# Steatohepatitic hepatocellular carcinoma: morphological diagnostic criteria and clinicopathological characteristics

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## Abstract

According to the WHO, steatohepatitic hepatocellular carcinoma (SH-HCC) is a distinct HCC subtype. Its prevalence and prognosis vary depending on the study, probably because of lack of a consensus pathological definition. The objectives of the study were to carefully describe the morphological features of SH-HCC to propose a relevant pathological definition and evaluate its impact on prognosis. We conducted a single-centre retrospective study including 297 surgically resected HCC. Pathological features of the tumor including SH criteria (steatosis, ballooning, Mallory-Denk bodies, fibrosis, and inflammation) were assessed. The diagnosis of SH-HCC was initially based upon the presence of at least 4 of the 5 SH criteria and the SH component represented > 50% of the tumor area. According to this definition, a total of 39 (13%) HCC cases corresponded to SH-HCC and 30 cases (10%) corresponded to HCC with SH component (< 50%). SH criteria were distributed as follows: ballooning :100% in SH-HCC *vs* 11% in non–SH-HCC, fibrosis:100% vs 81%, inflammation:100% vs 67%, steatosis :92% *vs* 8%, and Mallory-Denk bodies :74% vs 3%. Five-year recurrence-free survival (RFS) and 5-year overall survival (OS) were similar for SH-HCC and non–SH-HCC cases (p = 0.413; and p = 0.866, respectively). The percentage of SH component does not impact OS and DFS (p = 0.589; and p = 0.443). Based on these results, we propose a simplified definition of SH-HCC based on ballooning as a major criterion with at least 2 other SH criteria regardless the extent of SH component. Eighty-three HCC (28%) corresponded to SH-HCC according to this definition, including all HCC classified initially as SH-HCC and HCC NOS with SH contingent.

Finally, we confirm in a large cohort the high prevalence of SH-HCC and propose a simplified SH-HCC definition that could be easily applied in routine practice.


## Introduction

Over the past few years, in parallel with the increasing epidemic of obesity and insulin resistance (defining metabolic syndrome) in most developed countries, the incidence of non-alcoholic fatty liver disease (NAFLD) has increased incrementally as a risk factor of hepatocellular carcinoma (HCC) (1–6). Among the various HCC subtypes recognized according to the World Health Organization (WHO) classification (clear cells, macrotrabecular-massive, scirrhous, chromophobe, fibrolamellar carcinoma, neutrophil-rich, and lymphocyte-rich), the steatohepatitic HCC (SH-HCC) subtype is more often observed in patients with metabolic syndrome and NAFLD (7–9).

SH-HCC, first described in 2010, has similar diagnostic histologic features as non-tumoral liver with steatohepatitis (i.e., steatosis, hepatocellular ballooning, Mallory-Denk bodies, inflammation, and fibrosis) (10). The prevalence of SH-HCC is variable across studies, ranging from 13–35%, which probably reflects the different epidemiological features of studies but also the lack of consensus on pathological diagnosis. Indeed, the definition of SH-HCC differs among series, not only in number of histological criteria required (from 1 to 5 among steatosis, ballooning, Mallory-Denk bodies, inflammation and fibrosis) but also in the extent of tumor area with SH features (from > 5–50%) (11). Noteworthy, WHO classification did not provide accurate definition of this subtype. The prognosis of SH-HCC also remains unclear, described as better or similar compared to HCC not otherwise specified (NOS) in several published series (7–9, 12, 13). Of note, these studies assessed the prognosis of patients with SH-HCC, mainly treated by liver transplantation.

Molecularly, HCC defines a heterogeneous group of tumors. For instance, the G1-G6 molecular classification linked histological subtypes or pathological features of HCC to genetic alterations (14). According to this classification, SH-HCC is more frequently associated with the G4 group which does not displays specific mutations, especially involving *p53* or *CTNNB1* (14–17). From a transcriptomic point of view, SH-HCC frequently exhibits activation of *IL6/JAK/STAT* and hedgehog pathways as well as inhibition of carnitine palmitoyl transferase 2, which may suggest the role of inflammation and lipotoxicity in its carcinogenesis (16, 18, 19). While such molecular features could help to identify surrogate biomarkers specific to this histological subtype (especially inflammation markers like CRP and SAA). The aims of this study were to report in a large surgical series of HCC the morphological features of SH-HCC, describe this subtype and evaluate its prognostic impact to propose a relevant pathological definition.

## Materials And Methods

## Patients

In this retrospective, monocentric study, all HCC samples surgically resected from 2012 to 2019 were retrieved from the pathological archives (Department of Pathology, Beaujon Hospital). Patients who had undergone pre-operative treatment were included (chemoembolization, radiofrequency ablation) unless no residual tumor was observed on microscopic examination. Fibrolamellar HCC, which defines a specific subtype of HCC mostly observed in young patients without underlying chronic liver diseases were excluded. Written informed consent was obtained from all patients as required by French legislation. The study was approved by the local ethics committee (Institutional Review Board [00006477] of AP-HP.Nord, Paris University, no. CER-2021-88).

