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Transient elastography and biomarkers for liver fibrosis assessment and follow-up of inactive hepatitis B carriers

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SUMMARY

Background
Non invasive methods for fibrosis evaluation remain to be validated longitudinally in hepatitis B.

Aim
To evaluate longitudinally transient elastography (TE) and biomarkers for liver fibrosis assessment and follow-up of hepatitis B virus (HBV) inactive carriers.

Methods
Three hundred and twenty-nine consecutive HBeAg-negative HBV patients (201 inactive carriers) who underwent TE, Fibrotest and aspartate to platelet ratio index (APRI) the same day were studied.

Results
TE (median 4.8 vs. 6.8 kPa, $P < 0.0001$), Fibrotest (0.16 vs. 0.35, $P < 0.0001$) and APRI values (0.28 vs. 0.43, $P < 0.0001$) were significantly lower in inactive carriers than in the remaining patients whereas they did not differ among inactive carriers according to HBV DNA levels. In 82 inactive carriers with repeated examinations, although differences were observed among individual patients, TE values did not differ significantly over time (median intra-patient changes at end of follow-up relative to baseline: −0.2 kPa, $P = 0.12$). Conversely, significant fluctuations were observed for Fibrotest (+0.03, $P = 0.012$) and APRI (−0.01, $P < 0.05$). Eleven inactive carriers (5.5%) had initial elevated TE values (>7.2 kPa) confirmed during follow-up in two with significant fibrosis (F2 and F3) on liver biopsy.

Conclusion
Non-invasive tools, particularly TE, could be useful, in addition to HBV DNA and transaminase levels, for follow-up of HBV inactive carriers as well as better selection of patients who require a liver biopsy.

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INTRODUCTION

Chronic hepatitis B virus (HBV) infection with negative Hepatitis B e antigen (HBeAg) is becoming the predominant type of chronic HBV infection worldwide,[1] as well as in France.[2] The clinical spectrum of HBeAg-negative chronic HBV infection may range from the inactive chronic HBsAg carrier state, characterised by persistently normal alanine aminotransferase (ALT) values, low or undetectable viremia and no liver injury to active chronic hepatitis B (CHB) with elevated ALT activity, high HBV DNA levels and active liver histological lesions. It is important and sometimes difficult to distinguish true inactive carriers (IC) from patients with active HBeAg-negative CHB in whom phases of spontaneous remission may occur.[3] IC have a good prognosis with a very low risk of complications and need just to be followed up regularly.[1] Differential diagnosis with CHB is based largely on careful monitoring of ALT activity, serum HBV DNA levels and liver histology. Although liver biopsy remains the reference method for assessment of liver disease severity in chronic HBV infection, it is currently not recommended in IC.[4, 5] In addition, liver biopsy is a painful and an invasive procedure[6, 7] with rare but potentially life-threatening complications[8, 9] and prone to sampling errors.[10, 11] These limitations have stimulated the search for new non-invasive approaches.[12–14] A variety of methods including the measurement of liver stiffness, using transient elastography (TE) and biomarkers, ranging from routinely available nonpatented scores such as APRI to more complex patented algorithms such as the Fibrotest (FT), have been proposed for the non-invasive assessment of hepatic fibrosis, mainly in chronic hepatitis C.[15–20] Validation of these methods in hepatitis B is ongoing[21–24] but longitudinal data are still pending. The aim of this longitudinal study was to evaluate the value of TE and biomarkers (FT and APRI) for liver fibrosis assessment and follow-up of HBV IC.

PATIENTS AND METHODS

Patients

Between June 2003 and June 2009, 412 patients were referred to our centre for HBeAg-negative HBV infection. The diagnosis of chronic HBV infection was based on the presence in serum of HBsAg and anti-HBe antibodies for more than 6 months. Patients with other viral infection [HIV (n = 11), HCV (n = 7), HDV (n = 5)], other causes of liver disease (n = 17) and unsuccessful liver stiffness measurements (n = 43) were excluded from the study. Finally, 329 patients were analysed. Two hundred and one patients were considered as IC on the basis of persistently normal ALT and AST and HBV DNA $<10^5$ copies/mL (<20 000 IU/mL) on at least two determinations during the past 6 months which corresponded to the IC definition when the study was initiated.[25] The remaining patients (n = 128) were considered as HBeAg-negative chronic hepatitis (CHB). IC patients were also analysed according to the new HBV DNA threshold of 2000 IU/mL proposed by EASL guidelines in 2009.[4]

All patients were enrolled after giving their written informed consent to the study which was approved by the Local Ethics Committee.

Liver histology and staging of liver fibrosis

Liver biopsy was performed according to clinical needs by senior operators using the Menghini technique with a 1.6-mm-diameter needle (Hepafix; Braun, Melsungen, Germany). Biopsy specimens were fixed in formalin and embedded in paraffin. All biopsy specimens were analysed by the same trained pathologist blinded to the results of non-invasive tests.

