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Staging chronic hepatitis C in seven categories using fibrosis biomarker (FibroTest™) and transient elastography (FibroScan®)

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Background & Aims: FibroTest™ (FT) and Transient Elastography (TE) have been validated as non-invasive markers of METAVIR fibrosis stages from F0 to F4 using biopsy, and as prognostic markers of liver related mortality in patients with chronic hepatitis C. The aim was to extend the validation of FT and TE as markers of critical steps defined by occurrence of cirrhosis without complications (F4.1), esophageal varices (F4.2), and severe complications (F4.3): primary liver cancer, variceal bleeding, or decompensation (ascites, encephalopathy, or jaundice).

Methods: The updated individual data of 3927 patients (1046 cirrhotics) without complications at baseline were pooled from three prospective cohorts called "EPIC", "Paris", and "Bordeaux" cohorts.

Results: At 5 years, among 501 patients without varices at baseline (F4.1) varices occurred in 19 patients [F4.2 incidence of 4.0% (95% CI 2.2–5.8)]. The predictive performance (AUROC) of FT was 0.77 (0.66–0.84; p <0.001).

At 10 years severe complications occurred in 203 patients, [F4.3 incidence of 13.4% (9.6–17.1)], including primary liver cancer in 84 patients [6.4% (3.5–9.3)]. FT was predictive (Cox adjusted on treatment) of severe complications [AUROC 0.79 (76–82); p <0.0001], including primary liver cancer [AUROC 0.84 (80–87); p <0.0001]. Similarly TE was predictive of severe complications [AUROC 0.77 (72–81); p <0.0001], including primary liver cancer [AUROC 0.86 (81–90); p <0.0001].

Conclusions: FibroTest™ and TE increase were associated with the occurrence of all severe complications including hepatocellular carcinoma, hepatic insufficiency, and variceal bleeding. FibroTest™ increase was also associated with the occurrence of esophageal varices.

Introduction

Fibrosis progression is the earlier estimate of severity in patients with chronic hepatitis C (CHC) [1]. Before the stage of cirrhosis, the estimates of fibrosis progression mainly used transition rates between successive stages from normal liver (F0) to cirrhosis (F4), defined by histological score such as the METAVIR scoring system [1,2]. The last stage F4 included patients with a wide range of severity. As stated by Garcia-Tsao *et al.*, "there was a need to move beyond the characterization of cirrhosis as a single stage and instead, thinking of cirrhosis as a series of critical steps that culminate in hepatic decompensation" [3].

In CHC, FibroTest™ (FT) and transient elastography (TE) have been validated as markers of METAVIR fibrosis stages from F0 to F4 using biopsy [4–8], of liver fibrosis progression [9,10] or regression [10,11] but also validated as prognostic quantitative markers for occurrence of liver related complications, survival without liver related death, and overall survival [12–17]. In these studies the performances of FT and TE were at least similar to those of biopsy for predicting overall survival and liver related complications. However due to the low incidence of each severe complication at 5 years (Supplementary Table 1), more patients

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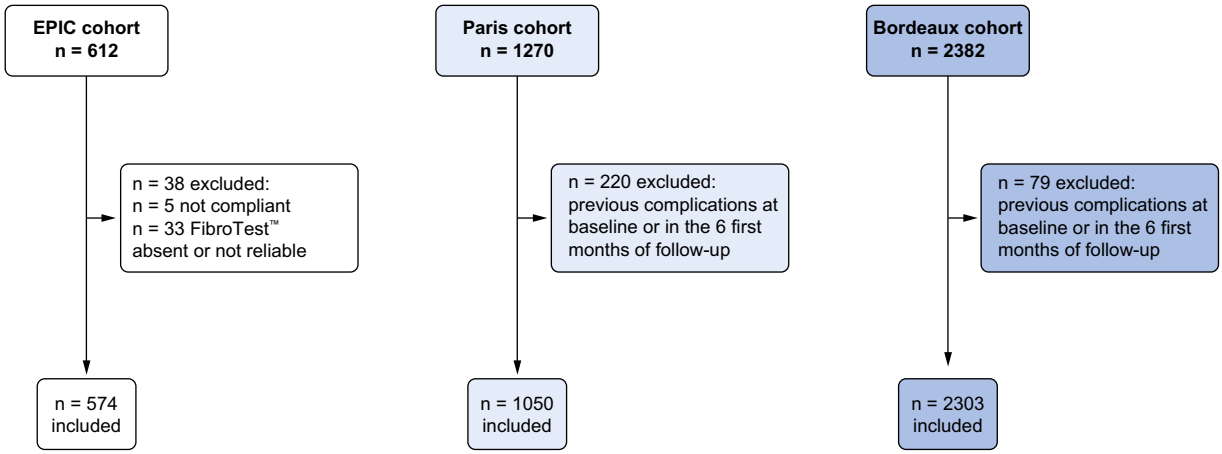

Fig. 1. Chart-flow of the study population.

and longer follow-up were needed to assess the specific performance of FT and TE for predicting separately the occurrence of varices, variceal bleeding, hepatic insufficiency (ascites, encephalopathy, or jaundice) and hepatocellular carcinoma.

The aim of the present study was to assess the performance of FT and TE for predicting the main steps in fibrosis progression, from F0 to death. The "seven stages spectrum", included the 4 non-cirrhotic stages and the 3 critical steps in cirrhosis [3]: F4.1 defined as cirrhosis without varices or severe events, F4.2 defined as the presence of varices without severe events and F4.3 defined by the occurrence of the first severe event. Previously, F4.3 was defined as the occurrence of the following severe events: variceal hemorrhage, and hepatic insufficiency (ascites, encephalopathy, or jaundice the three clinical signs of decompensation in the Child-Turcotte classification) [3]. As primary liver cancer (PLC including hepatocellular carcinoma or cholangiocarcinoma) is the main cause of death in CHC, even in sustained virological responders (SVR) [11], we included also PLC in the stage F4.3 definition.

