs or alcohol abuse (> 40 g of alcohol/day). Patients previously treated for hepatitis C were excluded.

Socio-demographic, epidemiological and clinical data

Baseline data were collected at the time of liver biopsy (in Groups 1 and 2) including: age, gender and length of time under haemodialysis (in group 1).

Laboratory data

Levels of aminotransferases (alanina-aminotransferase-ALT and aspartate-aminotransferase-AST), hemoglobin, platelets, and albumin were collected at the time of liver biopsy in groups 1 and 2. Parathormone (PTH) was measured in group 1.

Assay for anti-HCV antibodies was determined using enzyme immunoassay EIA III (Abbot Laboratories, North Chicago, IL, USA). For RNA extraction, blood HCV-RNA was detected by transcription followed by nested-RT-PCR using primers derived from the 5'-UTR non-coding region of the HCV. The genotype was determined through Restriction Fragment Length Polymorphism (RFLP) analysis of the PCR product.

Histological analyses

Liver samples were obtained by percutaneous ultrasound-directed hepatic biopsy. Two experienced pathologists examined (reviewed) liver fragments in a blinded manner (they knew the patients had hepatitis C but did not have any information about their clinical statuses). After disclosure of the diagnosis, the discordant cases were re-analyzed and a consensus was reached. The histological samples were analyzed using the METAVIR algorithm:[22] F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = few septa, F3 = numerous septa without cirrhosis, and F4 = cirrhosis. Stages of fibrosis were categorized as significant fibrosis (METAVIR ≥ F2) and absent or mild fibrosis (METAVIR < F2).

Plasma hyaluronic acid assay

HA plasma levels were assessed by a quantitative enzyme-linked antibody (HA-ELISA) and colorimetric detection with a commercially available test Kit (Echelon Biosciences®, Salt Lake City, USA). For this test, HA normal value was < 50 ng/mL.

Statistical analyses

Descriptive analyses were performed to determine the frequency of the categorical variables and the central tendencies of the continuous variables. Continuous variables were compared using the Students *t* test, or Kruskal-Wallis, as appropriate comparisons of groups. Categorical variables were compared using Chi-square or Fishers exact test. The relationships between HA levels and fibrosis stages were established using the Mann-Whitney test. Logarithmic transformation was used for HA levels. The accuracy of HA to predict the presence or absence of significant fibrosis (METAVIR ≥ F2) was tested by measuring the area under the receiver operating characteristic curve (AUROC). The best cutoff point was determined based on this curve. A p-value of less than 0.05 was considered statistically significant. Statistical analysis was performed by SPSS software version 12.0 (SPSS Inc., Chicago, IL).

RESULTS

Socio-demographic, clinical and laboratory characteristics

Socio-demographic, clinical and laboratory features of the patients are shown in Table 1.

A total of 136 patients were considered for enrollment in groups 1 and 2. However, the plasma samples were not collected within one year of liver biopsy in 20 and 12 in groups 1 and 2 patients, respectively. Other 22 patients of group 1 were not included because of the following reasons: age < 18 or > 70 years (n = 2), schistosomiasis (n = 7), hepatitis B (n = 3), use of hepatotoxic drugs (n = 6), and auto-immune hepatitis (n = 1). In group 2, 26 patients were not included because of the following findings: age < 18 or > 70 years (n = 2), schistosomiasis (n = 4), alcohol abuse (n = 8), use of hepatotoxic drugs (n = 4), hepatitis B (n = 4), previously treated for hepatitis C (n = 4).

Baseline characteristics (age, gender and time on haemodialysis in group 1) of patients who were excluded due to unavailable plasma sample collected at least one year of liver biopsy were compared to the characteristics of the included participants in groups 1 and 2. In group 1, excluded patients were significantly older than those included (p = 0.01), whereas in group 2 no difference was observed between included and excluded patients.

A total of 52 patients have been selected for this study: group 1 (Hepatitis C and ESRD, n = 23) and group 2 (Hepatitis C and no ESRD, n = 29).

