The role of real-time ultrasonography in predicting esophageal varices in hemophiliacs co-infected with hepatitis C and human immunodeficiency virus

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Background and Objectives. Endoscopic procedures are the gold standard for the diagnosis of esophageal varices but these invasive methods are complex to perform in hemophilic patients co-infected with hepatitis C virus/human immunodeficiency virus (HCV/HIV). Real-time ultrasonography has been reported to be an effective, non-invasive procedure able to monitor patients with chronic liver disease and to give useful information for the diagnosis of liver cirrhosis, portal hypertension and the presence of esophageal varices.

Design and Methods. Seventy patients with severe hemophilia were evaluated by esophago-gastro-duodenoscopy (EGDS) and ultrasonography; 40 had HCV/HIV co-infection and 30, comparable for age and HCV exposure time, were HCV⁺/HIV⁻. Hepatic longitudinal diameter, splenic longitudinal diameter, portal vein diameter and the average speed of portal flow were measured. The congestion index was calculated.

Results. Thirteen out of 40 (32.5%) HCV/HIV coinfected patients had esophageal varices. None out of 30 HCV⁺/HIV⁻ patients had esophageal varices ($p< 0.001$). Univariate analysis showed that the 13 HCV/HIV coinfected patients with esophageal varices had significantly higher hepatic longitudinal diameter ($p=0.006$), splenic longitudinal diameter ($p=0.0002$), portal vein diameter ($p=0.0005$) and congestion index ($p=0.0001$) than did the remaining 27 HCV/HIV coinfected patients. The stepwise logistic regression analysis indicated that, of the various ultrasonographic parameters evaluated, splenic longitudinal diameter and portal vein diameter had the greatest diagnostic efficiency in diagnosing a high proportion of patients with esophageal varices. The diagnostic efficiency of the combined criterion expressed by the area under the ROC curve was 0.8803.

Interpretation and Conclusions. Real-time ultrasonography, by evaluation of splenic longitudinal diameter and portal vein diameter, is an effective non-invasive technique able to classify correctly a large proportion of HCV/HIV co-infected hemophilic patients with esophageal varices.

Key words: ultrasonography, HCV/HIV, coagulopathic patients.

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Chronic liver disease related to hepatitis C virus (HCV) infection is considered an emerging problem in human immunodeficiency virus (HIV) positive, transfused hemophilic patients.[1-3] The progression of chronic hepatitis is indicated as one of the most important causes of death in this population because of the rapid progression of the HCV infection due to the HIV-related immunodeficiency status, which accelerates the natural course of the infection,[4-9] or because of the low therapeutic index of interferon therapy.[10,11] In addition, antiretroviral therapy with protease inhibitors reduces HIV replication and increases CD4⁺ lymphocyte counts but it does not result in lower HCV-RNA load.[12,13] Portal hypertension is one of the major sequelae in the general population with liver cirrhosis and is often associated with variceal bleeding and splenomegaly.[14,15] In the general population, varices appear in patients with a portal pressure gradient above 10 mmHg, and enlarge in 10-20% of these patients within 1-2 years; the incidence of bleeding in patients with a portal pressure gradient above 12 mmHg is about 10% per year and the mortality rate about 50%.[16-18]

In patients with severe hemophilia, the first variceal bleed is a real life-threatening event whose risk can be reduced by primary pharmacological prophylaxis.[18,19] Endoscopic procedures are the gold standard for monitoring chronic liver disease in the general population with HCV⁺ infection but these invasive methods are complex to perform in HCV/HIV co-infected hemophilacs. In fact, the diagnosis of liver cirrhosis, portal hypertension and esophageal varices is difficult in HCV/HIV co-infected hemophiliacs.

Severe hemorrhagic complications were reported in about 20% of patients with chronic liver disease who underwent invasive diagnostic procedures such as liver biopsy, hepatic vein catheterization and endoscopy.[4] Moreover, these procedures are very expensive in such patients because of the large amount of clotting factor concentrates needed to correct the coagulopathy.