# Clinical data

Clinical data systematically collected were age, sex, potential risk factors for chronic liver diseases (excessive alcohol consumption, metabolic syndrome, hepatitis B virus [HBV] or hepatitis C virus [HCV] infection, other risk factors, no factor). The definition of metabolic syndrome was based on the International Diabetes Federation (2006), defined by the association of central obesity and at least 2 other factors among increased triglycerides level, reduced high-density lipoprotein cholesterol level, high blood pressure (systolic ≥ 130 mmHg or diastolic ≥ 85 mmHg), high plasma glucose level or previously diagnosed type 2 diabetes (20).

# Pathological analysis

# Evaluated pathological criteria

For each patient, macroscopic data for the tumor (number of nodules, size, color, limitation, capsule, presence of macroscopic vascular invasion and satellite nodules) were collected from pictures and/or the pathological report. All haematoxylin-eosin-safran (HES)-stained slides for each case were reviewed by 2 pathologists (LT and AB). The microscopic criteria systematically assessed in the tumor included differentiation grade (Edmondson and WHO), architectural patterns (microtrabecular, macrotrabecular, pseudoglandular, and compact), presence of capsule, steatosis (graded 0, < 5%; 1, 5–32%; 2, 33–65%; 3, ≥ 66%), fibrosis (graded 0, absent; 1, mild; 2, moderate; 3, extensive, and by type: pericellular, septal, or mixed), inflammation (graded 0, absent; 1, mild; 2, moderate; 3, intense), type of inflammation (mixed, neutrophilic, lymphocytic), ballooning (graded 0, absent; 1, few; 2, moderate; 3, numerous), necrosis (percentage of area), microvascular invasion (present/absent), and satellite nodule (present/absent).

In a first step, and according to the WHO classification, HCC cases were divided into specific subtypes (steato-hepatitic, clear cells, macrotrabecular massive, scirrhous, chromophobe, neutrophil-rich, and lymphocyte-rich) or HCC NOS (11). For a diagnosis of SH-HCC, the following criteria assessed in all tumors were steatosis (> 5%), ballooning, Mallory-Denk bodies, fibrosis, and inflammation. The percentage of the SH component was specified for each case. Then, HCC cases were defined as SH following the most commonly used criteria (presence of at least 4 of 5 morphological criteria covering at least 50% of the total viable tumor surface area) (7, 9, 10). If the SH component was < 50%, the tumor was classified as “HCC NOS with SH component < 50%”. HCC cases showing steatosis > 5% of the tumor area without other SH-HCC criteria were identified as “steatotic HCC”.

Secondly, the diagnostic criteria for SH-HCC were analysed individually and in combination to establish a relevant new diagnostic definition. Based on this new definition, a group of HCC called “newly SH-HCC” was constituted and compared with non-SH-HCC.

To better appreciate the tumor architecture, reticulin staining on whole slides representative of the tumor was analysed for 38 SH-HCC cases (one case not analysed due to lack of material). The stained slides were evaluated qualitatively according to the framework (preserved, decreased or absent). The presence of a pericellular distribution was also assessed.

Review of HES- and picrosirius-stained slides of the underlying non-tumoral liver also allowed for systematic evaluation of fibrosis, steatosis and activity (based on METAVIR score; steatosis, activity, and fibrosis [SAF] score; and NAFLD Activity Score [NAS]) according to the risk factor) (21–23).

When available, pre-operative biopsies of resected HCC samples (SH-HCC and HCC NOS with SH component) were reviewed and diagnostic criteria for SH-HCC were evaluated.

# Tissue microarray (TMA) and immunohistochemistry

Immunohistochemical analysis involved tissue microarrays regrouping 38 SH-HCC (1 case not analysed due to lack of material), 15 steatotic HCC and 71 non–SH-HCC cases. Three to four 1-mm punches from each tumor in the SH and non-SH areas if present and one 1-mm punch from each non-tumoral liver were collected. Paraffin sections were stained for C-reactive protein (CRP; Y284, 1:100, Abcam, Cambridge, UK), serum amyloid A (SAA; mc1, 1:25, Agilent, Santa Clara, USA), glutamine synthetase-6 (1:500, BD Biosciences, San Jose, USA), glypican 3 (1G12, 1:100, Diagomics, Blagnac, France), heat shock protein 70 (HSP70; 2A4, 1:500, Abcam, Cambridge, UK) and cytokeratin 8/18 (35BH11, 1:200, Diagomics, Blagnac, France). The expression was defined as the percentage of immunostained cells and the intensity of staining (1+, 2+, 3+). Because of the small tissue area examined on the TMA blocks, we chose not to set a minimum threshold of marked cells.

# Statistical analysis

Continuous variables were compared by Student *t* test and categorical variables by chi-squared and Fisher's exact tests. Overall survival (OS) was defined as the time from the date of surgery to the date of death or the date of last follow-up for patients who were still alive. All causes of death, including postoperative deaths, were considered for estimating OS. Five-year OS was defined as the time from the date of surgery to the date of death or the time of five years for patients who were still alive. Recurrence-free survival (RFS) was defined as the time from the date of surgery to the date of HCC recurrence or the date of last follow-up for patients who did not show recurrence. Five-year RFS was defined as the time from the date of surgery to the date of death or the time of five years for patients without recurrence. Kaplan-Meier curves were used to compare 5-year RFS and 5-year OS after resection by using the log-rank test. $P < 0.05$ was considered statistically significant. Variables achieving statistical significance at $p = 0.1$ on univariate analysis or variables of particular interest (such as percentage of SH contingent) were considered for multivariate analysis. Multivariate survival analysis involved Cox proportional-hazards models, estimating hazard ratios (HRs) and 95% confidence intervals (CIs). Statistical analyses were performed with Medcalc and Prism software.