Materials and methods

To assess the prognostic values of FT and TE on a large sample of CHC with a wide severity spectrum we analyzed the updated individual data of three prospective cohorts called "EPIC", "Paris", and "Bordeaux" cohorts (Fig. 1). The cohorts' characteristics were detailed in previous publications [11–13,18] and in the Supplementary data.

The cohorts' protocols were approved by the appropriate institutional review boards, regulatory agencies and conducted in accordance with principles of Good Clinical Practice. They were declared to US clinical trial registry. All patients provided written informed consent before cohort entry. All co-authors had access to the study data and had reviewed and approved the final manuscript.

The EPIC[3] program included an open-label randomized study ("EPIC cohort") of patients with CHC and cirrhosis who had failed to respond to interferon alfa plus ribavirin (Clinical trial registry number: NCT00048724) [18]. All patients had compensated cirrhosis with no evidence of HCC. Patients received peginterferon alfa-2b (0.5 μg/kg/week; n = 311) or no treatment (controls, n = 315) for a maximum period of 5 years or until 98 patients had a clinical event (hepatic decompensation, HCC, death, or liver transplantation). The primary measure of efficacy was time until the first clinical event. Clinic visits were scheduled every 3 months and included hematology and AFP measurements. Esophagogastroduodenoscopy was conducted at baseline, as needed during treatment, and at the completion of treatment. FT measurements were assessed retrospectively on remaining frozen serum at baseline and during follow-up. All patients had confirmed cirrhosis on liver biopsy as assessed by a central pathologist. Patients with liver disease because of reasons other than CHC or with human immunodeficiency virus or hepatitis B coinfection were excluded. Additional exclusion criteria included decompensated liver disease, and history of HCC. Patients receiving medication known to decrease portal hypertension were excluded.

The inclusion criteria were the same in the Paris and the Bordeaux cohort: all consecutive patients aged older than 18 years with chronic hepatitis C of any severity and at least one FT, were included. The determination of CHC was made using standard diagnostic criteria: serological detection of hepatitis C antibodies and positive serum HCV-RNA by polymerase chain reaction for >6 months. Patients who had a liver transplantation before the period of follow-up were not included. Exclusion criteria were chronic hepatitis B virus infection and all other causes of chronic liver disease. Patients with human immunodeficiency virus (HIV) infection were included. HCV treatment was given according to the successive guidelines. Patients with cirrhosis were followed according to guidelines, abdominal ultrasounds were proposed every 6 months and esophagogastroduodenoscopy was proposed at least every 2 years. Mortality and cause of death were assessed using the centralized French mortality office updated in October 2012.

The Paris cohort (FIBROFRANCE-HCV) belongs to FIBROFRANCE, a program organized in 1997 to assess the burden of chronic liver diseases in France (Clinical trial registry number: NCT01927133). The aim of the Paris cohort was to assess the prevalence, progression of fibrosis, morbidity, and mortality in consecutive patients addressed for CHC to the Pitié-Salpêtrière hospital in Paris, France [11]. During the follow-up, the change in risk factors, the liver complications, liver transplantation or death were collected. Follow-up consultations and non-invasive biomarkers were scheduled at least every 2 years, if possible in the center clinic. When not present, patients were contacted by phone or letter or obtained information through their physicians. Liver stiffness measurement (LSM) by TE was introduced in June 2005. The end of the follow-up was October 2012.

The Bordeaux cohort (Bordeaux-HCV cohort) belongs to a prospective hospital-based cohort of the Hepatology Unit of Haut-Lévêque Hospital (University Hospital of Bordeaux, Pessac, France) (Clinical trial registry number: NCT01241227). The Bordeaux cohort is the consecutive population of patients presenting to this center with chronic hepatitis C and for whom FT and TE was performed at least once. Follow-up consultations and examinations were targeted every 6 or 12 months or closer. The end of the follow-up was June 2013.

Definition of endpoints

Only EPIC was designed to assess prospectively both the incidence of varices at baseline (Stage F4.2) and the incidence of stage F4.3 during follow-up. In the Paris and Bordeaux cohorts the endoscopy was not prospectively organized and was performed according to each physician.

The three cohorts were designed to assess prospectively, the first severe clinical event (F4.3) defined as the occurrence of liver decompensation: variceal bleeding, ⩾grade 2 hepatic encephalopathy, jaundice (total bilirubin >50 μmol/L), ascites requiring therapeutic paracentesis, and/or additional therapy, and development of HCC. Death (liver related or not) and liver transplantation were also recorded. In the EPIC cohort, all severe clinical events were adjudicated by an independent committee of experts blinded to treatment. In the Paris and Bordeaux cohorts, HCC was diagnosed by histologic examination of liver tissue

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obtained by liver biopsy, or at autopsy, or if 1 or more hepatic space–occupying lesions observed at ultrasonography or computed tomography were shown to have vascular patterns typical of HCC by angiography, dual phase spiral tomography, or magnetic resonance imaging. Variceal bleeding was diagnosed on the basis of endoscopic findings in patients presenting with upper gastrointestinal hemorrhage.

Estimates of fibrosis

FibroTest™

FT measurement was predetermined and performed according to the recommended pre-analytic and analytic methods [5], on fresh serum in Paris and Bordeaux cohorts, on frozen samples (−80 °C) in EPIC. FT includes serum α_2-macroglobulin, apolipoprotein A1, haptoglobin, total bilirubin, and γ-glutamyltranspeptidase (GGT), adjusted for age and gender. FT scores range from zero to 1.00. Patients without high-risk profile of false positive/negative were considered as non-reliable [19].

Liver stiffness measurement by TE

The same methodology was used in Paris and Bordeaux cohorts. TE was performed according to published recommendations, using the M probe of FibroScan®. The results were expressed in kilopascals (kPa). Only procedures with at least 10 validated measurements and more than 60% success rate and an interquartile range inferior to 30% of the median value were considered reliable [20]. The standard 12.5 kPa for the diagnosis of cirrhosis and 7.1 kPa for F2F3F4 were used [21]. A significant decrease/increase in fibrosis was defined as a decrease/increase of 4 kPa.

