# Hepatocellular Carcinomas in Patients With Metabolic Syndrome Often Develop Without Significant Liver Fibrosis: A Pathological Analysis

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**Metabolic syndrome (MS) is a newly identified risk factor in chronic liver disease (CLD) and hepatocellular carcinoma (HCC). The aim of this study was to analyze the pathological characteristics of HCC and nontumoral liver in patients with MS as the only risk factor for liver disease in comparison with those that developed in the course of other CLDs in order to provide further insight into the physiopathology of HCC associated with MS. HCC patients with features of MS as the only risk factor for liver diseases (MS group, n = 31) were compared to HCC patients with overt causes of CLD (CLD group, n = 81) or without causes of CLD (cryptogenic group, n = 16) who underwent surgical resection during the same period of time. Among the patients of the MS group, there were 30 males and 1 female. In comparison with the patients with HCC of the CLD group, the patients with MS were older (mean age: 67± 7 versus 59 ± 14 years, $P < 0.01$), and the background liver was significantly more often free of significant fibrosis (F0-F2: 65% in the MS group versus 26% in the CLD group, $P < 0.001$). In addition, HCCs associated with MS were more often well differentiated (65% versus 28%, $P < 0.001$). Five HCCs, all from the MS group, developed on a preexisting liver cell adenoma, with three of them showing typical histological features of telangiectatic adenoma. *Conclusion:* This study shows that HCCs in patients with features of MS as the only risk factor for liver disease have distinct morphological characteristics and mainly occur in the absence of significant fibrosis in the background liver. In addition, some of them arise through malignant transformation of a preexisting liver cell adenoma.** (HEPATOLOGY **2009;49:851-859.**)

Hepatocellular carcinoma (HCC) ranks as the fifth most common malignancy worldwide and has become a major concern in Western countries because of its dramatically increasing incidence.[1] To a great extent, this increase has been attributed to hepatitis C virus (HCV) epidemic infection, but it also parallels the increasing incidence of obesity and type 2 diabetes.[2,3] Indeed, obesity and type 2 diabetes are two conditions clearly associated with the development of nonalcoholic fatty liver disease (NAFLD), which is currently recognized as one of the leading causes of chronic liver disease (CLD).[4,5]

NAFLD encompasses a full spectrum of metabolic fatty liver disorders, including simple steatosis and nonalcoholic steatohepatitis (NASH), that may progress to fibrosis and cirrhosis.[6-9] Although steatosis is recognized as a benign liver condition with limited risk of progression, NASH is clearly associated with further development of fibrosis, which will evolve into cirrhosis in approximately 20% of cases.[7,10] Accordingly, several studies have demonstrated that a significant percentage of patients considered to have cryptogenic (CG) cirrhosis probably have NAFLD, supporting the notion that NAFLD is a significant cause of CG end-stage liver disease.[11-13] Liver complications related to NASH-induced cirrhosis have been

*Abbreviations: BMI, body mass index; CG, cryptogenic; CLD, chronic liver disease; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; LCA, liver cell adenoma; MS, metabolic syndrome; NAFLD, nonalcoholic fatty liver disease; NAS, nonalcoholic fatty liver disease activity score; NASH, nonalcoholic steatohepatitis; NS, not significant; TA, telangiectatic adenoma.*

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documented, including rare cases of HCC.[3,13-16] Although, as in other CLDs, the presence of cirrhosis may promote development of HCC in such patients per se, diabetes and obesity also appear to be independent risk factors in liver carcinogenesis.[17,18]

NAFLD/NASH is now regarded as the liver manifestation of metabolic syndrome (MS). The definition of MS, which has evolved over time, is based on the presence of several clinical and biological parameters reflecting dyslipidemia, insulin resistance, hypertension, and obesity.[19] Clear-cut recognition of this syndrome now enables more accurate study of patients with liver diseases related to MS, especially HCC. Indeed, characteristics of HCC occurring in the setting of NAFLD have been poorly described, and very few have been reported in patients without cirrhosis.[20] Therefore, the purpose of our study was to analyze a series of HCCs that arose in patients with features of MS as the only risk factor for CLD and to compare their clinicopathological characteristics to those of HCCs that developed in the setting of other CLDs in order to provide further insight into the physiopathology of HCCs associated with MS.