## Results

# Patients

Between 2012 and 2019, 312 patients underwent liver surgical resection for HCC (Fig. 1), and among them, 297 fulfilled inclusion criteria. The median age was 64 years (range 21–99) with a male predominance (77%, n = 229). The underlying risk factors for chronic liver diseases included metabolic syndrome (n = 93, 31%), HBV infection (n = 77, 26%), HCV infection (n = 78, 26%), and excessive alcohol consumption (n = 52, 17%). Overall, 35% (n = 104) of patients had cirrhosis. Non-tumoral livers exhibited NAFL and non-alcoholic steatohepatitis (NASH) in 15% (n = 45) and 15% (n = 45) of patients, respectively.

According to the WHO classification, 180 HCC (61%) were classified as HCC NOS, 39 (13%) SH-HCC, 22 (7%) macrotrabecular-massive HCC, 5 (2%) clear cell HCC, 4 (1%) lymphocyte-rich HCC, and 1 (0.3%) scirrhous HCC (Fig. 1). Among HCC NOS cases, 30 (10%) showed SH features covering less than 50% of the tumor area and 16 (5%) were defined as steatotic HCC.

# Prevalence of elementary features of SH-HCC in overall cohort

In the whole cohort (n = 297), 7% of HCC cases (n = 15) had none of the 5 SH criteria and 32% (n = 95) had ≥ 3/5 criteria. The most frequent criteria present in the overall series were fibrosis and inflammation (n = 246, 83% and n = 213, 72%, respectively) (Fig. 2), suggesting their poor specificity for HCC subtyping.

Among SH-HCC cases according to the initial definition (n = 39), 13 (33%) had 4/5 SH criteria, and 26 (67%) had all 5 diagnostic criteria in > 50% of the tumor area. As compared with non–SH-HCC cases, all SH-HCC cases showed ballooning, fibrosis and inflammation (100% *vs* 11%, $p < 0.001$; 100% *vs* 81%, $p = 0.003$; and 100% *vs* 67%, $p < 0.001$, respectively) (Fig. 2). Also, steatosis was found in 36 SH-HCC cases (92% *vs* 8%, $p < 0.001$) and Mallory-Denk bodies in 29 (74% *vs* 3%, $p < 0.001$). Fibrosis was mixed (pericellular and septal) in 30 SH-HCC cases (77% *vs* 4% non–SH-HCC, $p < 0.001$) and was moderate or extensive in 51% of SH-HCC cases (*vs* 22%, $p < 0.001$). Inflammation was moderate or intense in 31% (*vs* 17%, $p = 0.05$), polymorph in 51% of SH-HCC cases (*vs* 7%, $p < 0.001$) and lymphocytic in 49% SH-HCC cases (*vs* 60%, $p = 0.201$). Ballooning was moderate or high in most cases (74%, n = 29). Steatosis was mild in most cases (49%, n = 19) and moderate in 12 (31%) (Fig. 3).

In the non–SH-HCC group, most HCC cases (n = 202, 89%), had < 3 criteria and 26 (12%) had 3/5 criteria. In this group, ballooning was focal (15/15 cases, 100%), inflammation was mostly mild (14/22 cases, 63%) and fibrosis was only septal (25/25 cases, 100%).

# Characteristics of SH-HCC

As compared with non–SH-HCC patients, SH-HCC patients were older (66 *vs* 63 years, p = 0.004), had more frequently metabolic syndrome (56% *vs* 26%, p < 0.001) (Table 1). They had also more frequently cirrhosis (49% *vs* 32%, p = 0.047), NAFL (23% vs 12%, p = 0.056), and NASH (33% *vs* 11%, p < 0.001).

Table 1
Clinical and pathological features of steatohepatitic hepatocellular carcinoma (SH-HCC) versus newly defined SH-HCC and non–SH-HCC.