Liver fibrosis and necroinflammatory activity were evaluated semi-quantitatively according to the METAVIR scoring system.[26, 27] Fibrosis was staged on a 0–4 scale, as follows: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis and few septa; F3, numerous septa without cirrhosis; F4, cirrhosis. Activity was graded as follows: A0, none; A1, mild; A2, moderate; A3, severe.

Two clinically relevant end points were chosen: significant fibrosis ($F \geq 2$) and cirrhosis (F4). The presence of significant fibrosis in HBV patients is considered a hallmark of a progressive liver disease and an indication for antiviral treatment and the presence of cirrhosis triggers screening for complications such as oesophageal varices and hepatocellular carcinoma.[4]

Liver stiffness measurement

Liver stiffness measurements were performed using TE (FibroScan; Echosens, Paris, France). Details of the technical background and examination procedure have been previously described.[28] Ten successful measurements were performed on each patient. The success rate was calculated as the number of validated measurements divided by the total number of measurements. The results were expressed in kilopascals (kPa). The median value of successful measurements was considered representative of the liver stiffness in a given patient, according to the manufacturer's recommendations [interquartile range (IQR) <30% of the median value and success rate >60%]. Unsuccessful results were defined as

either failure (no valid measurement) or unreliable results (valid measurements <10 or success rate <60% or IQR >30% of median value).[29] The cut-offs used for diagnosing significant fibrosis and cirrhosis were those proposed by Marcellin *et al.*[21] in French patients (7.2 and 11.0 kPa respectively).

Liver stiffness measurements were repeated over time (every 6–12 months) since 2005 in IC patients, particularly in those with initial elevated liver stiffness values (>7.2 kPa). In case of elevated liver stiffness values on at least two examinations, a liver biopsy was proposed.

Serum biomarkers

The parameters allowing the calculation of FT and APRI were determined in the same laboratory on blood sampled at the time of TE. The FT score was purchased from Biopredictive website (http://www.biopredictive.com). The APRI was calculated according to the original formula as follows: AST levels divided by its upper normal limit/platelet count (10^9/L) × 100.[18] The cut-offs used for diagnosing significant fibrosis and cirrhosis were those from original publications: FT values >0.48 and >0.74 respectively;[19] APRI values <0.5 or ≥1.5 and <1 or ≥2 respectively.[18]

Statistical analysis

Patient characteristics are given as mean ± s.d. or as median and range as appropriate. Comparisons between groups were performed using nonparametric tests, including the Mann–Whitney test (two groups) or the Kruskal–Wallis test (three groups). Comparisons between groups for qualitative data were performed using Chi-squared test or Fisher's exact test when necessary.

Table 1 | Characteristics of the 329 HBeAg-negative patients at the time of fibrosis evaluation according to their status: inactive carriers and chronic active hepatitis (CHB) patients

	HBeAg-negative Total (n = 329)	Inactive carriers (n = 201)	HBeAg-negative CHB patients (n = 128)	P
Gender (male)	62%	54%	76%	<0.0001
Age (years)	39 ± 14	36 ± 12	44 ± 16	<0.0001
BMI (kg/m^2)	24.0 ± 3.9	23.7 ± 3.8	24.4 ± 4.1	N.S.
ALT (IU/L) (n < 50)	46 ± 70	27 ± 11	75 ± 106	<0.0001
AST (IU/L) (n < 50)	37 ± 41	27 ± 8	55 ± 61	<0.0001
Platelets (10^9/L)	227 ± 72	237 ± 67	213 ± 79	0.004
HBV DNA (IU/mL)	2.7 ± 17.1 × 10^6	1.5 ± 2.7 × 10^3	7.4 ± 27.9 × 10^6	0.0002

BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBV, hepatitis B virus.

Table 2 | Diagnostic performances of transient elastography (TE), Fibrotest (FT) and APRI in the 60 patients with a liver biopsy

Method	AUROC (95% CI)	Endpoint	Cut-offs	Se (%)	Sp (%)	PPV (%)	NPV (%)	+LR	−LR	Correctly classified (%)
TE	0.76 (0.63-0.90)	F ≥ 2	>7.1 kPa*	68	63	83	42	1.84	0.51	67
FT	0.71 (0.58-0.85)		>0.48	61	81	90	43	3.21	0.48	67
APRI	0.66 (0.50-0.82)		<0.5	62	64	38	64	1.72	0.59	
			≥1.5	14	100	100	30	Infinite	0.86	27
TE	0.89** (0.80-0.98)	F4	>9.6 kPa*	87	80	59	95	4.35	0.16	82
			>11.0 kPa	73	87	65	91	5.31	0.31	83
FT	0.74 (0.58-0.90)		>0.74	47	91	67	84	5.20	0.58	80
APRI	0.79 (0.67-0.91)		<1.0	47	80	44	82	2.35	0.66	
			≥2.0	13	96	50	76	3.25	0.90	63

* Optimised cut-offs in our population.