Real-time ultrasonography (US) associated with pulsed and color Doppler ultrasound has been indicated as a non-invasive, inexpensive technique able to give substantial help in diagnosing liver cirrhosis and portal hypertension in the general population with chronic liver disease.[20-24] Increased hepatic longitudinal diameter, splenic longitudinal diameter, and portal vein diameter have been associated with the diagnosis of liver cirrhosis and portal hypertension in patients without congenital bleeding disorders with chronic liver disease.[20] Moreover, real-time US has been suggested to be a non-invasive technique able to predict the presence of esophageal varices in patients without congenital bleeding disorders with liver cirrhosis; in fact, a significant correlation was found between the congestion index and the presence and size of varices and severity of liver cirrhosis.[25]

To our knowledge, no data have been reported on the role of US in predicting the presence of esophageal varices diagnosed by esophago-gastro-duodenoscopy (EGDS) in HIV+ severe hemophiliacs with HCV-related liver disease. Therefore, the aim of this prospective study was to compare the ultrasonographic parameters in HCV/HIV co-infected hemophiliacs with and without esophageal varices diagnosed by EGDS and to select those parameter able to classify HCV/HIV co-infected hemophilic patients with esophageal varices.

Design and Methods

A prospective study was performed among severe hemophilic patients attending the Hematology Department of University La Sapienza in Rome. Congenital severe coagulopathy was defined by plasma levels of factor VIII:C, IX:C and VII:C below 2%. The HCV/HIV co-infected patients who fulfilled the following criteria were evaluated: (i) severe hemophilic patients who had received not virus-inactivated clotting factors concentrates; (ii) absence of HBsAg; (iii) no interferon treatment and (iv) absence of portal vein thrombosis and/or reversed flow in the portal vessels and/or US detectable porto-systemic shunts. Out of 60 HCV/HIV co-infected hemophilic patients, 40 fulfilled the entry criteria. Among 105 HCV+/HIV− severe hemophiliacs 30 of those who fulfilled the entry criteria, were comparable for age and

HCV exposure time. All 70 patients underwent gastroscopy, a double contrast esophagogram and a real-time digital esophagogram. Esophageal varices were classified according to the endoscopic rules of the Japanese Research Society for Portal Hypertension.[26]

HCV/HIV-RNA loads, ultrasonographic and biochemical parameters were evaluated. The CD4+ lymphocyte absolute count was measured in all 40 co-infected patients. Informed consent was obtained from all evaluated patients.

Ultrasonographic evaluation

Liver longitudinal diameter, splenic longitudinal diameter, portal vein diameter, presence of ascites and collateral vessels were evaluated by the ultrasonographic method using an electronically focused 3.75 MHz sectorial transducer (Toshiba SSH-140A). Portal vein diameter was measured as the largest antero-posterior diameter at the crossing point with the hepatic artery, during suspended respiration.[22] Duplex and color Doppler hemodynamic evaluations were performed in order to evaluate the average speed of portal flow and the spectrum characteristics of the flow in the suprahepatic vein. The average speed of portal flow was calculated positioning the sample volume at the crossing point of the vein with the hepatic artery[21] and was considered normal when ≥18 cm/sec.

The congestion index (CI) of the portal vein, ratio of cross-sectional area and the blood flow velocity in the portal vein were calculated according to the modified Moriyasu's formula using the mean velocity instead of the maximal flow velocity.[27] CI is considered a significant parameter reflecting the pathophysiological hemodynamics of the portal venous system in portal hypertension because of its correlation with the portal venous pressure. All measurements were performed in the early morning after overnight fasting by one observer blind to the diagnosis of esophageal varices.

Date of HCV infection

Because no stored sera were available to define dates of seroconversion for any of the patients, the date of HCV infection was assumed to correspond to the date of first exposure to not virus-inactivated clotting factor concentrates prepared from pooled donation.[8]

Date of HIV seroconversion

For each individual, the date of HIV seroconversion was calculated as the median value of the seroconversion interval between the last HIV negative test and the first HIV positive test under a Weibull seroincidence distribution, as previously described.[28,29] In detail, as for date of HIV seroconversion, the limit-

ing dates (L and R) of the overall interval in which patients with congenital coagulation disorders had been at risk of HIV infection due to clotting factor concentrates, were chosen on the basis of epidemiological evidence. Specifically, HIV diffusion in Italian hemophiliacs could be assumed to have started no earlier than 1 January 1979 (L), while due to HIV screening of donors and virucidal heat treatment of clotting factors no shorter infections could be expected after the date of 31 December 1987(R). Subsequently, the *last negative HIV test date* (L) and the *first positive HIV test date* (R) were collected for each patient, using the limiting dates of the overall interval in the case of missing data.