The two groups were similar regarding age and gender. None of the included patients had clinical manifestations of liver failure. There was a predominance of genotype 1, being of 95.7% in group 1 and of 79.0% in group 2. ALT and AST levels were significantly lower in patients with hepatitis C and ESRD , when compared to patients without ESRD. These patients also had a lower level of albumin and hemoglobin (Table 1).

Table 1. Distribution of socio-demographic, clinical and laboratory features of 52 patients included in groups 1 (hepatitis C and end-stage renal disease) and 2 (hepatitis C alone)

Characteristics	Group 1 (Hepatitis C and ESRD) n = 23	Group 2 (Hepatits C alone) n = 29	p-value*
Age (mean ± SD years)	43.47 (± 12.37)	46.07 (± 12.39)	0.74
Gender			
Male n (%)	11 (48.1)	18 (62.1)	0.45
Female n (%)	12 (51.9)	11 (37.9)	-
Skin color			
White n (%)	07 (30.4)	13 (44.8)	-
Black n (%)	05 (21.7)	01 (3.4)	0.67
Others n (%)	11 (47.8)	15 (51.7)	-
Time of haemodialysis (years, mean ± SD)	9.67 ± 5.00	-	-
ALT** (U/L median)	37.0	74.0	0.00
AST*** (U/L median)	29.0	53.3	0.00
Haemoglobin (g/dL median)	11.5	15.6	0.00
Platelets (/mm^3 median)	194,000	212,000	0.51
Albumin (g/dL median)	3.7	4.3	0.00
PTH**** (pg/L mean)	381.04	-	-

*Student *t* test, Fishers exact test, Chi-square test, as appropriate comparisons of groups.
**ALT: alanine-aminotransferase.
***AST: aspartate-aminotransferase.
****PTH: Parathormone (normal value: below 69 pg/L).

Histological data

The mean number of portal tracts in the biopsies was 9 in both groups. Steatosis was observed in 55.2% of group 2 patients, and hepatic siderosis in 56.5% of group 1 subjects. Histological examination revealed significant fibrosis in 56.5% in patients of group 1 and in 44.3% in group 2. However, the frequency of advanced fibrosis and cirrhosis (METAVIR ≥ F3) was low in both groups (Figure 1).

Variables associated with significant fibrosis

Median of plasma HA levels in groups 1 and 2 were: 1546.3 ng/mL and 160.8 ng/mL, respectively ($p < 0.00$) (Table 2; Figure 2). In group 1, higher plasma levels of HA were correlated to significant liver fibrosis: the median of HA was 822.1 ng/mL in patients without significant fibrosis (METAVIR < F2), and 3,402.6 ng/mL in patients with significant fibrosis ($p = 0.01$). In group 2, the median of HA plasma levels

Figure 1: Frequency distribution of histological fibrosis stage according to the METAVIR score in 23 patients with end-stage renal disease (ESRD) and hepatitis C (group 1) and 29 patients with hepatitis C alone (group 2).

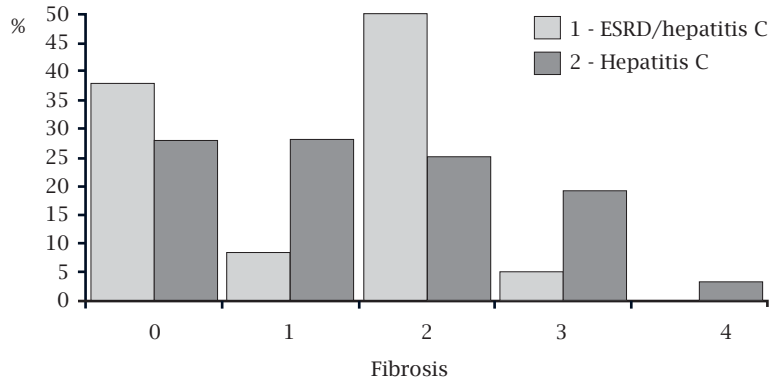


Figure 2: Box plot of logarithmic hyaluronic acid (HA) level in relation to study groups. There was significant difference between HA levels in groups 1 and 2 ($p = 0.00$).