** P < 0.03 vs. FT.

Intra-group comparisons were made using Wilcoxon's test for paired data. Tests were two-tailed and *P*-values <0.05 were considered significant.

Receiver operating characteristics (ROC) curves were constructed. Sensitivity (Se), specificity (Sp), positive and negative predictive values (PPV and NPV), positive likelihood ratio (+LR) and negative likelihood ratio (−LR) were calculated using cut-offs previously described for significant fibrosis and cirrhosis for TE, FT and APRI. Also for TE, cut-offs were established in our population according to ROC curve in order to maximise Se and Sp. Areas under ROC curve (AUROC) were calculated using the trapezoidal rule. Comparisons of AUROCs were done using the method described by Hanley and McNeil for correlated data.[30] Initially, we compared all AUROCs, and in case of rejection of the null hypothesis (all AUROCs are equal), differences were searched for by two-by-two comparisons, using Bonferroni adjustment for multiple pairwise comparisons. Analyses were performed using SPSS software (Statistical Systems, Kayville, UT, USA) and STATA V8.0 (Stata Statistical Software, release 8.0; StataCorp, College Station, TX, USA).

RESULTS

Patients

The baseline characteristics of the 329 patients are shown in Table 1. There were 205 men (62%), and their mean age was 39 ± 14 years. Among these patients, 60 underwent a liver biopsy. The mean liver biopsy length was 22.6 ± 8.0 mm. As expected, IC were significantly younger (36 ± 12 vs. 44 ± 16 years respectively, $P < 0.0001$), were less often men (54% vs. 76% respectively, $P < 0.0001$) and had lower HBV DNA ($1.5 \pm 2.7 \times 10^3$ vs. $7.4 \pm 27.9 \times 10^6$ IU/mL respectively, $P = 0.0002$) than CHB patients.

Cross-sectional study

Comparative diagnostic performance of TE and biomarkers for fibrosis staging. In the 60 patients who underwent a liver biopsy, activity grade and fibrosis score were as follows: A0–A1 ($n = 25$); A2 ($n = 22$); A3 ($n = 13$) and F0–F1 ($n = 16$); F2 ($n = 16$); F3 ($n = 13$); F4 ($n = 15$). Diagnostic performances of TE and biomarkers are shown in Table 2.

TE, FT and APRI had similar performance for F ≥ 2 [AUROC (95% CI): 0.76 (0.63–0.90), 0.71 (0.58–0.85) and 0.66 (0.50–0.82) respectively, P = N.S.]. Conversely, for F4 TE had better performance than FT [0.89 (0.80–0.98) vs. 0.74 (0.58–0.90) respectively, $P = 0.03$] but not different from APRI [0.89 (0.80–0.98) vs. 0.79 (0.67–0.91) respectively, P = N.S.]. The TE cut-offs optimised in our population were: 7.1 kPa for significant fibrosis (F ≥ 2) and 9.6 kPa for cirrhosis (F4) respectively.

Comparison of TE and biomarkers between IC and HBeAg-negative disease. IC had significantly lower liver stiffness values [median: 4.8 (4.1–5.8) vs. 6.8 (4.9–9.5)