Liver biopsies

In EPIC all patients had a biopsy and a diagnosis of cirrhosis. In Paris and Bordeaux patients had liver biopsies, mainly before 2007 when FT and TE were approved by French Health authorities and after 2007 mainly in case of discordances between FT and TE. Centralized pathologist in each cohort, unaware of the biochemical markers, evaluated the stage of fibrosis and grade of activity according to the METAVIR scoring system. Fibrosis was staged on a scale of 0–4: F0, no fibrosis; F1, portal fibrosis without septa; F2, few septa; F3, numerous septa without cirrhosis; F4, cirrhosis.

Statistical methods

The FT performance for the diagnosis and prediction of F4.2 at 5 year was assessed using the EPIC, using endoscopy at baseline and the occurrence of varices during follow-up among cirrhotic patients without varices at baseline. The FT performance for the diagnosis and prediction of F4.3, was assessed using the three cohorts. The TE performance for predicting F4.3 used Paris and Bordeaux cohorts. The incidence of events defining stage F4.2 and F4.3 as well as the FPR were calculated using time-dependent methods, Kaplan-Meir and the cumulative hazard function [11,22]. Comparison used log-rank test, and proportional hazard regression multivariate analysis. FPR (in METAVIR equivalence fibrosis stage per year) was calculated as the difference between last and first FT (or TE) divided by the time elapsed.

The predictive values of FT and TE were assessed semi-quantitatively and quantitatively. The semi-quantitative analysis used predetermined cut-offs equivalent to the standard cut-offs for non-cirrhotic METAVIR stages; for FT: F0 (0 to $\leqslant$0.28), F1 (>0.28 to $\leqslant$0.48), F2 (>0.48 to $\leqslant$0.58) and F3 (>0.58 to $\leqslant$0.74) [5]; for TE: F0 (0 to $\leqslant$5 kPa), F1 (>5 to $\leqslant$7.1 kPa), F2 (>7.1 to $\leqslant$9.5 kPa), and F3 (>9.5 to $\leqslant$12.5 kPa) [21]. For the new cirrhotic stages the previously suggested "5 year mortality cut-offs" [12–14] were predetermined; for FT: F4.1 (>0.74 to $\leqslant$0.85), F4.2 (>0.85 to $\leqslant$0.95) and F4.3 (>0.95–1.00); for TE: for F4.1 (>12.5 to $\leqslant$20 kPa), for F4.2 (>20 to $\leqslant$50 kPa) and for F4.3 (>50–75 kPa).

The quantitative method used proportional hazard regression in uni- and multivariate analysis standardized on treatment and the prognostic area under the ROC curves [14,22]. Survival rate or complications rates (one minus survival) were calculated from the first FT or TE to the date of death, transplantation or complications according to the circumstances. Fibrosis progression rates were calculated using hazard function (HR), which is the probability that a subject experiences the event of interest (in this case, progression of fibrosis from one stage to a higher stage) during a small time interval given that the individual has survived up to the beginning of the interval. To avoid overestimation of complications' incidence, only events occurring at least 6 months after baseline inclusion were taken into account. To avoid overestimation of patient's effect, all cases contributed only to one group; NT were patients never treated during all the follow-up; NR were patients who never achieved SVR. To take into account the impact of treatments, treatment responses (3 classes) were included in the multivariate analyses. The prognostic value of FT, and TE were assessed using the AUROC. NCSS8.0 Software was used [23].

Results

Characteristics of patients (Table 1)

A total of 3927 patients without complications at baseline were pooled.

In the EPIC cohort and in comparison with the two other cohorts, patients had twice more advanced fibrosis, were all previous non-responders, older, and with more genotype 1. Their characteristics were similar to those of the EPIC overall group, and also without significant difference on primary endpoints between randomized groups [16], 286 received maintenance therapy and 288 were observed.

In the Paris cohort and in comparison with Bordeaux, patients were more often male, with more HIV co-infection, more diabetes, and less Caucasian ethnicity. These two cohorts were very similar for fibrosis spectrum, history of previous liver events, treatment during follow-up and applicability (non-failure and reliable results) of FT and TE [19,20]. More PLC were observed in Paris than in the Bordeaux cohort.

Main endpoints

Predictive value of FT for varices (F4.2) in EPIC

FT was associated with varices occurrence [Regression coefficient (RC) = 11 (4–18); $p = 0.001$] independently of treatment. The predictive AUROC was 0.77 (0.66–0.84; $p < 0.001$). At baseline 73 patients F4.2 (with varices) had higher FT [0.82 (95% CI 0.79–0.86)] than 501 patients F4.1 (without varices) [0.77 (95% CI 0.75–0.78); $p = 0.007$]. The AUROC for presence of varices was 0.60 (0.52–0.66; $p = 0.008$). According to FT cut-offs the predictive value rose from 0% when $\leqslant$0.74 to 4.5% (0.6–8.9), 6.1% (2.4–9.7) between 0.74 to 0.95, 13.6% (1.1–26.0) above 0.95.

Predictive value of FT for severe complications (F4.3) (Table 2 and Fig. 2)

Severe complications (F4.3) occurred in 203 patients (incidence 13.6% at 10 years), including primary liver cancer in 84 patients (6.4%; only one cholangiocarcinoma); the total number of liver related events was 256 (24.2%) including 39 transplantations (2.9%) and 52 (4.3%) liver related deaths.

FT was predictive of severe complications [AUROC 0.79 (76–82); $p < 0.0001$], including primary liver cancer [AUROC 0.84 (80–87); $p < 0.0001$], independently of treatment response.

According to the predetermined FT cut-offs the probability of severe complications increased from 2.2% (0.7–3.6) in 1127 FT $\leqslant$0.28 (F0) to 60.3% (38.4–82.2) in 94 FT >0.95 (F4.3).

Among presumed cirrhotic patients, FT predetermined cut-offs (0.74, 0.85, and 0.95) were validated for predicting decreasing 10-year survival without complications, hepatocellular carcinoma, liver related events and death, between stage F4.1 *vs.* F4.2 and between F4.2 *vs.* F4.3 (Fig. 2). FPR did not add significant prognostic values to FT. Similar FT performances were observed

for each non-cancer complication: ascites, jaundice-encephalopathy-Child-C and bleeding, and according to each cohort (Supplementary Tables 2–4).