## Patients and Methods

***Study Design.*** We retrieved pathological files of patients who had undergone liver resection for HCC between 1995 and 2007 at Beaujon Hospital (Clichy, France). Patients who received chemotherapy or percutaneous therapy before surgery and patients who underwent transplantation were ruled out. For all patients, any potential causes of CLD, including HCV and hepatitis B virus (HBV) infection (serological tests), genetic hemochromatosis (positive genetic testing or hepatic iron index $>$ 1.9), autoimmune liver diseases (serum autoantibodies), and excessive alcoholic consumption (defined by consumption higher than 40 g/day), were screened. In addition, we collected the main metabolic risk factors, including diabetes mellitus (fasting plasma glucose $>$ 6.1 mmol/L), obesity [body mass index (BMI) $>$ 30 or waist to hip ratio $>$ 0.9 in males or $>$ 0.85 in females], dyslipidemia (triglycerides $\geq$1.7 mmol/L or high-density lipoprotein cholesterol $<$ 0.9 mmol/L in males or $<$ 1 mmol/L in females), and hypertension (blood pressure $>$140/90 mm Hg). According to the Adult Treatment Panel III definition, 26 patients displaying at least three metabolic risk factors were defined as having a definite diagnosis of MS.[21] We also included in this group five patients having only two criteria of MS but without any other risk factors for CLD.

A total of 128 patients with complete work-up were available and further studied. Among them, we defined three groups of patients according to the etiology of liver disease: presence of an overt cause of CLD (CLD group, n = 81), presence of features of MS as the only risk factor for CLD (MS group, n = 31), and no identified risk factors (CG group, n = 16). Patients with an obvious cause of CLD and superimposed features of MS were included in the CLD group. For all patients, written informed consent was available. Alpha-fetoprotein levels were available and were collected in all cases.

***Histological Analysis.*** For each case, resected liver specimens were reviewed (E.B. and V.P.), and pathological analysis was performed on paraffin-tissue sections stained with hematoxylin-eosin, Masson's trichrome, and reticulin staining. Tumor characteristics, including size, number of nodules, presence of a capsule, satellite nodules, vascular invasion, grade of differentiation, and presence of necrosis or hemorrhage, were reported. Histological analysis of the adjacent liver was also systematically performed. Steatosis was scored as absent, mild, moderate, or severe when it involved less than 5% (0), 5% to 33% (1), 33% to 66% (2), or more than 66% (3) of hepatocytes, respectively. For cases associated with MS, fibrosis was staged according to Kleiner et al.[22] as follows: no fibrosis (stage 0), zone 3 perisinusoidal or portal fibrosis (stage 1), perisinusoidal and portal fibrosis without bridging (stage 2), bridging fibrosis (stage 3), and cirrhosis (stage 4). The diagnosis of NASH was based on the NAFLD activity score (NAS), which is defined as the unweighted sum of the scores for steatosis (0-3), lobular inflammation (0-3), and ballooning (0-2), ranging from 0 to 8, according to Kleiner et al. Cases with an NAS of 0 to 2, 3 to 4, or $>$5 were considered nondiagnosed, probable, or diagnosed as NASH, respectively. For cases with viral hepatitis, staging of fibrosis was performed with the METAVIR score.[23]

***Immunohistochemistry.*** $\beta$-Catenin (BD Transduction Laboratories; 1:200 dilution) expression was studied by immunohistochemistry on selected paraffin sections of the liver tumor in all cases. $\beta$-Catenin–positive immunostaining corresponded to nuclear and/or cytoplasmic staining, whatever the number of tumor-stained hepatocytes.

***Statistical Analysis.*** Categorical data were analyzed after multiple $2 \times 2$ contingency tables were set up with the chi-square test or Fisher's exact test when appropriate. Continuous parametric data were analyzed with the Student *t* test, and nonparametric data were analyzed with the Mann-Whitney *U* test. Normally distributed data are reported as the mean $\pm$ standard error of the mean. Data not normally distributed are reported as medians. $P <$ 0.05 was considered statistically significant. Statistical

**Table 1. Main Clinical Data for Patients with Hepatocellular Carcinoma According to Background Liver Disease in the Three Groups of Patients**

| | MS Group (n = 31) | CLD Group (n = 81) | CG Group (n = 16) | *P* (MS Versus CLD) |
|---|---|---|---|---|
| Age (year) | 67.4 ± 7.5 | 59.4 ± 14.1 | 53.4 ± 15.0* | <0.01 |
| Sex (male/female) | 30/1 | 75/6 | 13/3 | NS |
| Body mass index (kg/m$^2$) | 29.7 ± 4.6 | 25.0 ± 3.6 | 22.9 ± 2.7*† | <0.0001 |
| < 25 | 6 (19%) | 46 (57%) | 13 (81%) | |
| 25–30 | 10 (32%) | 25 (31%) | 3 (19%) | |
| >30 | 15 (48%) | 10 (12%) | 0 (0) | |
| Diabetes mellitus | 24 (77%) | 10 (12%) | 1 (6%) | <0.0001 |
| Hypertension | 26 (84%) | 13 (18%) | 2 (12.5%) | <0.0001 |
| Dyslipidemia | 20 (65%) | 5 (6%) | 1 (6%) | <0.0001 |
| Incidental diagnosis | 17 (55%) | 12 (15%) | 2 (12.5%)‡ | <0.0001 |
| Alpha-fetoprotein level (ng/mL) | 1515 ± 6516 | 8339 ± 38,439 | 12,367 ± 41,425 | <0.001 |

The MS group included patients with features of MS; the CLD group included patients with an overt cause of CLD; and the CG group included patients with no evident causes of liver disease or MS.