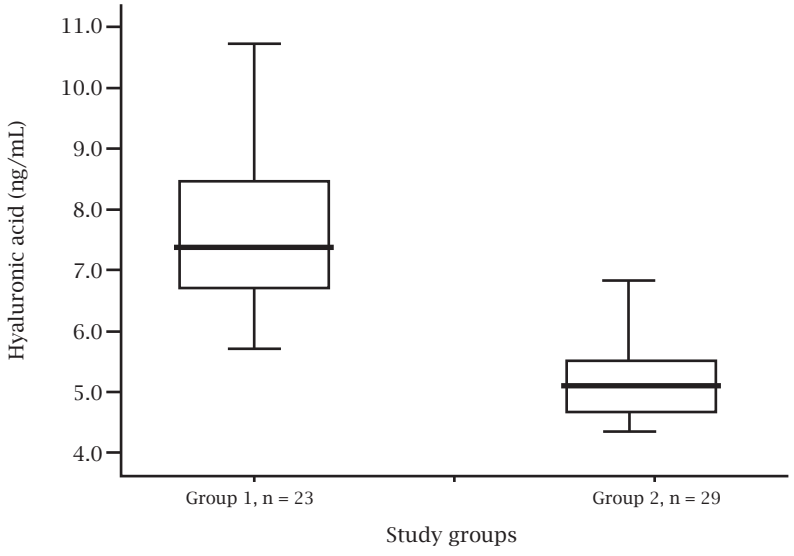


Table 2. Hyaluronic acid levels in groups 1 (hepatitis C and end-stage renal disease) and 2 (hepatitis C alone)

		Hyaluronic acid (ng/mL)					
Study groups	**n**	**Mean**	**Median**	**Minimal**	**Maximum**	**SD**	**p-value***
1 (ESRD**/hepatitis C)	23	5002.8	1546.3	302.5	3922.3	9455.9	0.00
2 (Hepatitis C)	29	268.3	160.8	80.9	1029.3	255.3	-

*Kruskal-Wallis.
** ESRD: end-stage renal disease.

was 141.0 ng/mL in patients without significant fibrosis and 277.6 ng/mL in patients with significant fibrosis (p = 0.02).

In group 1, only HA levels were correlated with the degree of liver fibrosis by METAVIR algorithm. There was no significant association between age, gender, AST and ALT levels with degree of fibrosis. In group 2, HA and ALT levels were significantly associated with degree of liver fibrosis (Table 3).

In Group 1, the HA cutoff applied to discriminate significant fibrosis was 984.8 ng/mL, with accuracy (AUROC), sensitivity and specificity of 80.8%, 83.0%, and 70.0%, respectively. In group 2, the HA cutoff was 222.3 ng/mL, with accuracy, sensitivity and specificity of 74.5%, 70.0%, and 94.0%, respectively (Figure 3).

Variables associated with plasma levels of hyaluronic acid in patients on hemodialysis