| | *SH-HCC n = 39(%)* | *Newly defined SH-HCC n = 83(%)* | *Non-SH HCC n = 228(%)* | *p (SH vs non-SH HCC)* | *p (Newly SH- vs non-SH HCC)* |
|---|---|---|---|---|---|
| Age (median) | 66 [38–79] | 65 [21–80] | 63 [21–99] | **0.004** | 0.170 |
| Gender (male) | 34 (87) | 65 (78) | 174 (76) | 0.121 | 0.711 |
| *Etiologies* | | | | | |
| BMI > 30 | 11 (28) | 21 (25) | 40 (17) | 0.114 | 0.127 |
| MS | 22 (56) | 35 (42) | 60 (26) | **< 0.001** | **0.007** |
| OH | 11 (28) | 20 (24) | 34 (15) | **0.039** | 0.058 |
| HCV | 9 (23) | 17 (20) | 64 (29) | 0.527 | 0.177 |
| VHB | 5 (13) | 12 (14) | 68 (30) | **0.029** | **0.006** |
| Other etiologies | 5 (13) | 11 (13) | 18 (8) | 0.349 | 0.151 |
| No etiologie | 2 (5) | 11 (13) | 31 (14) | 0.345 | 0.937 |
| *Underlying liver* | | | | | |
| No steatosis | 15 (38) | 38 (46) | 154 (68) | **< 0.001** | **< 0.001** |
| NAFL | 9 (23) | 20 (24) | 27 (12) | 0.056 | **0.008** |
| NASH | 13 (33) | 21 (25) | 25 (11) | **< 0.001** | **0.001** |
| Cirrhosis | 19 (49) | 36 (43) | 74 (32) | **0.048** | 0.07 |
| *Macroscopic features* | | | | | |
| Tumor size (cm) | 4 [0.6–25] | 4 [0.6–25] | 6.9 [0.5–25] | **< 0.001** | 0.08 |
| > 1 nodule | 9 (23) | 14 (17) | 29 (13) | 0.102 | 0.348 |
| Yellowish | 22 (56) | 49 (59) | 76 (33) | **0.005** | **< 0.001** |
| White | 18 (46) | 24 (29) | 29 (13) | **< 0.001** | **< 0.001** |
| Polychrome | 1 (3) | 10 (12) | 39 (17) | **0.015** | 0.278 |
| Well limited | 32 (82) | 62 (75) | 169 (74) | 0.288 | 0.918 |
| Macroscopic capsule | 21 (54) | 45 (54) | 164 (72) | **0.023** | **0.003** |
| *Histoprognostic factors* | | | | | |
| Satellites nodules | 5 (13) | 22 (26) | 55 (24) | 0.118 | 0.666 |
| Macroscopic vascular invasion | 2 (5) | 5 (6) | 31 (14) | 0.189 | 0.063 |

*Data are n (%) unless otherwise indicated.*

*MS, metabolic syndrome; OH, chronic alcohol intake, HCV, hepatitis C virus; HBV, hepatitis B virus; NASH, non-alcoholic steatohepatitis; NAFL, non-alcoholic fatty liver; RFS, recurrence-free survival; OS, overall survival.*

**Metabolic syndrome as defined by the International Diabetes Federation (2006)*

| | *SH-HCC n = 39(%)* | *Newly defined SH-HCC n = 83(%)* | *Non-SH HCC n = 228(%)* | *p (SH vs non-SH HCC)* | *p (Newly SH- vs non-SH HCC)* |
|---|---|---|---|---|---|
| Microscopic vascular invasion | 15 (39) | 36 (43) | 108 (47) | 0.302 | 0.532 |
| Well differentiated | 16 (41) | 25 (30) | 72 (31) | 0.239 | 0.806 |
| Moderatly differentiated | 23 (59) | 57 (69) | 135 (59) | 0.978 | 0.129 |
| Poorly differentiated | 0 (0) | 1 (1) | 21 (9) | 0.052 | **0.014** |
| *Data are n (%) unless otherwise indicated.* | | | | | |
| *MS, metabolic syndrome; OH, chronic alcohol intake, HCV, hepatitis C virus; HBV, hepatitis B virus; NASH, non-alcoholic steatohepatitis; NAFL, non-alcoholic fatty liver; RFS, recurrence-free survival; OS, overall survival.* | | | | | |
| **Metabolic syndrome as defined by the International Diabetes Federation (2006)* | | | | | |

# Macroscopic and microscopic features of SH-HCC

Macroscopically, SH-HCC were smaller than non–SH-HCC (mean size 4 cm *vs* 7 cm, p < 0.001). Overall, 54% of SH-HCC versus 72% of non–SH-HCC had a macroscopic capsule (p = 0.023). SH-HCC and non–SH-HCC cases did not differ in terms of histo-prognostic factors.

The reticulin framework was more frequently preserved in SH-HCC versus non–SH-HCC (24% *vs* 7%, p = 0.071), with a pericellular pattern distribution (50% *vs* 26%, p = 0.010) (Fig. 3).

Among the 69 SH-HCC, 10 pre-operative biopsies were available. The diagnostic agreement between the biopsy and surgical specimen was 70% (n = 7/10).

# Immunohistochemical analysis

Inflammation markers (CRP and SAA) were significantly more expressed in SH-HCC compared to non–SH-HCC (82% of SH-HCC versus 14% of non–SH-HCC, p = < 0.001). SAA expression gradually increased with number of SH criteria, whereas CRP expression was mostly present with the observation of ≥ 2 SH criteria (Figs. 4 and 5). Strong and diffuse expression of glutamine synthetase in the tumor was less frequent in SH-HCC than non–SH-HCC (18% *vs* 31%, p = 0.157), whereas cytokeratin 8/18 (highlighting Mallory-Denk bodies) was more frequent in SH-HCC (82% *vs* 63%, p = 0.049). Immunostaining for glypican and HSP70 was similar in the two groups; 71% (*vs* 65%, p = 0.508) and 89% (*vs* 80%, p = 0.218) of SH-HCC were positive.

# Follow-up and prognosis of SH-HCC

The median follow-up in the overall series was 24 months (range 0–94). The prognosis was similar between SH-HCC and non-SH-HCC, with a 5-year overall rate of 13% *versus* 18% (p = 0.413, HR: 1.163 [0.79–1.72]) and a 5-year RFS rate of 41% versus 43% (p = 0.866, HR: 1.11 [0.64–1.92]) (Fig. 6).

Factors associated with OS and RFS in univariate and multivariate analysis were summarized in Table 2. The SH subtype based on the “classical definition” and the percentage of SH contingent were not associated with RFS or OS.