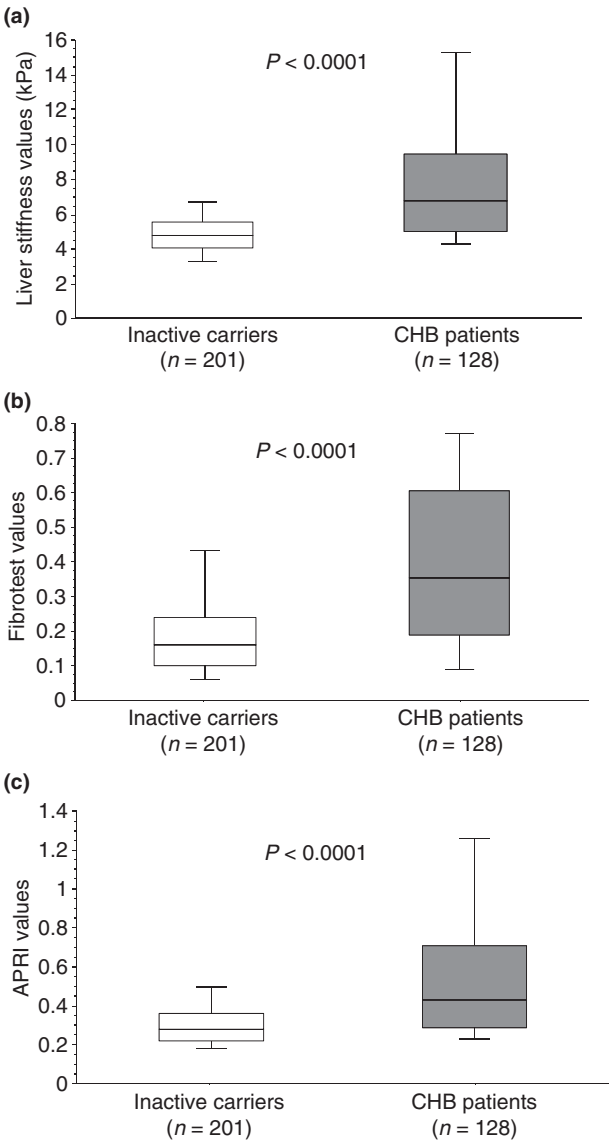

Figure 1 | Box plots of liver stiffness (a), Fibrotest (b), and APRI (c) values in the 201 IC patients and the 128 CHB patients. The top and bottom of the boxes are the first and third quartiles respectively. The length of the box thus represents the IQR within which 50% of the values were located. The line through the middle of each box represents the median. The error bars show the minimum and maximum values (range).

Table 3 | Characteristics of the 11 inactive carriers patients with baseline elevated liver stiffness values (>7.2 kPa) with repeated measurements

Patients	Gender/age	BMI (kg/m^2)	ALT B-line (IU/L)	HBV DNA B-line (IU/mL)	LSM B-line (kPa)	FT B-line	APRI B-line	Delay B-line and EOF (days)	LSM second (kPa)	LSM third (kPa)	LSM fourth (kPa)	FT second	FT third	FT fourth	APRI second	APRI third	APRI fourth	ALT EOF (IU/L)	HBV DNA EOF (IU/mL)	LB
1	M/25	21.6	33	7536	7.4	0.12	0.24	460	7.3	6.5	7.8	0.09	0.16	0.20	0.17	0.23	0.23	35	7325	F2
2	F/25	19.7	24	<12	7.8	0.17	0.13	182	4.1	5.8	–	0.22	0.14	–	0.19	0.17	–	32	<12	No
3	M/49	27.1	35	<12	7.8	0.20	0.24	179	6.8	–	–	0.18	–	–	0.26	–	–	35	<12	No
4	M/31	20.5	18	315	7.9	0.13	0.25	253	5.9	–	–	0.22	–	–	0.32	–	–	23	<12	No
5	M/52	23.4	47	1600	8.0	0.43	0.08	236	5.4	4.8	–	0.33	0.28	–	0.10	0.09	–	34	973	No
6	M/18	21.8	24	662	8.2	0.11	0.41	395	9.8	9.5	12.7	0.12	0.08	0.06	0.28	0.35	0.36	21	372	F3
7	M/59	24.9	29	<12	8.6	0.34	0.60	247	6.3	–	–	0.30	–	–	0.51	–	–	20	<12	No
8	M/22	24.6	25	1782	8.8	0.16	0.54	100	5.9	–	–	0.15	–	–	0.54	–	–	29	1239	No
9	M/45	23.4	38	774	9.4	0.14	0.31	186	6.1	6.7	6.6	0.10	0.27	0.20	0.35	0.44	0.38	38	118	No
10	M/23	19.6	21	9380	9.5	0.06	0.21	416	6.9	6.8	–	0.13	0.12	–	0.17	0.20	–	9	9216	No
11	M/26	26.4	40	566	11.6	0.41	0.36	329	5.8	5.3	4.9	0.30	0.42	0.58	0.43	0.35	0.46	42	715	No

BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBV, hepatitis B virus; M, male gender; F, female gender; LSM, liver stiffness measurement; FT, Fibrotest; LB, liver biopsy: B-line, baseline; EOF, end of follow-up.

kPa respectively; $P < 0.0001$], FT values [median: 0.16 (0.10–0.25) vs. 0.35 (0.19–0.60) respectively; $P < 0.0001$] and APRI values [median: 0.28 (0.22–0.35) vs. 0.43 (0.29–0.72) respectively; $P < 0.0001$] than CHB patients (Figure 1).

Interestingly, 11 IC (5.5%) had elevated liver stiffness values (≥ 7.2 kPa) suggestive of significant fibrosis (Table 3). All were offered follow-up and their characteristics are detailed below in the longitudinal study chapter.