Among presumed non-cirrhotic patients, liver cancer occurred in 0% of patients' stage F0, 0.9% F1 ($p = 0.008$ *vs.* F0), 1.5% F2 ($p = 0.003$ *vs.* F0, $p = 0.35$ *vs.* F1,) and 12.0% in F3 [$p < 0.0001$ *vs.* F0, $p = 0.005$ *vs.* F1, $p = 0.19$ *vs.* F2, $p = 0.04$ *vs.* F4.1 (16.8%), $p < 0.0001$ both *vs.* F4.2 (26.1%) and F4.3 (30.8%).

Predictive value of TE for severe complications (F4.3) (Table 3 and Fig. 3)

TE was predictive of severe complications [AUROC 0.77 (72–81); $p < 0.0001$], including primary liver cancer [AUROC 0.86 (81–90); $p < 0.0001$], independently of treatment response. According to the predetermined TE cut-offs the probability of severe complications increased from 1.6% (0.3–2.8) in 790 TE $\leqslant$5 kPa (F0) to 71.0% (26.7–100) in 17 FT >50 kPa (F4.3). Among presumed cirrhotic

Table 1. Characteristics of patients included and incidence of complications.

Included	EPIC n = 574	Paris n = 1050	Bordeaux n = 2303	All n = 3927
Characteristics at inclusion				
Sex male	389 (68%)	625 (60%)	1209 (53%)	2223 (57%)
Age median yr (95% CI)	52 (52-53)	47 (46-48)	49 (48-50)	49 (49-50)
Body Mass Index >27	261 (45%)	163 (16%)	496 (21%)	920 (23%)
HIV co-infection	0 (0%)	233 (22%)	304 (13%)	537 (14%)
IV-drug injector	n.a.	253 (24%)	711 (31%)	964/3353 (29%)
Transfusion	n.a.	199 (19%)	859 (37%)	1058/3353 (32%)
Genotype 1	511 (89%)	618 (59%)	1460 (64%)	2589 (66%)
Caucasian	476 (83%)	749 (71%)	2298 (96%)	3523 (90%)
Alcohol >30 g/day	n.a.	78 (7%)	153/2094 (7%)	231/3353 (7%)
Tobacco	n.a.	448 (43%)	1150/2124 (54%)	1598/3353 (48%)
Cannabis	n.a.	179 (17%)	189/2087 (9%)	368/3353 (11%)
Diabetes[1]	254 (44%)	154 (15%)	145/2362 (6%)	553 (14%)
Number of biopsies baseline	574 (100%)	412 (34%)	222 (10%)	1208 (31%)
Number of FibroTest™ baseline	574 (100%)	1050 (100%)	2303 (100%)	3927 (100%)
Reliable FibroTest™	574 (100%)	1050 (100%)	2303 (100%)	3927 (100%)
Number of TE baseline	0 (0%)	1050 (100%)	2303 (100%)	3353 (85%)
Reliable TE	n.a.	1050 (100%)	2066 (90%)	3116 (93%)
Stage presumed by FibroTest™				
F0	10 (1%)	333 (32%)	784 (34%)	1127 (29%)
F1	27 (5%)	254 (24%)	452 (20%)	733 (19%)
F2	46 (8%)	107 (10%)	224 (10%)	377 (10%)
F3	108 (19%)	180 (17%)	356 (15%)	644 (16%)
Cirrhosis (F4)	383 (67%)	176 (17%)	487 (21%)	1046 (27%)
Stage cirrhosis F4.1: no complication	310 (54%)	176 (17%)	487 (21%)	1046 (27%)
Stage cirrhosis F4.2: varices	73 (13%)	n.a.	n.a.	n.a.
Stage cirrhosis F4.3: complication	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Status at the end of follow-up				
Follow-up for death (years) median	5.2 (5.2-5.2)	5.7 (5.2-6.0)	5.8 (5.7-5.9)	5.2 (5.2-5.2)
Patients still at risk at 5 year	439 (76%)	575 (55%)	1401 (61%)	2415 (61%)
Patients still at risk at 8 year	0 (0%)	161 (15%)	309 (13%)	470 (12%)
Patients still at risk at 10 year	0 (0%)	60 (6%)	1 (0%)	61 (2%)
Treatment				
Sustained responders (SVR)	0	204 (20%)	383 (17%)	587 (15%)
Non-responders (NR)	286 (50%)	453 (43%)	942 (41%)	1681 (43%)
Not treated (NT)[2]	288 (50%)	393 (37%)	978 (42%)	1659 (42%)
Repeated FibroTest™	445 (78%)	925 (88%)	2248 (98%)	3618 (92%)
Duration between FibroTest™	2.1 (1.6-3.0)	5.3 (5.0-5.5)	5.3 (5.2-5.5)	4.8 (4.7-4.9)
Repeated TE	n.a.	963 (92%)	2052 (89%)	3015 (77%)
Duration between TE	n.a.	3.0 (2.9-3.1)	5.3 (5.2-5.5)	4.4 (4.2-4.5)

(*continued on next page*)

Table 1. (*continued*)