Abbreviations: CG, cryptogenic; CLD, chronic liver disease; MS, metabolic syndrome; NS, not significant.

*Statistical difference between the MS and CG groups: $P < 0.001$.

†Statistical difference between the MS and CG groups: $P < 0.0001$.

‡Statistical difference between the MS and CG groups: $P = 0.005$.

analysis was performed with SPSS 13.0 for Windows software.

## Results

***Clinicobiological Data of Patients with HCC.*** A total of 128 patients were included in the study. The MS group was composed of 31 Caucasian patients (30 males and 1 female) with a mean age of 67 ± 7 years (range: 50-81 years). The mean BMI was 29.7 ± 4.6 (range: 20-40). Detailed data are reported in Table 1. In the group of patients with HCC related to overt causes of CLD (n = 81), the etiology was related to HBV in 29 patients (36%), HCV infection in 24 patients (30%), chronic alcohol consumption in 12 patients (15%), and hemochromatosis in 7 cases (9%) and was mixed in 9 cases (11%). This group was composed of 75 males and 6 females with a mean age of 59 ± 14 years (range: 22-82 years). In this group, in addition to CLD, 19 patients (23%) displayed at least two features of MS, including 10 with diabetes. As expected, the mean BMI was significantly higher in 19 patients with CLD having superimposed features of MS compared to the 62 patients in the CLD group without additional MS features (28.3 ± 4.3 versus 23.9 ± 2.6, $P = 0.0001$). In the remaining 16 patients, no risk factors for CLD or MS were reported (CG group). There were 13 males and 3 females with a mean age of 53 ± 15 years (range: 25-75 years). The mean BMI was 22.9 ± 2.7 (range: 19-28), and only one patient was diabetic.

A comparison of clinical data for the MS group and the other two groups showed that patients of the MS group were significantly older than those in the CLD and CG groups, whereas the sex ratio was not different between any paired groups. A diagnosis of HCC was made incidentally in 17 of the 31 patients in the MS group (55%); this percentage was significantly higher than those in the other two groups (15% in the CLD group and 12.5% in the CG group, $P < 0.0001$). In the MS group, HCC was revealed by abdominal pain in 12 patients (39%), including tumor rupture in one case. Detailed data are reported in Table 1.

***Pathological Characteristics of HCC.*** In the MS group, 24 patients (78%) had a single nodule. In cases with several tumors (seven cases, 22%), only the largest one was considered for further analysis. The mean HCC size in this group was larger than the mean HCC size in the CLD group, but the difference did not reach significance (8.8 ± 6.0 cm versus 7.8 ± 6.3 cm, $P = 0.06$). HCC was significantly more often well differentiated in the MS group than in the CLD group (64.5% versus 28%, $P < 0.001$). In the MS group, the presence of a peritumoral capsule, microscopic vascular invasion inside the capsule or immediately outside the capsule, and the presence of satellite nodules were noted in 23 (74%), 14 (45%), and 11 (35.5%) cases, respectively. These features were not significantly different in comparison with the CLD group.

HCCs in the CG group were significantly larger than those in the MS group (12.8 ± 5.8 cm versus 8.8 ± 6 cm, $P < 0.01$) or the CLD group (7.8 ± 6.3 cm, $P < 0.01$). The presence of a peritumoral capsule was less common in the CG group than in the MS group (44% versus 74%,

**Table 2. Pathological Characteristics of Hepatocellular Carcinoma and Nontumoral Liver in the Three Groups of Patients**