Comparison of TE and biomarkers according to HBV DNA levels in IC. The distribution of serum HBV DNA levels among IC was as follows: undetectable (<12 IU/mL), 33 (16%); >12 IU/mL and <2000 IU/mL, 139 (65%) and >2000 IU/mL and <20 000 IU/mL, 39 (19%). Patients with HBV DNA levels <2000 IU/mL (81%) correspond to the recently proposed EASL definition for IC[4] whereas patients with HBV DNA levels <20 000 IU/mL correspond to the classical definition.[25] IC did not differ according to serum HBV DNA levels for baseline characteristics (age, gender, BMI, ALT and AST) as well as for liver stiffness values, FT and APRI values (Figure 2).

Longitudinal study

Among the 201 IC, 82 underwent repeated TE and biomarkers determinations: two determinations ($n = 82$, median interval of 11.5 months; range: 3.3–26.8); three determinations ($n = 48$, median 23.1 months; range: 10.1–34.7) and four determinations ($n = 27$, median 34.4 months; range: 21.6–49.1). When pooling the different time points for the 82 patients, taking into account the last available time point (end of follow-up), the median interval was: 21.7 months (range: 3.3–49.1). These 82 patients did not differ from the other IC for baseline characteristics (age, gender, BMI, ALT, AST, platelet count), FT and APRI except for liver stiffness values (median 5.0 vs. 4.6 kPa respectively, $P = 0.005$). Indeed, as mentioned before, eleven of these patients had liver stiffness values >7.2 kPa at first examination, suggestive of the presence of significant fibrosis. Their characteristics are detailed in Table 3. These patients did not differ from the other 190 IC for most baseline characteristics (age, gender, BMI, ALT, AST and platelet count), FT and APRI, and TE success rate, except for the IQR/median value ratio (mean 0.21 ± 0.08 vs. 0.16 ± 0.07 respectively, $P < 0.03$). During follow-up, liver stiffness values returned to values below 7.2 kPa in all patients except two. These two patients (number 1 and 6) underwent liver biopsy. Patient number 1 had moderate fibrosis (F2) whereas patient number 6 had severe fibrosis (F3). Both were offered antiviral treatment.

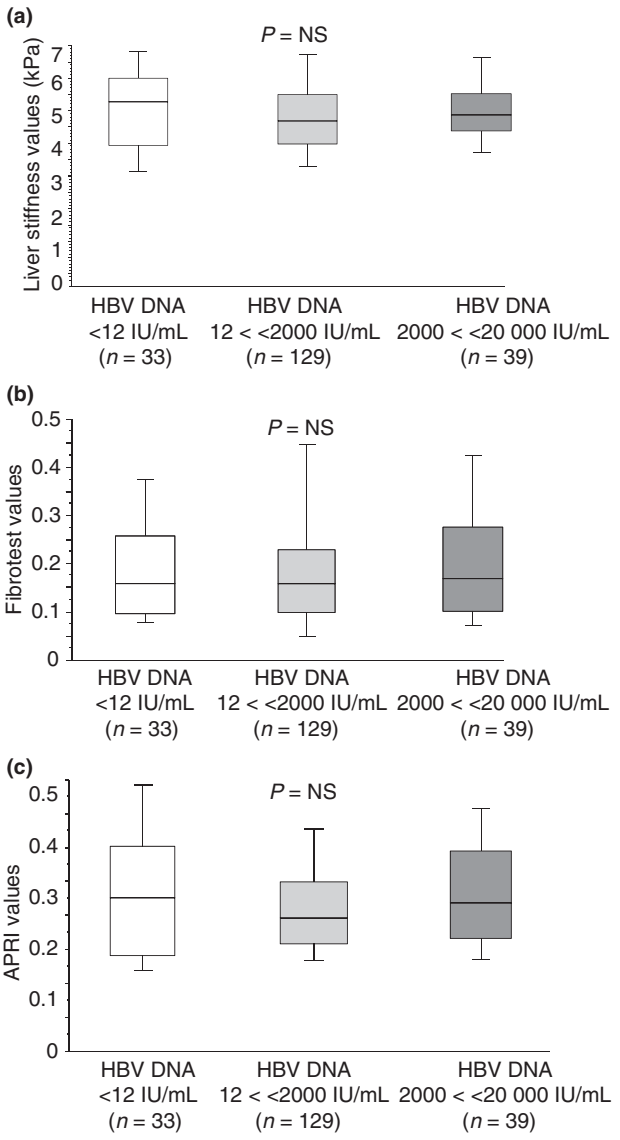

Figure 2 | Box plots of liver stiffness (a), Fibrotest (b), and APRI (c) values in the 201 IC patients according to HBV DNA levels. The top and bottom of the boxes are the first and third quartiles respectively. The length of the box thus represents the IQR within which 50% of the values were located. The line through the middle of each box represents the median. The error bars show the minimum and maximum values (range).