Included	EPIC n = 574	Paris n = 1050	Bordeaux n = 2303	All n = 3927
Incidence complications[3]				
Varices (F4.2)[4]	19/501 (4.0%)	n.a.	n.a.	n.a.
Severe complications (F4.3)[5]	55/574 (11.0%)	33/1050 (7%)	115/2303 (23.3%)	203/3927 (13.4%)
Ascites	22/574 (4.1%)	1/1050 (0.3%)	38/2303 (1.7%)	61/3927 (2.6%)
Primary liver cancer	24/574 (4.6%)	16/1050 (10.5%)	44/2303 (1.9%)	84/3927 (6.4%)
Bleeding	11/574 (2.0%)	2/1050 (0.4%)	12/2303 (1.1%)	25/3927 (1.1%)
Jaundice (bilirubin >50 μmol/L) or encephalopathy or Child-Pugh C	11/574 (2.0%)	14/1050 (2.6%)	47/2303 (13.5%)	72/3927 (5.5%)
No death, no transplantation	555/574 (96.5%)	1010/1050 (86.5%)	2157/2303 (88.8%)	3721/3927 (87.3%)
Death or transplantation	19/574 (3.5%)	40/1050 (13.5%)	147/2303 (11.2%)	206/3927 (12.7%)
Death	13/574 (2.4%)	31/1050 (10.1%)	124/2303 (9.0%)	168/3927 (10.1%)
Death, no transplantation	13/574 (2.4%)	30/1050 (10.5%)	124/2303 (9.0%)	167/3927 (10.1%)
Death after transplantation	0/574 (0%)	1/1050 (0.5%)	0/2303 (0%)	1/3927 (0.01%)
Transplantation	6/574 (1.1%)	10/1050 (3.4%)	23/2303 (2.3%)	39/3927 (2.9%)
Liver related death	6/574 (1.1%)	9/1050 (4.9%)	37/2303 (2.9%)	52/3927 (4.3%)
Liver related death or transplantation	12/574 (2.2%)	18/1050 (7.3%)	60/2303 (5.3%)	90/3927 (6.4%)
Liver related events: liver related death, transplantation, severe complication	58/574 (10.0%)	50/1050 (10.1%)	148/2303 (25.1%)	256/3927 (16.1%)
All events: death, transplantation, severe complication	61/574 (11.0%)	60/1050 (12.1%)	222/2303 (29.0%)	343/3927 (19.6%)

n.a., not available.
[1]In the 3 cohorts diabetes was defined as fasting glucose ⩾6.1 mmol/L, or treated diabetes. Data missing in 20 patients in Bordeaux.
[2]In EPIC patients were previously non-responders but not treated during 5 year follow-up.
[3]Incidences were calculated at 10 year for Paris and Bordeaux cohorts and at 5 year for the EPIC cohort.
[4]Varices were screened only in the EPIC cohort, among patients without baseline F4.2 or F4.3.
[5]Only patients without F4.3 at baseline (1050 in Paris and 2303 in Bordeaux); earliest event counted in case of multiple events.

patients, TE predetermined cut-offs (12.5, 20, and 50 kPa) were validated only for 12.5–20 (F4.1) *vs.* 20–50 kPa (F4.2) but not for 50–75 kPa (Fig. 3). FPR estimated using TE was not associated with significant prognostic value in multivariate analysis.

Similar TE performances were observed for ascites, jaundice-encephalopathy-Child-C and bleeding, and according to each cohort. However the power was not sufficient to estimate accurately TE performance for bleeding (Supplementary data).

Direct comparisons of FT, TE, and FT-TE combinations

There was no significant difference between 2332 contemporaneous FT and TE for the prediction (AUROC) of severe complication, primary liver cancer and overall mortality: 0.81 (0.76–0.85) *vs.* 0.79 (0.73–0.83), 0.86 (0.80–0.90) *vs.* 0.87 (0.81–0.91), 0.82 (0.78–0.86) *vs.* 0.82 (0.78–0.86) and 0.76 (0.71–0.83) *vs.* 0.78 (0.71–0.83). Combinations of FT and TE (ElastoFibroTest™) had slightly higher AUROCs: 0.82 (0.78–0.86), 0.88 (0.84–0.92), 85 (0.81–0.88), and 0.79 (0.73–0.84) respectively (Supplementary Table 5).

Discussion

In patients with chronic hepatitis C both FT and TE, have been extensively validated for the diagnosis of advanced fibrosis stages [4–13] and prediction of mortality [12–17]. Due to the low incidence of events in previous studies (Supplementary Table 1), larger cohorts and a longer follow-up were needed to assess the performance of biomarkers for each complication [24].

The strength of the present study is that it integrated a total of 3927 cases with a large spectrum of liver injury from 1127 without fibrosis to 1014 with cirrhosis, followed prospectively over 5 to 12 years. Multivariate analyses were performed to take into account the treatment effect and the heterogeneity between cohorts. It was possible for the first time to demonstrate that these biomarkers are not only an alternative to biopsy for staging fibrosis, but are also predictive of the incidence of complications, including the occurrence of varices (Stage F4.2) and severe complications (Stage F4.3). Complications were classified in three main classes: bleeding, primary liver cancer, and hepatic insufficiency (ascites, jaundice, encephalopathy or Child C). Therefore these biomarker can help clinicians adapt patient follow-up and management to the heterogeneity of cirrhosis [3].

Diagnosis of varices

The EPIC study analyzed prospectively the significant performance of FT for the diagnosis of varices. Despite modest results [AUROC = 0.60 (0.52–0.66; $p = 0.008$)], this performance was within range of the two other FT studies: 0.72 (0.60–0.85) in 70 patients with CHC [25], and 0.50 (0.41–0.59) in 166 patients with mixed causes [26]. The variability of these AUROCs was compatible with a spectrum effect underestimating the performance of biomarkers. In the EPIC cohort, all cirrhotics were previously treated. The cirrhotics patients in our pooled population had been selected and those with baseline complications were excluded, thus narrowing the spectrum of severity and therefore the risk of portal hypertension. Accordingly, the FT median values were

Table 2. FibroTest™ performance for the prediction of complications.