| | MS Group (n = 31) | CLD Group (n = 81) | CG Group (n = 16) | P (MS Versus CLD) |
|---|---|---|---|---|
| Tumor size (cm) | 8.8 ± 6 | 7.8 ± 6.3 | 12.8 ± 5.8* | 0.06 |
| Bilobar | 7 (23%) | 5 (6%) | 2 (12.5%) | 0.03 |
| Number of tumors | 1 (1-3) | 1 (1-5) | 1(1-2) | NS |
| Capsule | 23 (74%) | 51 (63%) | 7 (44%)† | NS |
| Macroscopic vascular invasion | 6 (19%) | 21 (26%) | 4 (25%) | NS |
| Differentiation | | | | |
| Well | 20 (64.5%) | 23 (28%) | 8 (50%) | |
| Moderate | 11 (35.5%) | 47 (58%) | 7 (44%) | |
| Poor | 0 (0) | 11 (14%) | 1 (6%) | <0.001 |
| Microscopic vascular invasion | 14 (45%) | 52 (64%) | 10 (62.5%) | NS |
| Satellite nodules | 11 (35.5%) | 36 (44%) | 6 (37.5%) | NS |
| Liver fibrosis | | | | |
| F0-F2 | 20 (65.5%) | 21 (26%) | 12 (75%) | |
| F3-F4 | 11 (35.5%) | 60 (74%) | 4 (25%) | <0.001 |
| Steatosis | | | | |
| 0 (<5%) | 6 (19%) | 47 (58%) | 15 (94%)‡ | |
| 1 (5%–33%) | 12 (39%) | 29 (36%) | 1 (6%) | |
| 2 (33%–66%) | 11 (35.5%) | 3(4%) | 0 (0) | |
| 3 (>66%) | 2 (6.5%) | 2(2%) | 0 (0) | <0.001 |

The MS group included patients with features of MS; the CLD group included patients with overt causes of liver disease; and the CG group included patients with no evident causes of liver disease or MS.

Abbreviations: CG, cryptogenic; CLD, chronic liver disease; MS, metabolic syndrome; NS, not significant.

*Statistical difference between the MS and CG groups: $P < 0.01$.

†Statistical difference between the MS and CG groups: $P < 0.05$.

‡Statistical difference between the MS and CG groups: $P = 0.0001$.

$P < 0.05$). Other criteria did not differentiate HCCs of the CG group from HCCs of the CLD and MS groups. Detailed data are reported in Table 2.

Interestingly, in the MS group, HCCs developed in a preexisting liver cell adenoma (LCA) in five cases, as shown by several foci of well-differentiated HCC in benign hepatocellular proliferation. Among these five adenomas, three were classified as telangiectatic adenomas (TAs) according to the presence of prominent vascular changes, including sinusoidal dilatation, peliosis, and abnormal portal tractlike remnants.[24] In addition, one LCA displayed cellular atypias, and one had no specific morphological features. No such lesion was observed in any HCCs of the CLD or CG group. When comparing patients of the CLD group with and without superimposed features of MS (19 versus 62 cases), we found no significant difference in the pathological characteristics of the tumors, including size, grade of tumor differentiation, and microscopic vascular invasion (data not shown).

Immunostaining for β-catenin was performed in all cases of HCCs. Overall, nuclear and cytoplasmic staining was observed in 18 of 128 HCCs (14%), whereas all other cases displayed normal membranous staining. Among them, 3 were observed in the MS group (3/31, 9.7%), whereas 18 were observed in control groups (3/16 or 18% in the CG group and 15/81 or 19% in the CLD group). Although frequency of nuclear staining was slightly lower in the MS group, the difference did not reach statistical significance. In the MS group, tumors with nuclear staining were well-differentiated HCCs with a mean size of 6.5 cm. In the CG group, β-catenin–mutated tumors were well and poorly differentiated in two cases and one case, respectively, with a mean size of 15 cm. In the CLD group, HCCs with nuclear positivity developed in cases with hemochromatosis (two cases), chronic alcohol consumption (one case), HBV infection (six cases), and HCV infection (six cases). The mean size of these tumors was 9.1 cm. The grade of tumor differentiation was well, moderate, and poor in four, nine, and two cases, respectively. Figure 1 illustrates β-catenin immunostaining in HCCs in patients with MS.

***Pathological Characteristics of Nontumoral Liver.*** Interestingly, HCCs in the MS group occurred most often in a background liver without significant fibrosis [11 cases (35%) with F0-F1, 6 cases (19%) with F2, 4 cases (13%) with F3, and 7 cases (22%) with F4]. In comparison with HCCs developing in the CLD group, the difference was highly significant (65% with F0-F2 in the MS group versus 26% in the CLD group, $P < 0.0001$). The same observation was made for HCCs of the CG group, which were also more commonly observed in patients with no or mild fibrosis (75% with F0-F2); this distribution was similar to that of the MS group but significantly different from that of the CLD group ($P < 0.001$). Not

surprisingly, steatosis was significantly more common in the MS group with HCC (25 cases, 81%) than in the CLD group (34 cases, 42%, $P < 0.001$) or CG group (1 case, 6%, $P < 0.0001$). Diagnosis of steatohepatitis was based on NAS. In the MS group, 19 of 31 cases (61%) displayed an NAS of 0 to 2 (not diagnosed as NASH), and 11 cases displayed an NAS of 3 or 4 (probable). Only one case had an NAS $\geq 5$ (NAS = 6, definite) according to Kleiner's criteria. Finally, in the MS group, the presence of steatosis and the diagnosis of steatohepatitis were not significantly different whether or not significant fibrosis was present in the background liver. No cases with large or small liver cell changes in nontumoral livers were observed in the MS group. The main features of nontumoral liver are reported in Table 2.