Table 4 shows the median intra-patient changes in liver stiffness, FT, APRI, AST, ALT and HBV DNA values at different time points relative to baseline in the 82 IC patients who underwent at least two determinations of non-invasive methods over time. Globally, the liver

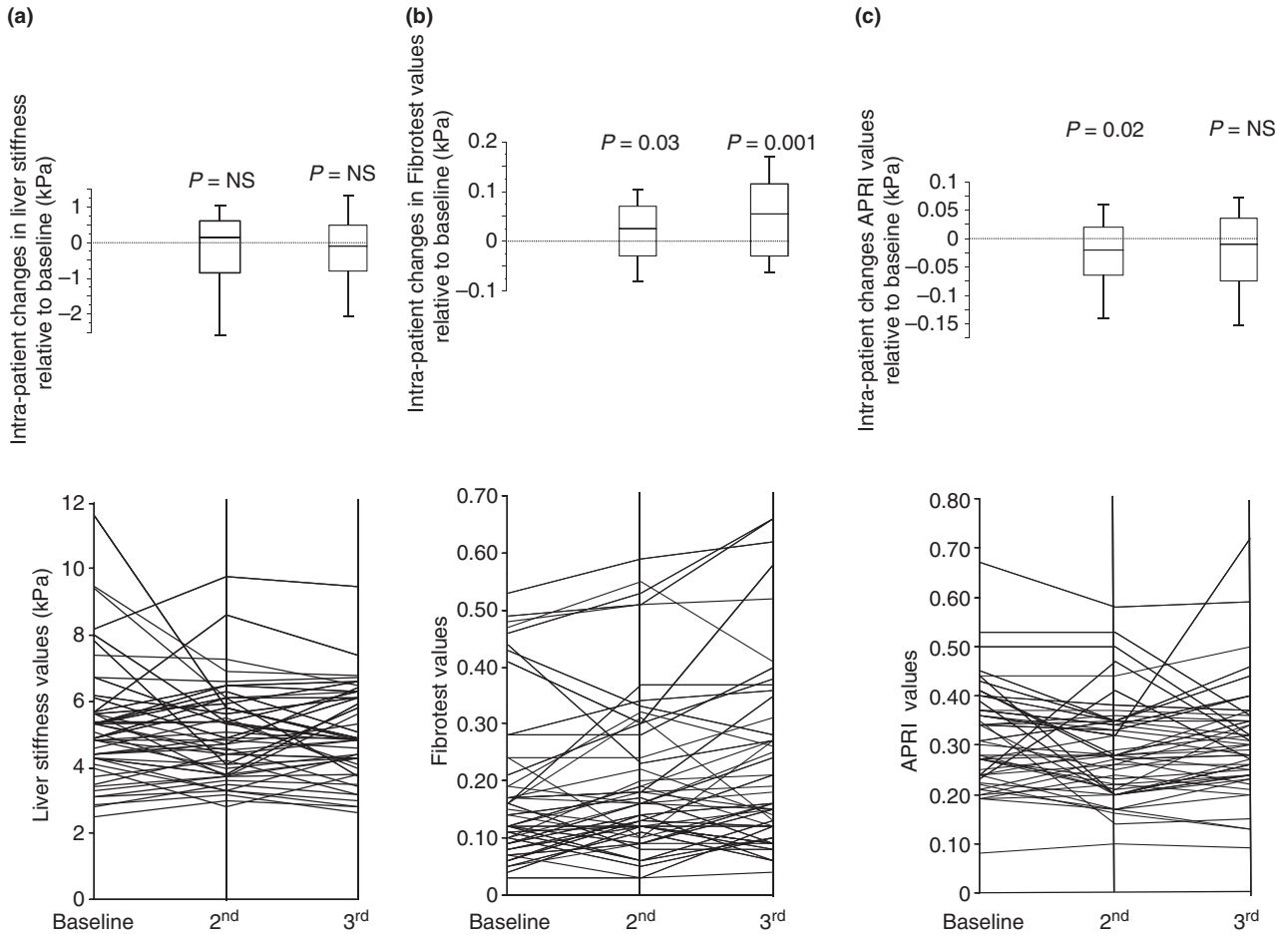

Figure 3 | Evolution over time of liver stiffness (a), Fibrotest (b), and APRI (c) values in the 48 inactive carriers patients who underwent three determinations over time (median: 23.1 months; range: 10.1–34.7). Median intra-patient changes at different time points relative to baseline (top) and individual data (bottom).

stiffness changes observed in 82 patients at the end of follow-up (last determination available) relative to baseline were not significant, although there was a trend towards a decrease in liver stiffness over time (median intra-patient changes at end of follow-up: −0.2 (−1.2 to +0.7) kPa, $P = 0.12$). As shown in Figure 3a, there were differences among individual patients, however, the median intra-patient liver stiffness changes relative to baseline were not significant: +0.1 (−0.9 to +0.5) kPa ($P = 0.41$) at second determination and −0.1 (−0.9 to +0.5) kPa ($P = 0.33$) at third determination (Table 4). Similarly, no significant change was observed for AST, ALT and HBV DNA levels at the end of follow-up relative to baseline (Table 4).