	Severe complications defining stage F4.3	Primary liver cancer	Liver related events	Death
All stages	203/3927 13.4% (9.6-17.1)	84/3927 6.4% (3.5-9.3)	256/3927 24.2% (19.5-30.3)	168/3927 10.1% (7.6-12.6)
FT predetermined cutoff for stages				
F0 ≤0.28	11/1127 2.2% (0.7-3.6)	0/1127 0.0%	12/1127 3.0% (0.9-5.0)	18/1127 2.8% (1.0-4.6)
F1 >0.28-≤0.48	14/733 4.1% (1.7-6.4)	4/733 0.9% (0-1.9)	16/733 7.2% (2.5-11.9)	20/733 5.6% (2.3-8.8)
F2 >0.48-≤0.58	9/377 4.8% (1.5-8.0)	4/377 1.5% (0-3.1)	10/377 6.8% (1.8-11.8)	12/377 5.8% (1.7-9.9)
F3 >0.58-≤0.74	30/644 7.7% (2.5-74.1)	13/644 12.0% (1.2-22.9)	45/644 36.8% (22.8-50.9)	32/644 16.9% (9.6-25.5)
F4.1 >0.74-≤ 0.85	46/515 36.4% (10.4-62.4)	20/515 16.8% (0.7-32.8)	58/515 48.3% (27.9-68.8)	31/515 14.4% (7.1-21.7)
F4.2 >0.85-≤0.95	60/437 46.8% (26.4-67.2)	27/437 26.1% (7.3-44.8)	78/437 73.6% (55.5-91.7)	37/437 29.1% (11.9-46.3)
F4.3 >0.95	33/94 60.3% (38.4-82.2)	16/94 30.8% (12.4-49.2)	37/94 87.9% (66.9-100)	18/94 53.1% (22.0-84.2)
Quantitative multivariate analysis		Regression coefficient (95% CI) *p* value		
Baseline FT[1]	203/3927 5.5 (4.7-6.2) *p* <0.0001	84/3927 7.3 (5.7-8.9) *p* <0.0001	256/3927 5.7 (5.0-6.4) *p* <0.0001	168/3927 3.4 (2.7-4.0) *p* <0.0001
FPR[2]	181/3618 5.8 (3.1-8.5) *p* <0.0001	75/3618 4.2 (-0.7-9.1) *p* = 0.10	256/3927 5.0 (2.1-7.9) *p* = 0.0007	126/3618 6.7 (3.6-9.8) *p* <0.0001
Prognostic performance (AUROCs) in paired subset		Number of events/total mean (95% CI) *p* value		
Baseline FT	181/3618 80 (76-83) *p* <0.0001	75/3618 84 (80-87) *p* <0.0001	223/3618 80 (77-83) *p* <0.0001	126/3618 74 (69-78) *p* <0.0001
FPR	181/3618 48 (43-52) *p* = 0.27	75/3618 42 (35-47) *p* = 0.03	223/3618 44 (40-48) *p* = 0.002	126/3618 49 (43-54) *p* = 0.67
FT and TRT	181/3618 79 (76-82) *p* <0.0001	75/3618 84 (80-87) *p* <0.0001	223/3618 79 (76-82) *p* <0.0001	126/3618 72 (68-76) *p* <0.0001
FPR and TRT	181/3618 59 (54-63) *p* = 0.001	75/3618 63 (56-69) *p* = 0.0002	223/3618 61 (57-65) *p* <0.0001	126/3618 53 (47-58) *p* = 0.30
FT and FPR	181/3618 81 (77-84)[3] *p* <0.0001	75/3618 84 (80-87) *p* <0.0001	223/3618 80 (77-83) *p* <0.0001	126/3618 75 (70-79) *p* <0.0001
FT, FPR and TRT	181/3618 80 (77-83) *p* <0.0001	75/3618 84 (80-87) *p* <0.0001	223/3618 80 (77-82) *p* <0.0001	126/3618 74 (70-78) *p* <0.0001

Kaplan Meir estimates were assessed at five year for EPIC and 10 year for Paris and Bordeaux cohorts.
[1]FT, FibroTest™. Cox Regression Coefficient adjusted on treatment.
[2]FPR, Fibrosis Progression Rate. Cox Regression Coefficient adjusted on baseline FibroTest™ and treatment.
[3]$p = 0.05$, combined FT FPR *vs.* FT.

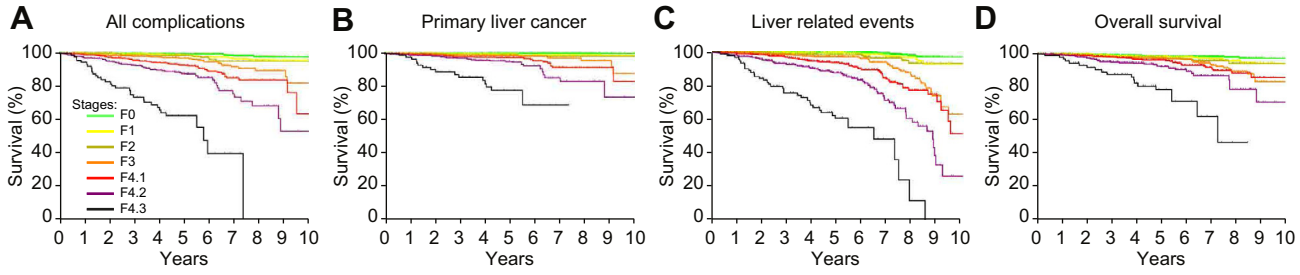

Fig. 2. Survival without liver complications according to baseline FibroTest™. Seven stages presumed by FibroTest™. Green line is stage F0 (FibroTest™ 0 to ⩽0.28); yellow is F1 (>0.28 to ⩽0.48); brown is F2 (>0.48 to ⩽0.58); orange is F3 (>0.58 to ⩽0.74); red is F4.1 (>0.74 to ⩽0.85); purple is F4.2 (>0.85 to ⩽0.95); and black is F4.3 (>0.95–1.00). (A) All complications. The following survival comparisons between adjacent stages were significant: F0 *vs.* F1 ($p = 0.047$), F2 *vs.* F3 ($p = 0.03$), F3 *vs.* F4.1 ($p = 0.004$), and F4.1 *vs.* F4.2 ($p = 0.002$), and F4.2 *vs.* F4.3 ($p < 0.0001$). (B) Primary liver cancer. The following survival comparisons between adjacent stages were significant: F0 *vs.* F1 ($p = 0.008$), F3 *vs.* F4.1 ($p = 0.04$), and F4.1 *vs.* F4.2 ($p = 0.01$), and F4.2 *vs.* F4.3 ($p < 0.0001$). (C) Liver related events. The following survival comparisons between adjacent stages were significant: F0 *vs.* F1 ($p = 0.04$), F2 *vs.* F3 ($p = 0.003$), F3 *vs.* F4.1 ($p = 0.006$), and F4.1 *vs.* F4.2 ($p < 0.0001$), and F4.2 *vs.* F4.3 ($p < 0.0001$). (D) Overall survival. The following survival comparisons between adjacent stages were significant: F4.1 *vs.* F4.2 ($p = 0.02$), and F4.2 *vs.* F4.3 ($p < 0.0001$). (This figure appears in colour on the web.)

low for a cirrhotic population: 0.74 in EPIC and 0.75 in the Paris and Bordeaux cohorts respectively.