***Features of HCC According to Nontumoral Liver in the MS Group.*** Because the absence of significant fibrosis in adjacent liver tissue seems to be a relevant characteristic of HCCs in the MS group, we compared clinicopathologic criteria of HCCs according to the presence or absence of significant fibrosis in this group of patients. In the absence of significant fibrosis, HCCs were larger (10.1 $\pm$ 6.7 cm in F0-F2 versus 6.6 $\pm$ 3.5 cm in F3-F4, $P = 0.05$) and more often well differentiated [15/20 cases (75%) in F0-F2 versus 5/11 cases (45%) in F3-F4]. However, the latter difference was not statistically significant. All HCCs occurring in livers with significant fibrosis were encapsulated versus 74% (23/31) of HCCs in livers with no significant fibrosis ($P < 0.05$). No differences were observed concerning the presence of satellite nodules or vascular invasion.

Interestingly, all HCCs that developed from a preexisting LCA were observed in the group of patients without significant fibrosis. Characteristics of HCCs did not differ according to the presence of significant steatosis or NASH in nontumoral liver. Figures 2 and 3 illustrate representative cases of HCCs arising in each subgroup of patients in the MS group.

Among the three cases that displayed an abnormal $\beta$-catenin expression pattern in the tumor, two were associated with significant fibrosis in the background liver.

## Discussion

HCC is a common complication of end-stage CLD, and it is generally agreed that, in most cases, carcinogenesis follows a sequential process, with cirrhosis as an intermediate key step.[25] Although the incidence of HCC arising in cirrhosis ranges from 2% to 5% yearly according to etiology, both the risk of HCC in patients with MS and the carcinogenic pathway are poorly understood in the absence of large prospective follow-up studies. A retrospective clinical study recently reported that 7 of 129 patients (5.4%) with NAFLD developed end-stage liver disease, including 3 patients with HCC, suggesting that the occurrence of HCC might be substantial in that context.[26] Regarding pathogenesis, it has been proposed that steatosis and several factors associated with MS, such as obesity, diabetes, and insulin resistance, may predispose to HCC in patients with cirrhosis.[26-28] Indeed, multivariate analysis has shown that obesity is a statistically significant independent risk factor for HCC in patients with CG cirrhosis.[18] Nevertheless, it remains to be clarified whether HCC occurring in patients with features of MS results from the endpoint of the fibrogenic process or is driven by underlying metabolic factors.

Until now, in the context of MS, HCC has been described as being a late complication of NASH-related cirrhosis.[3,5,13-16] Those reports were mainly based on retrospective analyses of series of patients with HCC in CG cirrhosis that, upon review, showed the presence of risk factors for MS.[13,29] That approach underestimated potential cases of HCC developing in noncirrhotic livers. By retrospectively assessing all patients who underwent liver resection for HCC at our institution, independently of the aspect of the background liver, we show that most HCCs associated with features of MS as the only risk factor for CLD develop in nonfibrotic liver. Indeed, only 35.5% of HCCs in this group of patients occurred in bridging fibrosis or cirrhosis. This approach has limitations because we selected only HCCs accessible to surgical management, thus probably overestimating the number of patients with preserved liver function and consequently less advanced liver disease. However, the same should hold true for other etiologies in our study; it is noteworthy that 75% of HCCs in patients from the CLD group displayed significant bridging fibrosis or cirrhosis. Thus, our results suggest that well-recognized multistep progression, that is, fibrosis-cirrhosis-HCC, may not be the main carcinogenic pathway in the context of MS. For staging fibrosis, we used either Kleiner's scoring system or the METAVIR scoring system according to the underlying liver disease. These two well-accepted scoring systems are adapted, at best, to either NASH or chronic hepatitis, respectively, and diverge only in the definition of initial stages (0 to 2) according to the different patterns of fibrogenesis characteristic of each disease. However, the two scoring systems use similar definitions for advanced stages of fibrosis (F3 and F4); thus, the comparison of patients with F3 or F4 versus patients with early fibrosis in the present study was not impacted by the use of two different scoring systems according to the etiology.