Conversely, a significant increase in FT values was observed over time: median of intra-patient changes in FT values at the end of follow-up relative to baseline [+0.03 (−0.04 to +0.09), $P = 0.012$; Table 4). Patients had significantly higher values at the end of follow-up than at baseline [median 0.19 (0.12–0.32) vs. (0.16 (0.10–0.24), $P = 0.012$]. Five patients (6%) had at baseline FT values suggestive of significant fibrosis (>0.48) but none of cirrhosis (>0.74). During follow-up, FT values remained comprised between 0.48 and 0.74 in these five patients. As shown in Figure 3b, there were important differences among individual patients and FT values fluctuated over time: as a result, median intra-patient FT changes relative to baseline were significant: +0.03 (−0.03 to +0.07) ($P = 0.03$) at second determination and +0.05 (−0.03 to +0.11) ($P = 0.001$) at third determination (Table 4).

As for APRI, there were also differences among individual patients and APRI values fluctuated over time (Figure 3c). A significant decrease was observed over time: median of intra-patient changes at the end of follow-up relative to baseline [−0.01 (−0.07–+0.03), $P < 0.05$; Table 4].

Table 4 | Median (IQR) intra-patient liver stiffness, Fibrotest, APRI, AST, ALT and HBV DNA values changes at different time points relative to baseline in inactive carriers patients with at least two determinations of non-invasive methods over time (EOF: last determination available)

Patients	Time point	Liver stiffness (kPa)	*P*	Fibrotest	*P*	APRI	*P*	AST (IU/L)	*P*	ALT (IU/L)	*P*	HBV DNA (IU/mL)	*P*
2 time points (n = 82)	EOF	−0.2 (−1.2 to +0.7)	0.12	+0.03 (−0.04 to +0.09)	0.012	−0.01 (−0.07 to +0.03)	<0.05	0.0 (−4.0 to +4.0)	0.99	0.0 (−6.0 to +5.0)	0.64	0.0 (−461 to +290)	0.98
3 time points (n = 48)	Second	+0.1 (−0.9 to +0.5)	0.41	+0.03 (−0.03 to +0.07)	0.03	−0.02 (−0.06 to +0.02)	0.02	0.0 (−5.5 to +3.0)	0.28	−2.0 (−9.0 to +2.0)	0.10	117.0 (−509 to +3230)	0.14
	Third	−0.1 (−0.9 to +0.5)	0.33	+0.05 (−0.03 to +0.11)	0.001	−0.01 (−0.08 to +0.04)	0.41	0.5 (−3.8 to +5.0)	0.99	0.0 (−5.0 to +4.0)	0.80	0.0 (−770 to +848)	0.82
4 time points (n = 27)	Second	+0.2 (−0.8 to +0.9)	0.98	0.00 (−0.04 to +0.06)	0.55	0.00 (−0.06 to +0.04)	0.45	2.0 (−4.0 to +4.0)	0.60	0.0 (−3.0 to +7.0)	0.90	117.0 (−422 to +4076)	0.18
	Third	−0.1 (−1.0 to +0.5)	0.50	+0.07 (0.00 to +0.13)	0.002	+0.02 (−0.06 to +0.06)	0.71	2.0 (−2.0 to +6.0)	0.22	2.0 (−3.0 to +7.0)	0.09	−70.0 (−1786 to +91)	0.21
	Fourth	+0.1 (−1.1 to +0.9)	0.88	+0.05 (−0.02 to +0.09)	0.08	+0.01 (−0.05 to +0.05)	0.75	3.0 (−1.0 to +5.0)	0.07	3.0 (−1.3 to +9.0)	0.03	4.0 (−1806 to +244)	0.89

EOF, end of follow-up; *P*-values are for intra-patients changes relative to baseline.

DISCUSSION

The results of the present study, based on a large series of consecutive HBeAg-negative HBV patients, suggest that non-invasive tools for liver fibrosis assessment, particularly TE, may be useful for liver fibrosis assessment and follow-up of IC.