The Paris and Bordeaux cohorts were not designed to assess the performance of FT and TE for the diagnosis of varices in

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Table 3. Transient elastography performance for the prediction of first severe complications defining cirrhosis stage F4.3.

	Severe complications defining stage F4.3	Primary liver cancer	Liver related events	Death
All stages	128/3031 13.0% (8.6-17.4)	53/3031 6.5% (3.1-10.8)	167/3031 41.0% (25.9-56.1)	104/3031 7.4% (5.9-9.4)
TE predetermined cutoff				
F0 0-≤5 kPa	7/790 1.6% (0.3-2.8)	0/790 0% (0-0)	10/790 2.5% (0.9-4.1)	10/790 3.7% (0.9-6.5)
F1 >5-≤7.1 kPa	25/1049 5.0% (2.8-7.3)	7/1042 1.1% (0.2-1.9)	26/1049 10.5% (5.4-15.7)	19/1049 4.2% (1.9-6.6)
F2 >7.1-≤9.5 kPa	14/505 11.0% (1.7-20.2)	3/505 2.1% (0-4.7)	21/505 40.6% (3.4-77.8)	10/505 3.5% (1.2-5.7)
F3 >9.5-≤12.5 kPa	8/276 25.7% (0-58.8)	5/276 24.6% (0-58.2)	15/276 19.6% (6.0-33.2)	14/276 11.8% (4.8-18.9)
F4.1 >12.5-≤20 kPa	27/210 23.4% (13.9-75.8)	13/210 12.7% (4.5-21.0)	32/210 62.1% (35.8-88.5)	15/210 20.3% (8.6-32.0)
F4.2 >20-≤50 kPa	42/184 55.9% (36.0-75.8)	22/184 33.6% (13.6-53.5)	56/184 77.1% (60.1-94.2)	34/184 30.3% (20.4-40.0)
F4.3 >50-175 kPa	5/17 71.0% (26.7-100)	3/17 58.7% (0.5-100)	7/17 100% (26.0-100)	2/17 14.8% (0-34.0)
Quantitative multivariate analysis		Regression coefficient (95% CI) *p* value		
Baseline TE[1]	128/3031 3.6 (3.1-4.2) *p* <0.0001	53/3031 4.1 (3.3-4.9) *p* <0.0001	167/3031 3.5 (3.0-3.9) *p* <0.0001	104/3031 3.5 (2.9-4.0) *p* <0.0001
FPR by TE[2]	128/3015 1.3 (0.3-2.3) *p* = 0.008	53/3015 0.61 (-1.9-3.1) *p* = 0.61	No convergence	No convergence
Prognostic performance (AUROCs) in TE paired subset		Number of events/total mean (95% CI) *p* value		
Baseline TE	128/3015 78 (73-82) *p* <0.0001	53/3015 87 (82-90) *p* <0.0001	167/3015 79 (75-83) *p* <0.0001	104/3015 75 (69-80) *p* <0.0001
FPR	128/3015 59 (53-64) *p* <0.0001	53/3015 57 (48-65) *p* = 0.11	167/3015 57 (51-62) *p* = 0.05	104/3015 59 (51-66) *p* = 0.01
TE and FPR	No convergence	No convergence	No convergence	No convergence
Baseline TE and TRT	128/3015 77 (72-81) *p* <0.0001	53/3015 86 (81-90) *p* <0.0001	104/3015 71 (65-76) *p* <0.0001	104/3015 75 (69-80) *p* <0.0001
FPR and TRT	No convergence	53/3015 67 (59-73) *p* <0.0001	No convergence	No convergence
TE, FPR and TRT	No convergence	No convergence	No convergence	No convergence

Follow-up is five year for EPIC and 10 year for Paris and Bordeaux cohorts.
[1]TE, Transient Elastography. Cox Regression Coefficient adjusted on treatment.
[2]FPR, Fibrosis Progression Rate. Cox Regression Coefficient adjusted on baseline TE and treatment.