Development of HCC in the absence of cirrhosis has also been observed in etiologies other than MS, especially in patients with chronic HBV infection.[30] It is of note that

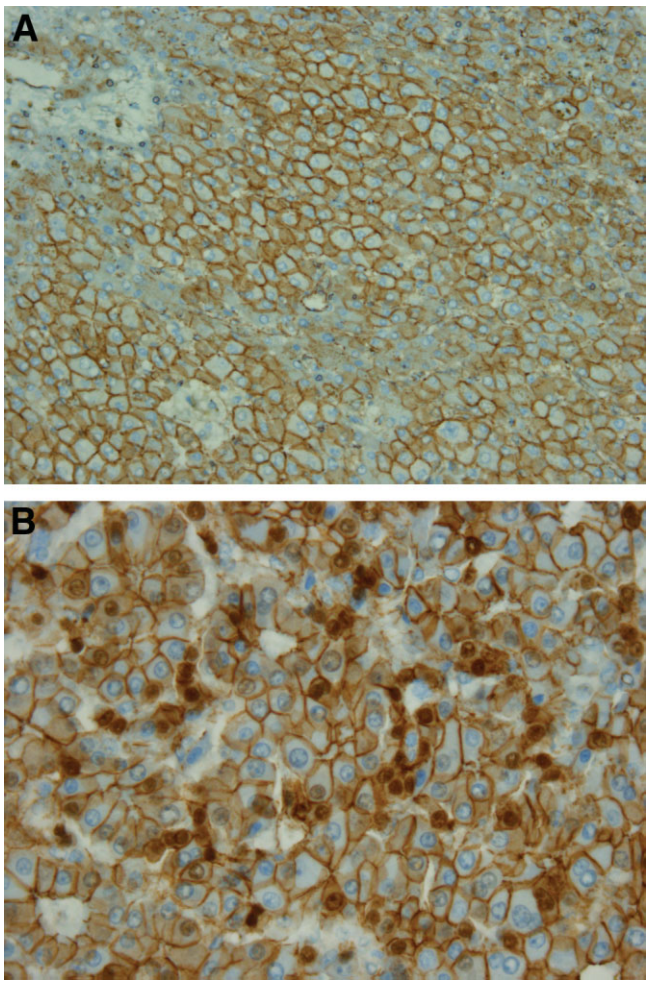


Fig. 1. β-Catenin immunostaining in hepatocellular carcinomas in patients with metabolic syndrome. (A) Case with normal β-catenin staining: all tumoral cells display a membranous staining pattern. (B) Case with abnormal β-catenin staining: some tumoral cells display nuclear and cytoplasmic staining.

HBV has direct oncogenic potential secondary to integration of HBV DNA into the genome of hepatocytes.[31] In line with this observation, it is suggested that MS per se could also have a direct oncogenic effect. Among the different parameters that define MS, some have already been recognized as being significant risk factors for the development of many types of malignancies, including gastrointestinal cancers. Thus, relative risks of HCCs reported in cohorts of obese patients have ranged from 2 to 4.5, with a higher risk in male patients.[28,32,33] In addition, an increased risk of HCCs has been found in patients with diabetes, mostly type 2 diabetes.[17,34] This is in line with our results because, in the MS group, all patients except one were males and most had a high BMI (79%) and diabetes (75%). Among potential carcinogenic mediators associated with NASH in MS, insulin, lipid peroxidation, and free radical oxidative stress are being actively investi-