The estimation of the seroconversion time by the mid-point of the seroconversion interval (L,R) is based on the assumption of a uniform distribution of seroconversion within the overall interval (L,R). This assumption is not realistic because HIV infection due to contaminated clotting factors, as any other infection, is supposed to follow the three phases of initial diffusion, rapid and large expansion, and sharp decline. Since in our population the median width of the seroconversion intervals was 77 months (range: 1-108 months), the use of the mid-point could lead to strong biases in the estimation of the seroconversion dates.

For this reason, we assumed an underlying Weibull distribution of seroconversion times within the interval (L,R) and estimated its shape and size parameters by applying the maximum likelihood method to all the seroconversion intervals. Finally, for each patient we estimated the seroconversion time as the mid-point of the seroconversion interval under the estimated Weibull distribution.[28]

Virological methods

HBsAg, HBeAg, HBeAb, HBcAb and HBsAb tests were performed using standard techniques. HCV positivity was detected by a second generation ELISA test (Ortho diagnostics). The HCV-RNA viral load was measured using the Amplicor HCV Monitor test (Roche Diagnostic System Inc.). Extraction, amplification and detection were performed according to the manufacturer's instructions starting from 100 µL of serum. This test has a sensitivity of 1,000 HCV-RNA copies/mL. HIV positivity was detected by the ELISA test and confirmed by a Western blot method. The HIV-RNA viral load measurement was performed by the NASBA QT system (Organon Teknica). This test has a sensitivity of 1,000 HIV-RNA copies /mL. The HCV-RNA and HIV-RNA loads were measured in a virology laboratory (Istituto Superiore di Sanita', Rome, Italy) certified by World Viral Quality Control (VQC).

Table 1. Patients' characteristics according to HIV status.

	HIV positive	HIV negative
Number	40	30
Age (years)*	35±12	34±11
HCV exposure time (years)*	23±4	22±4
Male/Female	37/3	30/0
Hemophilia A	36	27
Hemophilia B	1	3
Factor VII:C deficiency	3	0

means ± standard deviations.

Statistical analysis

Statistical analysis was performed using the BMDP statistical software (Ed. 1990, Berkley, California). Mean values and standard deviations of all evaluated variables were calculated for each group of patients. Student's t-tests and Mann-Whitney's tests were performed to evaluate the significance of the differences in ultrasonographic, virological and hematologic parameters between co-infected patients with esophageal varices and without, and co-infected patients without esophageal varices versus HCV+/HIV- patients. By adopting the Bonferroni's correction with an type I error probability of 0.05, each comparison was considered significant for p<0.025. Pearson's "r" coefficient was calculated for the correlation analysis. Stepwise logistic regression analysis was applied to identify the ultrasonographic parameters which significantly and independently contributed to correctly classifying patients with esophageal varices. The estimated ROC curve was plotted and the area under this curve was calculated as an overall measure of diagnostic efficiency of the selected criterion. Sensitivity (Se), specificity (Sp), positive and negative predictive values (PPV and NPV, respectively) were computed.

Results

Thirteen out of 40 (32.5%) HCV/HIV co-infected patients had esophageal varices while none out of 30 HCV+/HIV- patients had esophageal varices (*p* <0.001).

The patients' characteristics according to HIV status are reported in Table 1. Twelve out of 13 patients with esophageal varices had small varices (grade 1) and 1 patient had grade 2 varices without the red color sign. Moreover, no patients suffered from bleeding complications due to rupture of esophageal varices prior to entering the study. Univariate analysis showed that the 13 HCV/HIV co-infected patients with esophageal varices had a significantly higher hepatic longitudinal diameter (*p*=0.006), splenic longitudinal diameter (*p*=0.0002), portal vein diameter

Table 2. Ultrasonographic, virological and hematologic parameters according to HIV status and esophageal varices (EV).