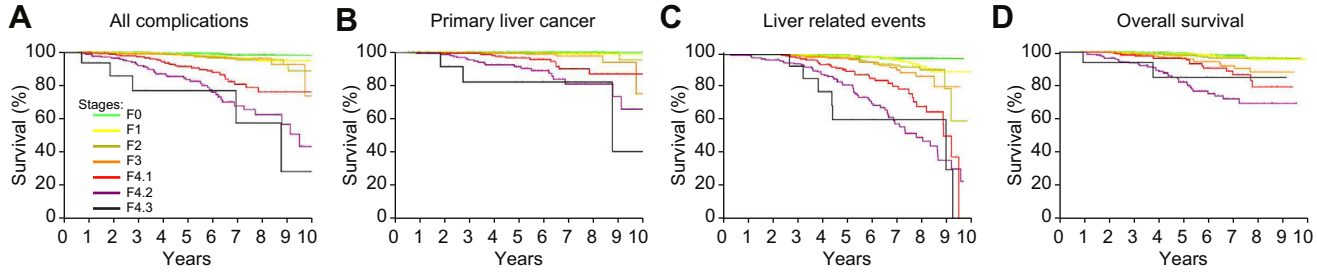

Fig. 3. Survival without liver complications according to baseline Transient Elastography. Stages presumed by transient elastography. Green line is stage F0 (stiffness 0 to ⩽5 kPa); yellow is F1 (>5 to ⩽7.1 kPa); brown is F2 (>7.1 to ⩽9.5 kPa); orange is F3 (>9.5 to ⩽12.5 kPa); red is F4.1 (>12.5 to ⩽20 kPa); purple is F4.2 (>20 to ⩽50 kPa) and black is F4.3 (>50–75 kPa). (A) All complications. The following survival comparisons between adjacent stages were significant: F0 *vs.* F1 ($p = 0.02$), F3 *vs.* F4.1 ($p = 0.0001$), and F4.1 *vs.* F4.2 ($p = 0.002$). (B) Primary liver cancer. The following survival comparisons between adjacent stages were significant: F0 *vs.* F1 ($p = 0.02$), F3 *vs.* F4.1 ($p = 0.02$) and F4.1 *vs.* F4.2 ($p = 0.01$). (C) Liver related events. The following survival comparisons between adjacent stages were significant: F3 *vs.* F4.1 ($p = 0.0006$), and F4.1 *vs.* F4.2 ($p = 0.002$). (D) Overall survival. The following survival comparisons between adjacent stages were significant: F2 *vs.* F3 ($p = 0.02$) and F4.1 *vs.* F4.2 ($p = 0.001$). (This figure appears in colour on the web.)

cirrhotic patients. Two external studies compared FT and TE for the diagnosis of varices in CHC and did not find significant differences. Interestingly, the same wide range of AUROCs was observed. The AUROCs, in the first study for all varices were 0.72 (0.60–0.85) *vs.* 0.84 (0.75–0.94), and for large varices 0.87 (0.77–0.97) *vs.* 0.75 (0.62–0.87) [25]; the second study had lower AUROCs for both FT and TE, suggesting a spectrum effect: 0.50 (0.41–0.59) *vs.* 0.53 (0.44–0.63) [26]. Three other studies also observed significant but heterogeneous AUROCs for TE: 0.76 (0.60–0.87) [27], 0.76 (0.62–0.90) [28] and 0.90 (0.85–0.95) [29].

Prediction of varices occurrence

Due to the low incidence (7%–8% per year) of varices and the burden of repeated endoscopies, validation studies are difficult [24].

The EPIC study was the first prospective study to validate the performance of FT for predicting varices occurrence. Despite a very low incidence (4%), FT had a significant performance (AUROC = 0.77). Based on these results, patients with FT remaining <0.74 do not need endoscopy and patients with FT >0.95 had 14% risk of developing varices at 5 years. No prospective study has assessed the value of TE for predicting varices occurrence.

Prediction of severe complications

We observed that both FT and TE predicted similarly the occurrence of each category of complications: primary liver cancer, variceal bleeding and the "hepatic insufficiency" complications (ascites, jaundice, encephalopathy). This is therefore the first demonstration of two indirect biomarkers of fibrosis predicting primary liver cancer, with similar hazard risk than for other liver complications. This highlights the usefulness of these biomarkers as surrogate markers of morbidity and mortality in CHC.

Our results validated the predetermined FT cut-offs in cirrhotics for critical clinical events: 0.74 to 0.85 for F4.1, 0.85 to 0.95 for F4.2, and >0.95 for F4.3. Pending further validation, this suggests that FT could be better than MELD alone for selecting candidates earlier for liver transplantation. On the contrary, the predetermined cut-offs for TE of 50 kPa were not optimal, as few patients were selected without discriminating between F4.3 and F4.2 for relevant endpoints.

There was a small but significant increase in performances for combined FT and TE, *vs.* these biomarkers alone, as already demonstrated for staging fibrosis [30]. However, as previously published [31] the major advantage of FT compared with TE is its applicability (absence of failure or non-reliable measurement): 98% for FT [19] and 80% for TE [20]. Proteome profiling has also identified other blood tests than FT components, which could be combined with TE such as YKL-40 (chondrex, cartilage glycoprotein-39) [32].

Limitations

Our population was recruited from tertiary centers and not from the general population, which could reduce the external validity. However the severity spectrum, including that of cirrhotic patients, was wide. The overall 10-year survivals, of our population of 1046 cirrhotics classified by biomarkers ranged from 86% for FTs of 0.74–0.85 to 47% for FTs >0.95. These rates were similar to those previously classified by clinical decompensation: 78% for compensated cirrhotics in a recent Danish population-based hospital registry and 45% in the U.K. General Practice Research Database [24].

The presence of varices is subject to significant observer variation particularly for small varices and the utility of a stage 4.2 is questionable [3]. FT and TE performance were not compared to the hepatic venous pressure gradient (HVPG), which is the best predictor of varices development and which also has predictive value for decompensation [3]. However the main advantages of FT and TE are the lower cost and the absence of adverse events. Furthermore, FT had a higher applicability both for failure and reliability, and a lower interobserver variability compared with TE and HVPG [31].

FT and TE were not compared to other non-invasive serum biomarkers [6] or quantitative liver function tests [33]. However FT has been validated much more frequently than liver function tests and other patented biomarkers [6], and it has a higher prognostic performance than non-patented biomarkers such as APRI and FIB4 [13,14].

Due to the burden of repeated endoscopy in patients with cirrhosis and the low incidence of esophageal varices, more data are needed to validate the performance of biomarkers in reducing the need for endoscopy. From the evidence-based data, endoscopy is useless in patients with FT $\leqslant$0.74 and mandatory for FTs >0.95.

Conclusion

In conclusion, predetermined cut-offs of FibroTest™ and transient elastography (when reliable) permits to rank the severity of chronic hepatitis C in "seven stages", from no-fibrosis to death, with three stages in cirrhotic patients with increasing morbidity and mortality.

Conflict of interest

TP is the inventor of patented FibroTest™, with a capital interest in BioPredictive the company marketing this test (FibroSure™ in USA). The patents belong to the French Public Organization "Assistance Publique Hopitaux de Paris".

YN, MM, OD are full employees of BioPredictive, the company marketing FibroTest™.

The other authors have nothing to declare.

Authors' contribution

TP: Study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; statistical analysis; obtained funding, technical, or material support; study supervision.

JV, YN, MM, WM, MC, VT, ES, CB, JA, MR, OD, PL, and DT: Acquisition of data; analysis and interpretation of data.

VR: Acquisition of data; analysis and interpretation of data; critical revision of the manuscript for important intellectual content.

VDL: Study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhep.2013.11.016.

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