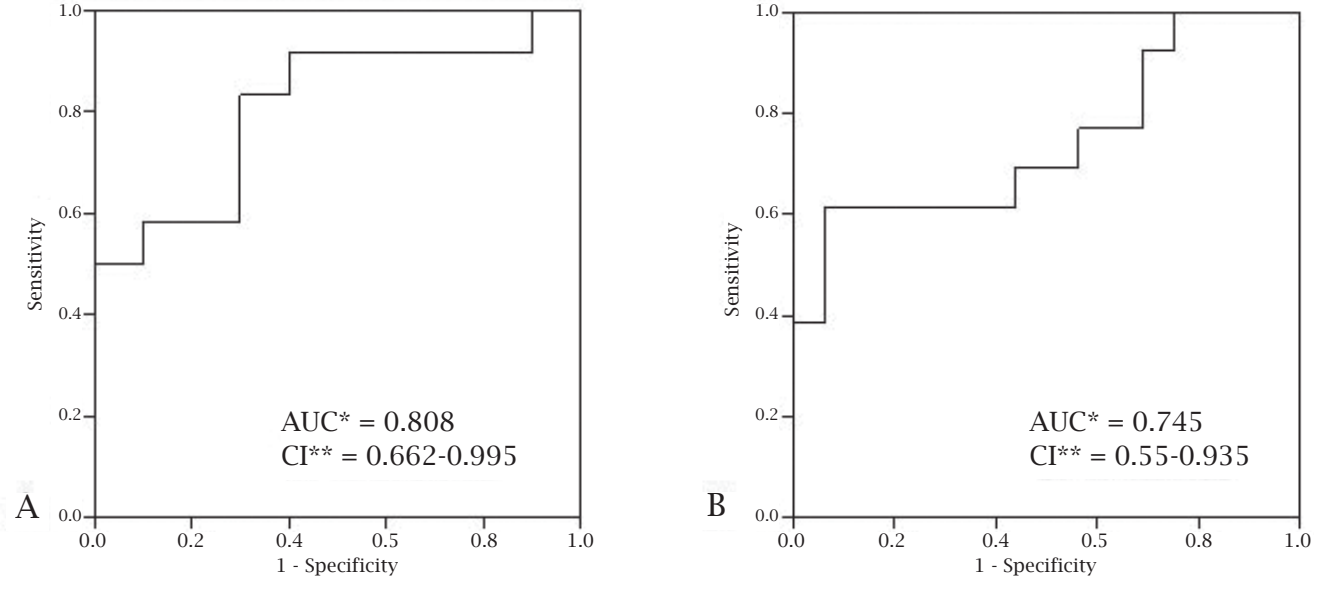
In group 1, only HA levels were correlated with the degree of liver fibrosis by METAVIR algorithm (p = 0.01). There was no significant correlation among age, gender and time of haemodialysis in this group. Hyperparathyroidism (PTH > 69 pg/L) was found in 95.7% of group 1 patients, but there was no significant correlation between serum parathormone (PTH) and HA levels.

Table 3. Variables associated with the degree of liver fibrosis (absent or mild: METAVIR < F2 or significant: METAVIR ≥ F2, in groups 1 (hepatitis C and end-stage renal disease) and 2 (hepatitis C alone)

Variables	Group 1 (n = 23)			Group 2 (n = 29)		
	METAVIR < F2	METAVIR ≥ F2	p-value	METAVIR < F2	METAVIR ≥ F2	p-value
Gender						
Male	3 (27.3%)	8 (72.8%)	0.21*	7 (38.9%)	11 (61.1%)	0.05*
Female	7 (58.3%)	5 (41.7%)	-	9 (81.8%)	2 (18.2%)	-
Age (years)†	41.5	46.0	0.44**	44.0	47.0	0.66**
AST (U/L)††	21.0	29.0	0.55**	44.0	49.0	0.07**
ALT (U/L)††	35.0	36.0	0.83**	56.0	91.0	0.01**
Hyaluronic acid (ng/mL)†	822.1	3402.6	0.01**	141.0	277.6	0.02**

† Median ; †† Mean.
* Fisher's exact test; ** Mann-Whitney test.

Figure 3: Receiver operating characteristic curves of hyaluronic acid for the prediction of significant fibrosis (METAVIR ≥ F2). A: group 1 (patients with end-stage renal disease with hepatitis C). B: group 2 (Hepatitis C alone).



*AUC: Area under the curve. **CI: Confidence interval.

DISCUSSION

In this study, plasma hyaluronic acid (HA) level was a good noninvasive marker of significant fibrosis in patients with hepatitis C on haemodialysis. For hepatitis C without ESRD, our findings are comparable to those reported previously.[14-17] For patients with ESRD, this is much more important than for patients with hepatitis C without co-morbidities. The frequency and severity of complications of liver biopsy in patients with renal failure has been documented.[11-13] Therefore, HA levels may be used to define when treatment for hepatitis C should be considered, especially in subjects with coagulation dysfunction caused by renal failure. Besides, it may be used as a screening test before a decision in favor of liver biopsy is to be presented to the patient.

Our findings contrast with the observations of a recent study.[22] In the latter, the diagnostic value of hyaluronic acid (HA) as a noninvasive marker of liver fibrosis was evaluated in 185 ESRD HCV-infected patients. For the prediction of significant fibrosis, the AUROC (0.798) of the regression model, including AST, platelet count and HA, was significantly higher than the AUROC of HA (0.650).