| | HIV positive | | HIV negative | p value* | |
	EV pos (n=13)	EV neg (n=27)	EV neg (n=30)	HIV⁺/EV⁺ vs. HIV⁺/EV⁻	HIV⁺/EV⁻ vs. HIV⁻/EV⁻
Hepatic longitudinal diameter (mm)	149.8±8	138.8±12.6	136.4±8.7	0.0064	0.4083
Splenic longitudinal diameter (mm)	151.7±22.2	126.3±16.6	117.1±14.6	0.0002	0.0311
Portal vein diameter (mm)	13.7±0.9	12±1.5	11.8±1.4	0.0005	0.6412
Average portal vein flow speed (cm/sec)	16.1±4.4	18.6±1.9	19.1±1.8	0.0492	0.3460
Congestion index of portal vein	0.99±0.32	0.63±0.21	0.59±0.16	0.0001	0.4095
HCV-RNA load (copies/mL)	797.769± 839.538	556.887± 642.716	161.573 ±276.896	0.5068	0.0779
CD4+ lymphocyte count (n/µL)	69.2±80.2	277.3±159.9		0.0003	

By adopting Bonferroni's correction , each comparison is considered significant for p<0.025.

Figure 1. A receiver operating characteristic (ROC) curve is shown for different diagnostic criteria. The diagnostic efficiency of the criteria were: portal vein diameter (PVD) = 0.8348, splenic longitudinal diameter (SLD) = 0.8276, and the combination of PVD and SLD = 0.8803.

(p=0.0005) and congestion index (p=0.0001) than did the remaining 27 HCV/HIV co-infected patients (Table 2). The former group also showed a lower average speed of portal flow than the latter (p=0.049). The HCV-RNA load was similarly high in HIV⁺ patients of both groups (p=0.50)(Table 2).The CD4⁺ lymphocyte count was significantly lower in co-infected patients with esophageal varices than in co-infected hemophiliacs without (p=0.0003). The HIV-RNA load was significantly higher in co-infected patients with esophageal varices than in co-infected patients without (p=0.0029). No significant differences were found in HCV-RNA load between co-infected patients without esophageal varices and HCV⁺/HIV⁻ patients (Table 2).

Univariate logistic regression followed by ROC analysis, performed separately on each ultrasonographic parameter of HIV⁺ patients, showed that each evaluated parameter could predict the presence of esophageal varices. The diagnostic efficiencies, measured by the areas under the ROC curves, were 0.6923, 0.7877, 0.8276 and 0.80348 for congestion index, hepatic longitudinal diameter, splenic longitudinal diameter and portal vein diameter, respectively. When applying stepwise multivariate logistic regression to the same patients, only portal vein diameter and splenic longitudinal diameter were selected as
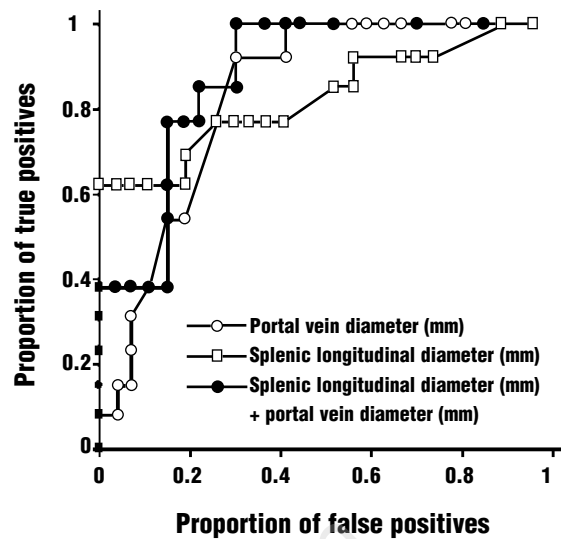
the ultrasonographic parameters able to classify, significantly and independently, the highest proportion of patients with esophageal varices, while congestion index and hepatic longitudinal diameter did not give any additional contribution to the classification. From the ROC analysis the diagnostic efficiency of the multivariate combined criterion of portal vein diameter and splenic longitudinal diameter was 0.8803, higher than the diagnostic efficiencies resulting from the univariate criteria based on the same parameters (Figure 1). The ROC curve can be used to choose the combination of sensitivity and specificity that satisfies clinical requirements, concerning the evaluation of the different risks associated with the misclassification of positive and negative patients. In particular, for the multivariate criterion we chose the combinations for which sensitivity and specificity were both higher than 70%. The three cut-points which maximized the sensitivity or the specificity were: 0.170 (Se=100% and Sp=70.4%, PPV=61.9% and NPV=100%), 0.280 (Se=84.6% and Sp=77.8%, PPV=64.7% and NPV=91.3%) and 0.510 (Se=76.9% and Sp=85.2%, PPV=71.4% and NPV=88.5%). The diagnostic rule according to the selected parameters is reported in Figure 2. Similar results were obtained when performing the analysis on data from all the 70 evaluated cases.