Table 2
Univariate and multivariate analysis of factors associated with overall survival and recurrence-free survival in the overall cohort (n = 297)

| | **Overall survival** | | | | | | **Recurrence-free survival** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Univariate analysis** | | | **Multivariate analysis** | | | **Univariate analysis** | | | **Multivariate analysis** | | |
| Variables | **HR** | **95% CI** | P | **HR** | **95% CI** | P | **HR** | **95% CI** | P | **HR** | **95% CI** | P |
| Cirrhosis | 0.86 | 0.51–1.46 | 0.593 | - | - | - | 1.33 | 0.90-1,95 | 0.133 | - | - | - |
| Hepatitis C | 0.91 | 0.53-1,60 | 0.755 | - | - | - | 0.82 | 0.54–1.23 | 0.351 | - | - | - |
| Metabolic syndrome | 0.69 | 0.41-1,16 | 0.185 | - | - | - | 0.82 | 0.56–1.19 | 0.308 | - | - | - |
| Chronic alcohol consumption | 1.48 | 0.77–2.84 | 0.179 | - | - | - | 1.30 | 0.81–2.10 | 0.235 | - | - | - |
| Hepatitis B | 0.84 | 0.48–1.47 | 0.554 | - | - | - | 0.92 | 0.61–1.40 | 0.70 | - | - | - |
| Tumor size | 1.53 | 0.94–2.50 | 0.09 | 1.10 | 1.03–1.86 | **< 0.001** | 1.61 | 1.12–2.31 | **0.009** | 1.09 | 1.05–1.12 | **< 0.001** |
| Number of nodules (> 1) | 2.34 | 1.16–4.70 | **0.002** | 1.50 | 1.18–1.92 | **0.001** | 1.76 | 1.00-3.10 | **0.014** | 1.52 | 1.20–1.93 | **< 0.001** |
| Macrotrabecular-massive HCC | 0.93 | 0.41–2.10 | 0,860 | - | - | - | 1.50 | 0.72–3.12 | 0.193 | - | - | - |
| SH-HCC | 0.81 | 0.39–1.67 | 0.586 | - | - | - | 1.09 | 0.63–1.88 | 0.75 | - | - | - |
| Percentage of SH contingent | 0.99 | 0.99–1.01 | 0.815 | 1.00 | 0.993–1.013 | 0.589 | 1.00 | 0.99–1.01 | 0.672 | 1.00 | 0.99,1.01 | 0.443 |
| HCC NOS with SH contingent < 50% | 1.02 | 0.48-2,15 | 0.962 | - | - | - | 0.63 | 0.37–1.09 | 0.156 | - | - | - |
| Macrovascular invasion | 3.13 | 1,36 – 7,18 | **< 0.001** | 6.43 | 2.90-14.26 | **< 0.001** | 2.23 | 1.16–4.28 | **0.001** | 3.98 | 2.08–7.63 | **< 0.001** |
| Microvascular invasion | 2.40 | 1.47-3,91 | **0.001** | 2.08 | 1.09-4,01 | **0.027** | 1.70 | 1.18–2.44 | **0.005** | 1.73 | 1.09–2.75 | **0.02** |
| Satellite nodule | 3.02 | 1.66-5,47 | **< 0.001** | 1.87 | 1.06-3,31 | **0.032** | 2.64 | 1.65–4.20 | **< 0.001** | 2.01 | 1.32–3.05 | **0.001** |
| WHO differentiation (poor vs well and medium) | 4.00 | 1,36-11.77 | **< 0.001** | 1.71 | 0.82–3.54 | 0.151 | 2.75 | 1.17–6.46 | **< 0.001** | 1.39 | 0.76–2.57 | 0.289 |

*WHO, World Health Organization; HR, hazard ratio; 95% CI, 95% confidence interval*

On multivariate analysis, factors associated with OS and RFS were tumor size (HR = 1.10 [95% CI 1.03–1.86], p < 0.001, and HR = 1.09 [1.05–1.12], p < 0.001 respectively), multiple nodules (HR = 1.50 [95% CI 1.18–1.92], p = 0.001, and HR = 1.52 [1.20–1.93], p < 0.001 respectively), macrovascular invasion (HR = 6.43 [95% CI 2.90-14.26], p < 0.001, and HR = 3.98 [2.08–7.63], p < 0.001 respectively), microvascular invasion (HR = 2.08 [95% CI 1.09–4.01], p = 0.027, and HR = 1.73 [1.09–2.75], p = 0.02 respectively) and satellite nodules (HR = 1.87 [1.06–3.31], p = 0.032, and HR = 2.01 [1.32–3.05], p = 0.001).

# HCC NOS with SH component < 50%

Overall, 10% of HCC cases (n = 30) had at least 4 of the typical features of SH-HCC but with SH component covering less than 50% of the total tumor area. In this subgroup, the SH component represented 17% of the tumor area on average (range 1–40%). Clinically, HCC NOS cases with SH component < 50% did not differ from SH-HCC cases showing SH features in > 50% (mean age of patients 65 years *vs* 66 years, p = 0.115) and risk factors were metabolic syndrome (37%, n = 11), excessive alcohol consumption (23%, n = 7), and the presence of NASH in background liver (20%, n = 6). Macroscopically, the size of HCC-NOS with SH component < 50% was significantly higher than that of SH-HCC (7 *vs* 4 cm, p = 0.013). Tumors were less commonly white (17% *vs.* 46%, p = 0.01) and more frequently polychrome (23% *vs* 3%, p = 0.018). Histoprognostic criteria were comparable except for satellite nodules, which were more often found in HCC-NOS with SH component < 50% than SH-HCC (37% *vs* 13%; p = 0.020).