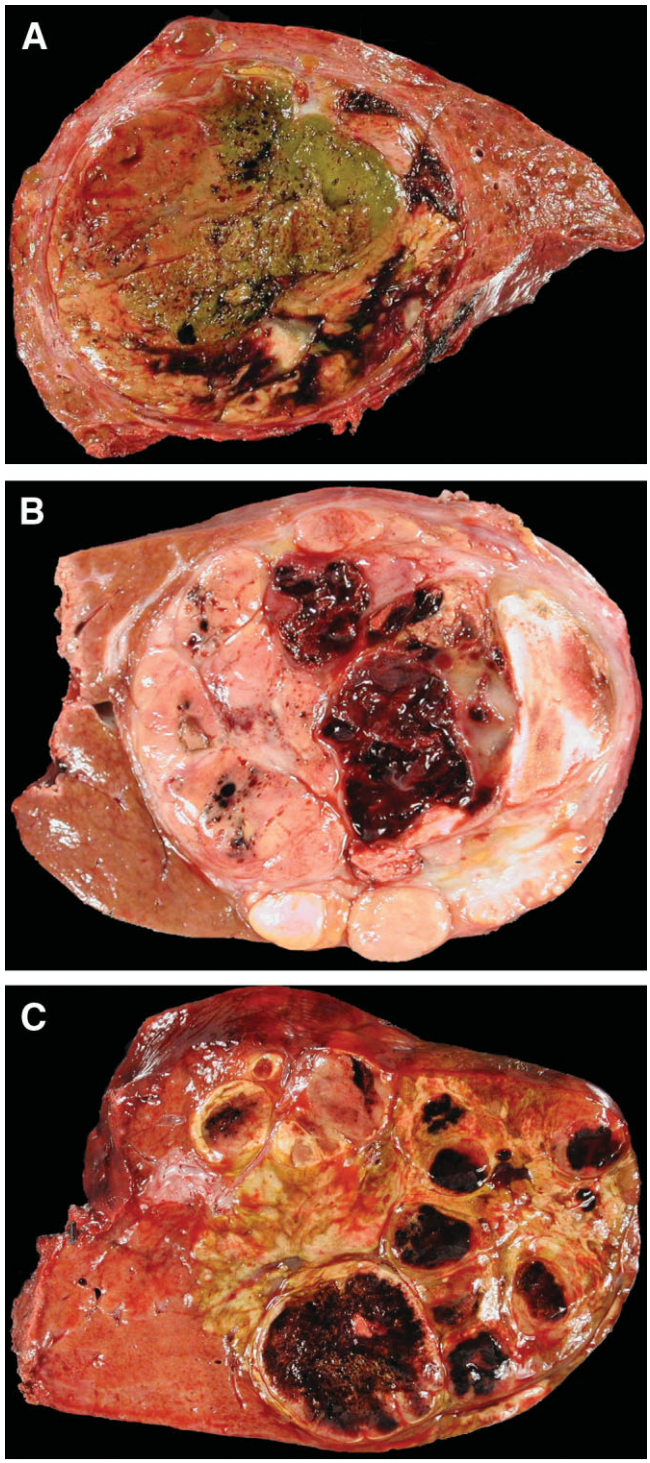


Fig. 2. Representative cases of hepatocellular carcinomas in patients with metabolic syndrome. (A) Macroscopic view of hepatocellular carcinoma that developed in a cirrhotic liver, showing an encapsulated polychrome tumor associated with satellite nodules. (B) Macroscopic view of hepatocellular carcinoma that developed on a liver without fibrosis, showing a partially encapsulated tumor with central necrotic and hemorrhagic changes. (C) Macroscopic view of hepatocellular carcinoma that developed on a preexisting liver cell adenoma, showing an unencapsulated tumor with hemorrhagic nodules. Malignant areas are marked with asterisks.