Splenic longitudinal and portal vein diameters were correlated (r=0.43, p<0.001). Moreover, an inverse correlation was found between splenic longitudinal diameter and average speed of portal flow (r=-0.46,

$$\text{SLD} > \frac{\ln\frac{(\text{CUTP})}{(1-\text{CUTP})} + 21.94 - 0.9238 \times \text{PVD}}{0.06573}$$
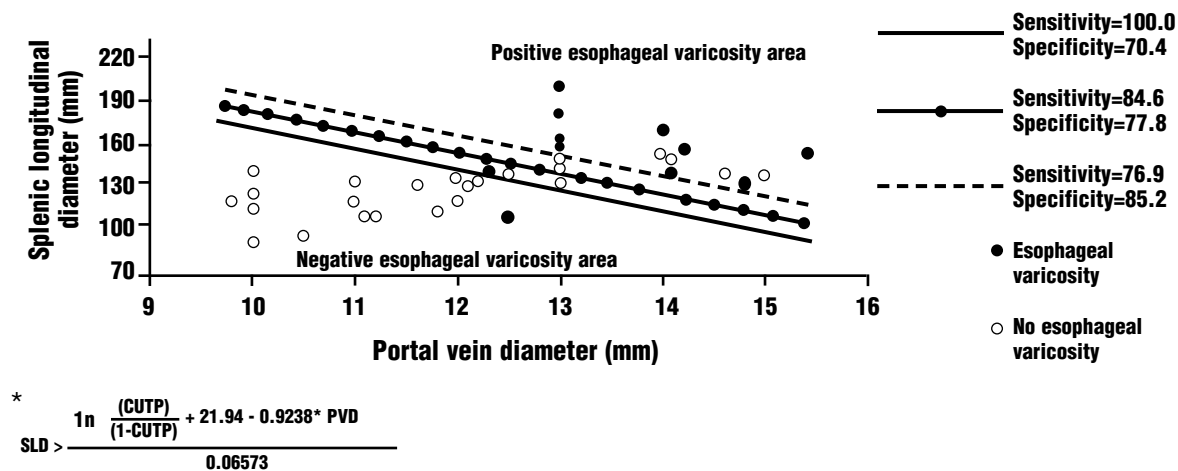
Figure 2. Diagnostic tests for different levels of sensitivity and specificity The curves represent the diagnostic test for different levels of sensitivity and specificity. Each curve divides the space into two areas, namely the negative esophageal varices area (below the curve) and the positive esophageal varices area (above the curve). Each patient can be represented as a point in this space according to his values of portal vein diameter (PVD) and splenic longitudinal diameter (SLD): if the point lies in the area above the selected curve, the patient has a high probability of having esophageal varices, whereas if the point lies in the area under the curve, the patient has a high probability of not having esophageal varices. Alternatively, according to the analytical expression of the diagnostic test, a patient has a high probability of having esophageal varices when (*see formula*)* where CUTP is the cut-point corresponding to the selected sensitivity and specificity (see text for choosing the cut-point value). The dots represent the 40 patients included in the ROC analysis.

$p< 0.001$) and between portal vein diameter and average speed of portal flow (r=-0.47, $p<0.001$). The congestion index was correlated with the splenic longitudinal diameter (r = 0.54, $p<0.001$).

Discussion

Chronic liver disease related to HCV infection is frequent in HCV/HIV co-infected transfused hemophiliacs[1-3] and the progression of chronic hepatitis is indicated as one of the most important causes of death in this population.[8,9]