The specificity of HA in detecting significant liver fibrosis was lower in group 1 than in group 2. It may result from other factors that increase HA levels in ESRD patients. High plasma levels of HA have already been described in ESRD.[24-26] The increase of plasmatic HA in ESRD has been attributed to an "inflammatory state" caused by haemodialysis itself.[24] Notwithstanding, as observed for patients in group 1, plasma HA levels reached even higher levels in the presence of hepatitis C infection, validating the test in the identification of patients with significant liver fibrosis.

Patients on haemodialysis usually develop secondary hyperparathyroidism and it was found in most group 1 patients (Table 1). Parathyroid hormone (PTH) is a key hormone regulating bone mineral homeostasis and it also stimulates the production of HA in cultures of bone cells[24, 25] Nevertheless, in comparing PTH levels with HA levels in group 1 (13 patients with fibrosis and 10 without) no statistical correlation was found (i.e. high HA levels were associated with liver fibrosis, but not with PTH values). Certainly, a larger number of patients should be evaluated to confirm whether PTH, due to its action in bone metabolism, has any influence in plasma HA levels. If HA is to be used alone as a marker of fibrosis, it may give a false positive test for fibrosis that may result in unnecessary and unwanted treatment for hepatitis C.

In the present study, women with ESRD had lower levels of plasma HA. It has been shown that estrogen decreases osteoclast number, decreasing therefore bone resorption.[26] Estrogen seems to regulate osteoclastogenesis by modulating cytokine production (interleukins 1 and 6 and tumor necrosis factor), and expression of adhesion molecules on bone marrow stromal cells. Sex steroids also act directly upon the parathyroid gland to increase PTH mRNA at physiological relevant doses.[26] The decrease of bone metabolism in estrogen producing women may explain the decrease in HA liberation by bone cells and their lower levels in patients with ESRD.

The longer the patient stays on haemodialysis, probably the stronger is the inflammatory process associated with chronic haemodialysis. It has been previously reported that the timespan on haemodialysis and certain markers of chronic inflammation, such as dialysis-related amyloid, correlate to HA serum levels.[16] Cytokine production may be stimulated by contact of the blood with bioincompatible dialysis membranes, and cytokines have been shown to stimulate the synthesis of HA. The length of time on haemodialysis did not correlate to HA levels in our study, and the reason for that is uncertain. However, two factors should be considered: (1) 12 out of our 23 patients (52.2%) on haemodialysis were women and estrogen decreases HA serum levels; and (2) the mean time on haemodialysis in the present study was of 9.7 years and a significant difference in HA levels can take more than 10 years to be detected.

Older people tend to have a series of disadvantages on haemodialysis. As survival has increased with dialysis, they are usually those who have endured haemodialysis for longer periods (and consequently they have spent more time in an environment with persistent chronic inflammation), and the chronic inflammatory process caused by dialysis may result in decreased albumin synthesis by the liver as a result of cytokines stimulation of acute-phase reactants.[21] In addition, as sex steroids decrease with aging, they may increase osteoporosis and the levels of plasma HA.

However, no statistical correlation was found between age, gender and time on haemodialysis in group 1. This finding indicates that liver fibrosis was the main factor associated with the increase of HA serum levels in group 1 patients. In addition, aminotransferase serum levels were not associated with liver fibrosis on haemodialysis patients with hepatitis C, as shown by others.[25]

The number of patients studied and the low frequency of advanced fibrosis and cirrhosis (METAVIR $\geq$ F3) were the main limitations of this study. As a matter of fact, patients with decompensated liver cirrhosis are not referred to our outpatient clinic for therapeutic decision because such patients are not candidates for hepatitis C treatment before kidney transplantation.

Time has come to start using the best tests, so far proven to be reliable, like HA levels, in the diagnosis of patients with hepatitis C on haemodialysis that are in need of treatment, and to start conventional therapy to evaluate their performance as markers of fibrosis regression. In fact, a recent trial has been reported using hyaluronic acid in patients treated for hepatitis C with promising results.[31]

In conclusion, plasma HA levels can predict the presence of significant fibrosis in patients with hepatitis C on haemodialysis with good sensitivity and specificity. It is desirable to start treatment for hepatitis C in this group of patients and to follow the behavior of hyaluronic acid levels after successful treatment to establish its value as a marker of fibrosis regression.

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