Among the different available non-invasive methods, we chose to evaluate two different and complementary approaches: (i) a physical approach based on the measurement of liver stiffness using TE; (ii) a biological approach based on serum biomarkers including a patented algorithm (FT) and a free nonpatented index (APRI).[31] These three non-invasive methods are by far the most widely used and validated.[32–34]

In the cross-sectional part of the study, we first validated the performances of TE, FT and APRI against liver biopsy for diagnosing significant fibrosis and cirrhosis in our population. TE had better diagnostic accuracy for cirrhosis (AUROC: 0.89) than for significant fibrosis (0.76), as previously reported in hepatitis C[32] and hepatitis B.[21, 35, 36] Interestingly, the TE cut-off we found for significant fibrosis (7.1 kPa) is similar to the one we proposed initially in chronic hepatitis C[16] and close to those recently proposed in CHB by Marcellin *et al.* (7.2 kPa),[21] as well as other groups.[35, 36] As for cirrhosis, our cut-off (9.6 kPa) is lower than in hepatitis C (12.5 kPa)[16] but close to those proposed in hepatitis B.[21, 23, 35, 36] The higher prevalence of macronodular cirrhosis in CHB than in hepatitis C as well as differences in the prevalence of cirrhosis in the different studied populations may account for these differences. As for FT and APRI, their performances were in keeping with those previously reported in hepatitis B.[24, 37–39] Interestingly, although performance did not differ among the three methods for the diagnostic accuracy of significant fibrosis, TE had better diagnostic accuracy than FT for cirrhosis, a finding consistent with our experience in patients with chronic hepatitis C.[40] In that respect, some authors have proposed algorithms combining TE with serum biomarkers to increase diagnostic accuracy for advanced fibrosis in patients with CHB.[41]

Although liver biopsy remains important for determining active disease,[42] it is not feasible or necessary to biopsy all HBeAg-negative patients with near normal ALT and relatively low HBV DNA levels.[43] Non-invasive tests could provide an additional adjunct for separating patients with inactive and minimal fibrosis from those for whom a liver biopsy could be mandated. Liver stiffness values in our series of 201 IC were low and similar to those reported in two recent series of healthy individ-

uals with[44] and without[45] liver biopsy as well as in patients with chronic hepatitis C and persistently normal ALT.[46] In addition, liver stiffness values were significantly lower than in CHB patients and consistent with those previously reported in IC.[36, 47] However, no serum fibrosis biomarker was evaluated in these two studies.[36, 47] Interestingly, both APRI and FT values were low in our IC as previously reported[38] and significantly lower than in CHB patients.

As a new definition of IC has been recently proposed,[4] we also examined the results of non-invasive tests according to HBV DNA levels. Approximately 80% of our IC patients had HBV DNA levels <2000 IU/mL, corresponding to this new definition, a finding in keeping with those of a recent series of 85 IC where 23% of patients had HBV DNA <2000 IU/mL.[48] Interestingly, TE, FT and APRI values did not differ according to HBV DNA levels in IC whereas they were significantly higher in patients with HBV DNA >20 000 IU/mL. Such findings are consistent with those of Papatheodoridis *et al.*[43] showing that in patients with persistently normal ALT and HBV DNA <20 000 IU/L, the 2000 IU/L cut-off does not allow discriminating between patients with and without significant fibrosis on liver biopsy.

In the second longitudinal part of the study, we thoroughly evaluated the evolution of TE, FT and APRI over time by measuring median intra-patients changes at different time points relative to baseline in 82 IC with repeated examinations (two to four examinations). Interestingly, although some differences were observed among individual patients (Figure 3), liver stiffness values were stable over time, as were AST, ALT and HBV DNA levels. Conversely, significant fluctuations were observed with FT and APRI.

We have no clear explanation for this latter finding. In the only study were FT was assessed longitudinally in 160 IC,[38] reproducibility was deemed excellent but no data were provided regarding the median or mean FT values over time. It is possible that some components of FT such as bilirubin levels or alpha-2-macroglobulin may fluctuate over time.[49] Similarly with APRI, AST and platelet may fluctuate over time. Concerning TE, it has been shown to be highly reproducible[50] and fluctuations of liver stiffness over time are mainly related to acute inflammation or ALT flares,[22, 36, 51] which are usually not observed in IC.

Interestingly, eleven out of 201 IC (5.5%) had baseline elevated liver stiffness values (>7.2 kPa), suggestive of the presence of significant fibrosis. During follow-up, liver stiffness values returned to values below 7.2 kPa in all patients but two. It should be noted that when compared to the other IC, these 11 patients had significantly higher TE IQR/median value ratio. The importance of this ratio for accuracy of TE results has been recently emphasised.[52] This finding suggests that when liver stiffness values are elevated (>7.2 kPa) in patients with a profile of IC, TE should be repeated before taking a liver biopsy, to make sure that IQR/median value ratio is satisfactory. Indeed, in the two patients with baseline elevated liver stiffness values confirmed on a second examination, liver biopsy revealed the presence of significant fibrosis in both cases (F2 and F3), suggesting that TE in this context is a sensitive tool for detection of significant fibrosis.

In conclusion, our results suggest that non-invasive tools for liver fibrosis assessment, particularly TE, could be useful, in addition to HBV DNA and transaminase levels, for follow-up of HBV IC as well as better selection of patients who require a liver biopsy.

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