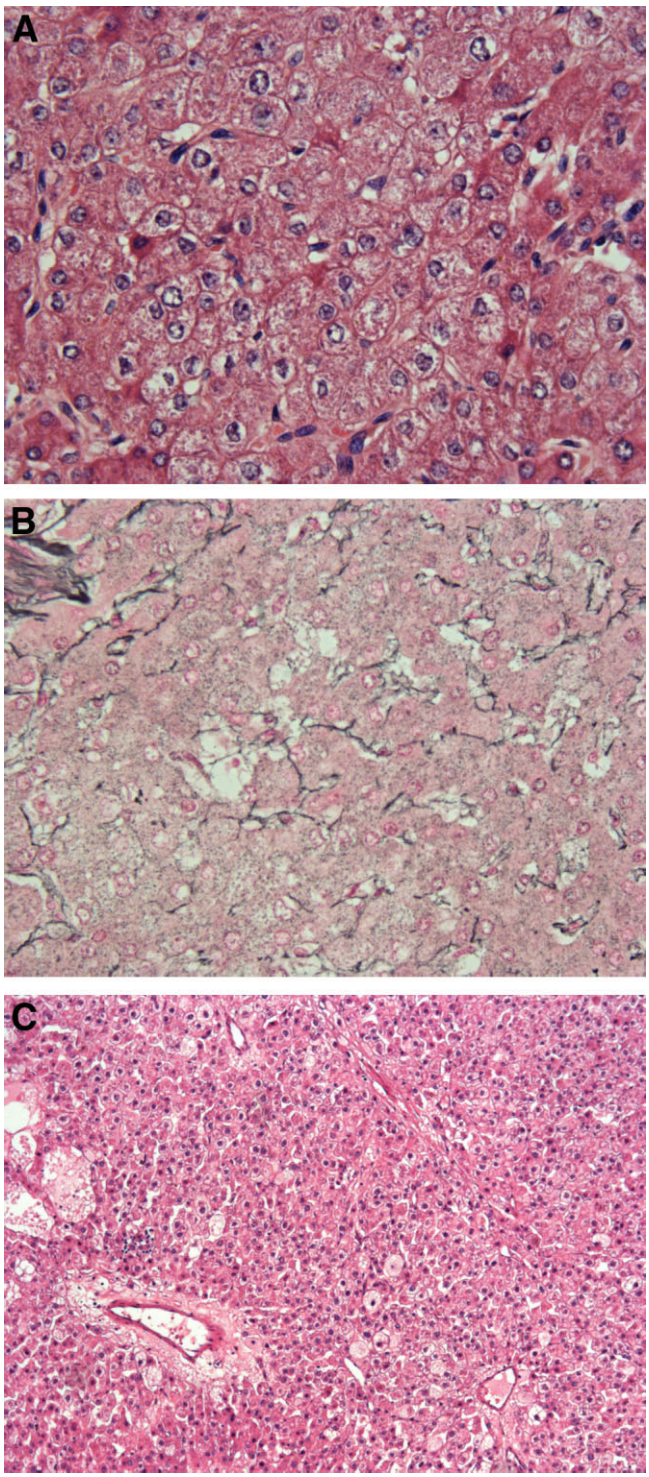


Fig. 3. Microscopic analysis of hepatocellular carcinomas in patients with metabolic syndrome. (A,B) Well-differentiated hepatocellular carcinoma developing in a nonfibrotic liver. (A) Hematoxylin-eosin staining shows trabecular hepatocellular proliferation with minimal cellular atypias. (B) Reticulin staining shows loss of reticulin pattern. (C) Liver cell adenoma. Hematoxylin-eosin staining shows thin liver plates with a few small arteries.

gated because they may stimulate cellular proliferation or favor epigenetic aberrations.[35-37] We also studied patients with HCC without any potential risk factors for CLDs, including features of MS (CG group). Interestingly, HCCs and nontumoral liver observed in such patients share some characteristics with those observed in patients from the MS group, including better tumor differentiation and a low prevalence of significant fibrosis.

Further molecular studies are clearly needed in order to provide additional insights into the physiopathology of HCCs developing in the context of MS. For that purpose, we evaluated β-catenin expression by immunohistochemistry. We observed nuclear and cytoplasmic β-catenin expression consistent with the presence of β-catenin mutations in only 3 of 31 cases of HCCs in the MS group. Those data indicated that WNT signaling pathway deregulation does not represent the main carcinogenic process involved in the context of MS. More importantly, in the present series, we observed in the MS group five cases of HCC associated with LCA, suggesting that these HCCs may arise from preexisting LCA. Whether HCC and LCA develop simultaneously or successively is difficult to prove. Interestingly, all of them belonged to the group of patients with no significant fibrosis in adjacent liver tissue. The prevalence of HCC arising within LCA might be even higher because most HCCs associated with MS are large; under this condition, the LCA remnant might have disappeared at the time of HCC diagnosis. We recently showed that TA, a subtype of LCA, occurs predominantly in overweight or even obese patients.[38] In the present study, three of the five LCAs associated with HCC were classified as TA according to a pathological analysis of the LCA remnant around HCC. Taken together, our results suggest that a significant percentage of HCCs that develop in the context of MS without significant fibrosis arise from malignant transformation of LCA, especially the TA subtype. Whether such well-differentiated hepatocellular proliferations developing in that specific context should be already considered overt HCCs deserves further investigations.

Pathological characteristics of HCCs in patients with features of MS have been reported in only a few studies. Those studies showed that the tumors were larger and better differentiated than HCCs arising in cirrhotic liver and induced by other etiologies.[3,19] Thus, in one surgical series comparing 18 HCC patients with CG cirrhosis secondarily related to NAFLD to HCC patients with alcohol-related or HCV-related cirrhosis, the grade of tumor differentiation was the only significant factor that distinguished the two groups.[29] In the present study, we confirm that most tumors (64.5%) arising in the context of MS are well differentiated; this percentage is significantly

higher than that for HCCs in the CLD group (28%). When splitting and comparing pathological features of HCCs in the MS group according to the presence or absence of significant fibrosis in nontumoral liver, we found that HCCs display different histopathological features such as size, differentiation, and encapsulation. Thus, HCCs that developed in nonfibrotic livers were more often well differentiated despite their larger size. Although a smaller size for HCC in patients with significant fibrosis and cirrhosis was expected because of earlier diagnosis related to the close follow-up of patients with cirrhosis, better differentiation of larger tumors in patients without significant fibrosis is unusual because it is commonly admitted that the grade of differentiation of HCC decreases with the size of the tumor.[39,40] This result reinforces the hypothesis suggesting that HCCs in fibrotic and nonfibrotic livers represent two distinct entities in terms of both pathogenesis and evolution.

In conclusion, this study demonstrates that the development of HCC in patients with features of MS as the only risk factor for CLD has distinct characteristics and occurs, in most cases, in the absence of significant liver fibrosis. In addition, in some cases, HCCs may arise from malignant transformation of a preexisting LCA. These results support the hypothesis that liver carcinogenesis related to MS often follows a specific molecular pathway of tumorigenesis different from the usual multistep process: fibrosis-cirrhosis-HCC. In that context, and if these results are confirmed in other studies, the high percentage of patients with HCC arising in nonfibrotic liver could lead to developing specific strategies for screening patients with MS independently of the presence of underlying chronic liver damage.

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