# Steatotic HCC

Five % (n = 16) of overall HCC cases were classified as steatotic HCC according to the proposed definition, namely HCC with steatosis > 5% but without other criteria of SH-HCC. Clinically, steatotic HCC occurred at a mean age of 68 years (*vs* 66 years for SH-HCC, p = 0.442) and was less frequently associated with the metabolic syndrome (19% *vs* 56%, p = 0.016) or the presence of NASH (13% *vs* 33%, p = 0.184). NAFL in the non-tumoral liver was observed in 44% of steatotic HCC and in 23% of SH-HCC (p = 0.191). Steatotic HCC and SH-HCC groups did not differ in histoprognostic factors [satellite nodule (13% *vs* 13%, p = 1.00); microvascular invasion (50% *vs* 39%, p = 0.431); well differentiation (38% *vs* 41%, p = 0.808); OS (median 20 *vs* 23 months, p = 0.731) or RFS (median 15 *vs* 14 months, p = 0.912)]. In the steatotic HCC subgroup, CRP and SAA were positive in 87% (n = 13) and 53% (n = 8) of cases (*vs* 97%, p = 0.189, and 82%, p = 0.046 in the SH-HCC group). Overall, 47% of steatotic HCC cases expressed both CRP and SAA (*vs* 82% of SH-HCC, p = 0.018).

Newly defined SH-HCC

Based on the distribution of each criterion in the overall cohort, ballooning was the most specific and sensitive criterion of SH-HCC according to the previous definition (). Thus, we propose a new simplified definition of SH-HCC based on ballooning as a major criterion with at least 2 other criteria among steatosis, Mallory Denk bodies, fibrosis and inflammation. According to the multivariate analysis described above, a diagnostic threshold > 50% does not seem relevant and a minimum percentage of 5% SH component should be sufficient for the diagnosis.

Eighty-three HCC (28%) corresponded to SH-HCC according to this new definition, includingall HCC classified initially as SH-HCC and HCC NOS with SH contingent (n = 69). The other 14 cases belonging to this new group were initially classified as HCC NOS (n = 12), steatotic HCC (n = 1) and clear cell HCC (n = 1). The comparison of this new group, in a clinico-pathological way, showed similar data to those found in the initial SH-HCC group, compared to non-SH-HCC (**Table. 1**). The prognosis was similar between “newly defined SH-HCC”, SH-HCC according to the classical definition and non–SH-HCC, with a 5-year overall rate of 17% vs 13% vs 18% (p = 0.708) and a 5-year RFS rate of 48% vs 41% vs 43% (p = 0.972) (Fig. 6).

# Discussion

Pathomolecular analysis, allowing the distinction of histological subtypes of HCC, is clinically useful as it is associated, at least in some subtypes, with specific prognosis, and possibly, response to systemic therapies (16, 24–26). Indeed, *TP53* mutations are associated with macrotrabecular-massive HCC and poor prognosis, whereas *CTNNB1* mutations are associated with microtrabecular HCC and better prognosis. In contrast, the prognosis of the SH subtype remains difficult to predict in the absence of an association with specific molecular genetic alterations of clinical interest and lack of a consensus pathological definition. Hence, we performed an exhaustive pathological analysis of SH-HCC based on one of the largest series of SH-HCC described in the literature, with 83 SH-HCC cases representing 28% of all HCC according to our new definition. This prevalence agrees with that found in previous studies (13–35%) (7–10). We also confirmed the association between SH-HCC and metabolic syndrome and, microscopically, with NAFLD in the underlying non-tumoral liver. Accordingly, the incidence of this subtype should grow in the future owing to the increased prevalence of metabolic syndrome leading to NAFLD, particularly in developed countries (3, 4). As suggested in other series (9, 12, 27), we also found excessive alcohol intake associated with metabolic syndrome and SH-HCC. The association of SH-HCC with metabolic syndrome and excessive alcohol intake could be explained by its carcinogenesis, with inflammation and lipotoxicity seeming to play a major role without specific genetic alterations (16, 18, 19).

Overall, SH-HCC tumors were smaller on average than non–SH-HCC tumors. They were well demarcated but less frequently encapsulated. As previously described, tumors had a yellow and/or white color, reflecting steatosis and fibrosis, respectively (7). Microscopically, SH-HCC consistently featured ballooning, fibrosis and inflammation. Ballooning was the most specific and sensitive criteria of SH-HCC. Importantly, ballooning was readily identifiable in all cases, cells. Compared to clear cells, ballooned tumor cells are usually larger, with indistinct cytoplasmic borders. In addition, fibrosis was highly specific when it had a pericellular distribution. While steatosis was also specific, its intensity was mild in most cases.