In the general population variceal bleeding due to portal hypertension is a major determinant of survival in cirrhosis.[17,18] Therefore, the prompt diagnosis of the presence of esophageal varices is essential in patients with chronic liver disease. Endoscopic procedures are the gold standard for diagnosing the presence and size of esophageal varices, but the diagnosis of these pathologies is rendered difficult in patients with severe hemophilia because hemorrhagic complications can occur in about 20% of these patients with chronic liver disease who undergo invasive diagnostic procedures such as liver biopsy, hepatic vein catheterization and endoscopy.[4] However, although hemophilic patients may be at increased risk of bleeding during these invasive procedures, several studies showed that when such patients received clotting factor concentrates at an appropriate dose prior to and after a procedure,

bleeding complications were no greater than in non-hemophilic patients.[30-33] In addition, hemophilic patients with inhibitors might be more difficult to control hemostatically, but with a mean CD4 count of 69.2/μL observed in our study, they may actually lose their inhibitors and, therefore, be able to receive factor VIII concentrates as usually given. However, although it is important to reduce invasive diagnostic procedures in HCV/HIV co-infected severe hemophilic patients it is also important to identify the majority of them with esophageal varices. In fact, approximately 25% to 30% of asymptomatic cirrhotic patients without congenital bleeding disorders with esophageal varices will bleed from ruptured varices, and 70% will do so within the first 2 years of follow-up;[16,17] mortality from a first bleed is around 50% and most survivors will have a rebleed, with an associated in-patient mortality of 30%.[17,18]

Real-time US associated with pulsed and color Doppler ultrasound has been indicated as a non-invasive inexpensive technique able to give a substantial contribution to the diagnosis of liver cirrhosis and portal hypertension.[20-24] In the general population portal hemodynamic parameters have been reported to have prognostic significance in patients with compensated liver cirrhosis.[25,34] In particular, the congestion index, a parameter directly correlated with the portal vein pressure,[27] has been indicated to be significantly correlated with the presence and size of esophageal varices and severity of liver cirrhosis in patients without congenital bleeding disorders.[25] To

our knowledge our study is the first in which ultrasonographic parameters have been evaluated in HCV/HIV co-infected hemophilic patients with and without esophageal varices diagnosed by EGDS in order to select those able to classify HCV/HIV co-infected hemophilic patients with esophageal varices correctly.

In accordance with previous studies on ultrasonography in chronic liver disease related to HCV infection performed in non-congenital coagulopathic patients,[20,22] our study showed significantly higher hepatic longitudinal diameter, splenic longitudinal diameter and portal vein diameter in HCV/HIV co-infected hemophiliacs with esophageal varices. Moreover, in this group of patients the congestion index, a parameter directly correlated with portal vein pressure,[27] was significantly higher. Stepwise logistic regression analysis selected splenic longitudinal diameter and portal vein diameter as the most effective ultrasonographic parameters that were able to classify a high proportion of patients with esophageal varices correctly.

It is interesting to point out that these parameters can be rapidly and precisely detected by ultrasonographic evaluation, while the measurement of the average speed of portal flow is more complex and also influenced by physical and instrumental factors.[35,36] In the diagnosis of esophageal varices, especially in patients with severe hemophilia, sensitivity is more relevant than specificity, due to the high risk of death related to the first variceal bleed in this population. In addition the risk of this life-threatening complication can be reduced significantly by a relatively low cost, long-term prophylactic pharmacological treatment.[17-19] For this reason, we suggest adopting the diagnostic test with a sensitivity of 100% and a specificity of 70%. In this way, all patients with a higher probability of esophageal varices would be identified and only these patients would undergo EGDS to confirm the presence of esophageal varices, thus reducing the proportion of patients who would undergo invasive diagnostic procedures. The risk of bleeding can be reduced by a primary pharmacological prophylaxis in all hemophiliacs with EGDS-confirmed esophageal varices.[18,19] Moreover, considering the high negative predictive value of this diagnostic test (100%), patients classified as negative could be monitored by real-time ultrasonography. Therefore, although endoscopic procedures are the gold standard for the diagnosis and size assessment of esophageal varices and for the evaluation of bleeding risk related to esophageal varices, in HCV[+] and in HCV/HIV co-infected patients in the general population and in hemophiliacs, the risk of bleeding complications related to EGDS in hemophiliacs and the high cost of recombinant factor concentrates could suggest the use of ultrasonographic procedures to identify the co-infected hemophilic patients who have a higher probability of esophageal varices.

In conclusion, real time ultrasonography, by evaluating the splenic longitudinal diameter and portal vein diameter, is an effective non-invasive technique able to classify correctly a large proportion of HCV/HIV co-infected hemophiliacs with esophageal varices.

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