According to our results, the 50% threshold, currently used, does not seem enough relevant to distinguish SH-HCC from HCC NOS since SH-HCC cases were clinically homogeneous, whatever the extent of the SH component (7). Furthermore, in multivariate analysis, the percentage of SH contingent had no prognostic impact. Thus, according to our results, a minimum percentage of 5% SH component should be sufficient to retain the diagnosis, as some authors have already suggested (8, 10, 12). Then, we propose a new diagnostic definition based on ballooning (major criterion) with two other criteria (among steatosis, Mallory Denk bodies, fibrosis and inflammation) in at least 5% of the tumour area. According to this new definition, 28% of HCC corresponded to SH-HCC and formed a group with similar clinico-pathological features and prognosis to those observed in the initial SH-HCC group. In this study, we assessed the prognosis of patients with SH-HCC undergoing partial liver resection. We observed a similar OS and RFS as compared with non–SH-HCC. These results are in accordance with most of the studies that evaluated the prognosis of patients with SH-HCC mainly treated by liver transplantation (7–10, 27). Moreover, histoprognostic factors such as tumor differentiation, vascular invasion and satellite nodules were similar to those with non–SH-HCC.

Overall, 5% of HCC cases had intra-tumor steatosis covering at least 5% of the tumor surface, without any other criteria of SH-HCC. Steatotic HCC does not seem associated with metabolic syndrome but is more frequently associated with NAFL in background liver rather than NASH as compared with SH-HCC, which is more associated with NASH. This similarity between underlying non-tumoral liver and tumor could suggest common pathomolecular mechanisms in the process of liver carcinogenesis. Some other features (e.g., tumor size and macroscopic appearance) tended to be similar to non–SH-HCC, as proposed by other studies (12, 28).

On immunostaining, SH-HCC was distinguished from other HCC cases by a more frequent expression of inflammation markers, such as CRP and SAA. The concomitant expression of CRP and SAA was correlated with the number of SH criteria within the tumor. CRP was less specific than SAA in the diagnosis of SH-HCC. These results extend previous data and highlight the potential role of the inflammation pathway in the carcinogenesis of SH-HCC (12, 16, 19, 29, 30). The less common overexpression of glutamine synthetase agrees with the absence of *CTNNB1* mutations in SH-HCC (16).

The excellent diagnostic agreement between biopsies and surgical resection in our cohort supports the use of biopsy to identify this subtype and to exclude differential diagnosis. Indeed, on biopsy, it may be challenging to distinguish SH-HCC and focal nodular hyperplasia with SH features (29, 31, 32). Reticulin stain, which is usually helpful for differentiating benign from malignant hepatocellular nodules, is less useful in this context because both lesions exhibit frequent preservation with a pericellular pattern (32, 33).

Because of the particular macroscopic and microscopic aspects of SH-HCC, imaging study would be of potential interest. A few imaging studies have assessed SH-HCC with a limited number of cases (34, 35). In a recent study, evaluation of the Liver Imaging Reporting and Data System did not differ between HCC subtypes, including SH-HCC (36). Thus, imaging studies of SH-HCC would be needed to evaluate the radiological characteristics of this subtype.

This was a monocentric and retrospective study in a referral centre for hepatobiliary pathology. It included only patients who were eligible for partial surgical resection, with an inherent good prognosis. Nevertheless, this homogeneous selection allowed for better highlighting the possible prognostic impact of a given histological subtype. The results from the subgroup analysis and preoperative biopsies need to be confirmed in larger series.

To conclude, our results suggest that the diagnosis of the SH-HCC subtype can be based on the association of ballooning as major criterion with at least two other minor criteria (among steatosis, Mallory Denk bodies, fibrosis and inflammation) regardless the extent of SH component. The prognosis of SH-HCC is similar to that of non–SH-HCC.

## Declarations

**Ethics approval and consent to participate:** Written consent was obtained from all patients as required by French legislation. This study was approved by the local ethics committee (Institutional Review Board -00006477- of AP-HP.Nord, Paris University, no. CER-2021-88).

**Author contributions:**

L.T., A.B., F.C., C.H. and T.C. conceived and designed the study and acquired data. L.T, A.B., and V.P. analysed and interpreted data and performed statistical analysis and writing. V.P., M.L., F.C., C.H., and M.B reviewed and revised the paper; M.A. provided technical and material support. All authors read and approves the final paper.

**Conflict of interest:** The authors have no conflicts of interest to report.

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**Data availability statement:** The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Conflict of interest:** The authors have no conflicts of interest to report.

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# Figures

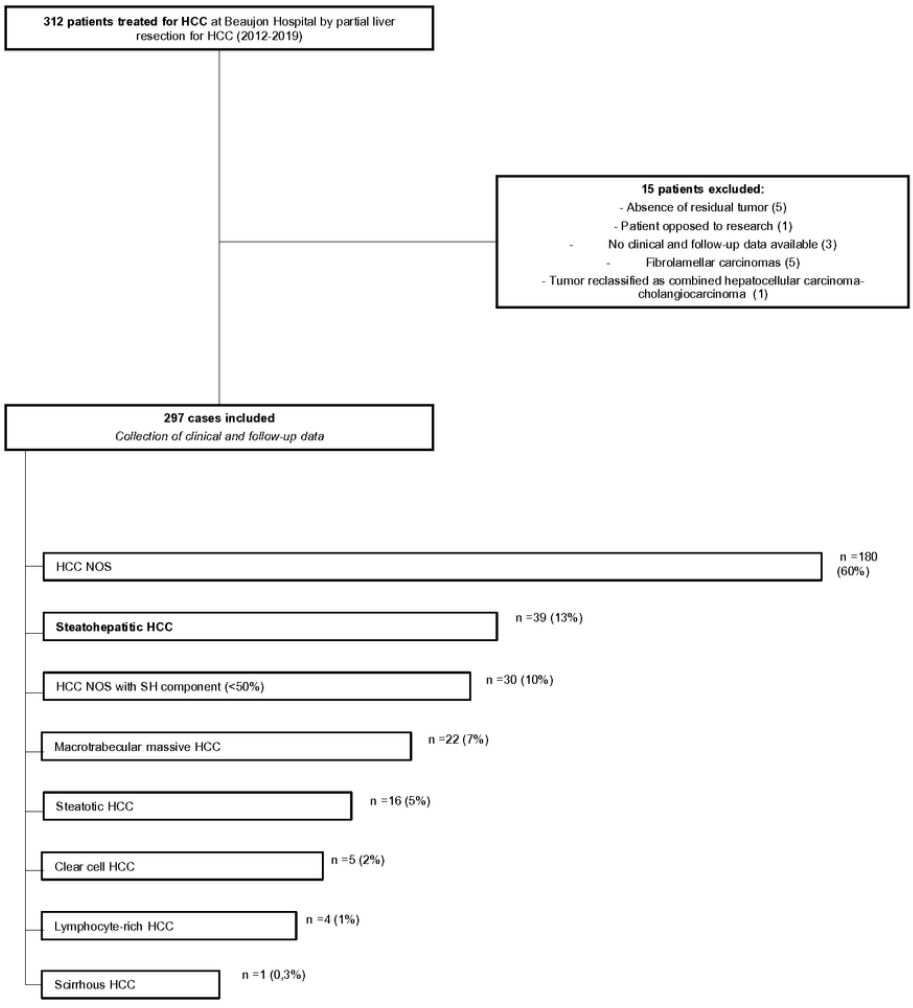


**Figure 1**

**Flowchart of the participants in the study and prevalence of hepatocellular carcinoma (HCC) subtypes**

*NOS, not otherwise specified*

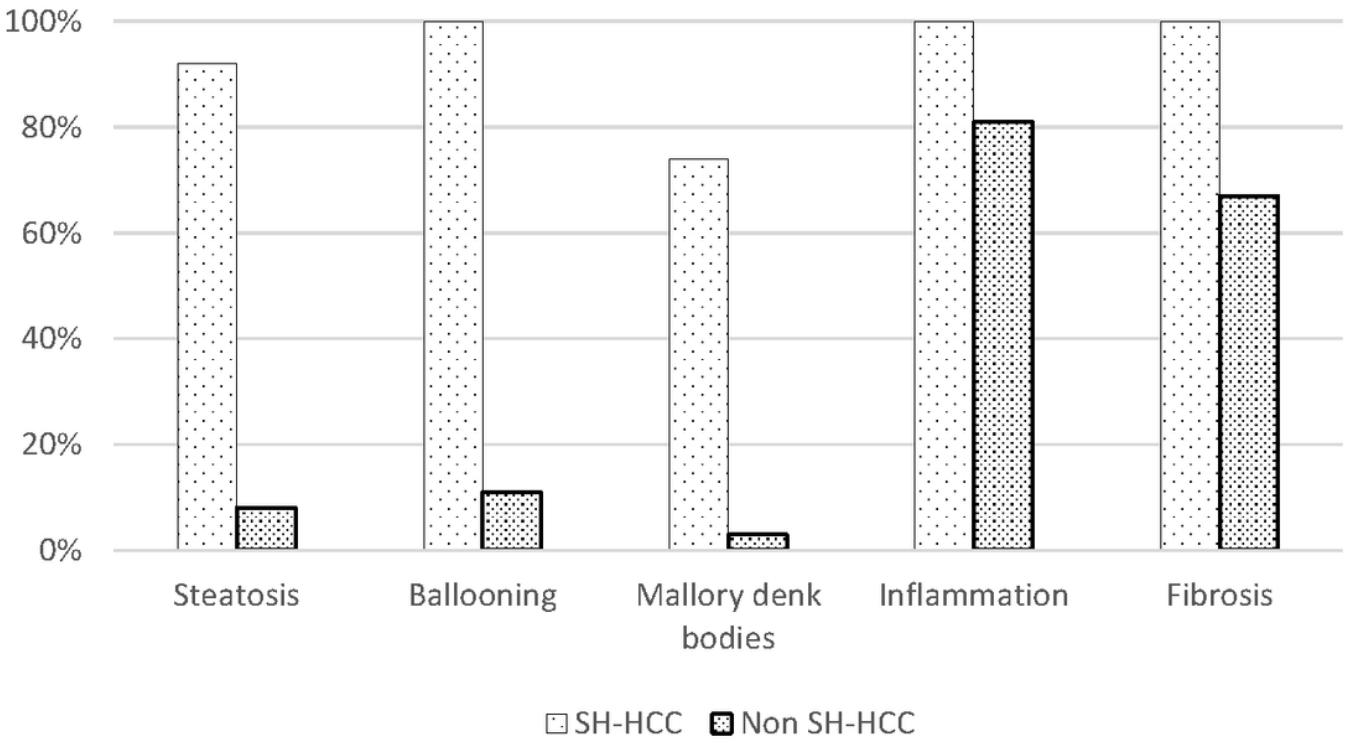


Figure 2

Distribution of SH criteria in SH-HCC and non−SH-HCC

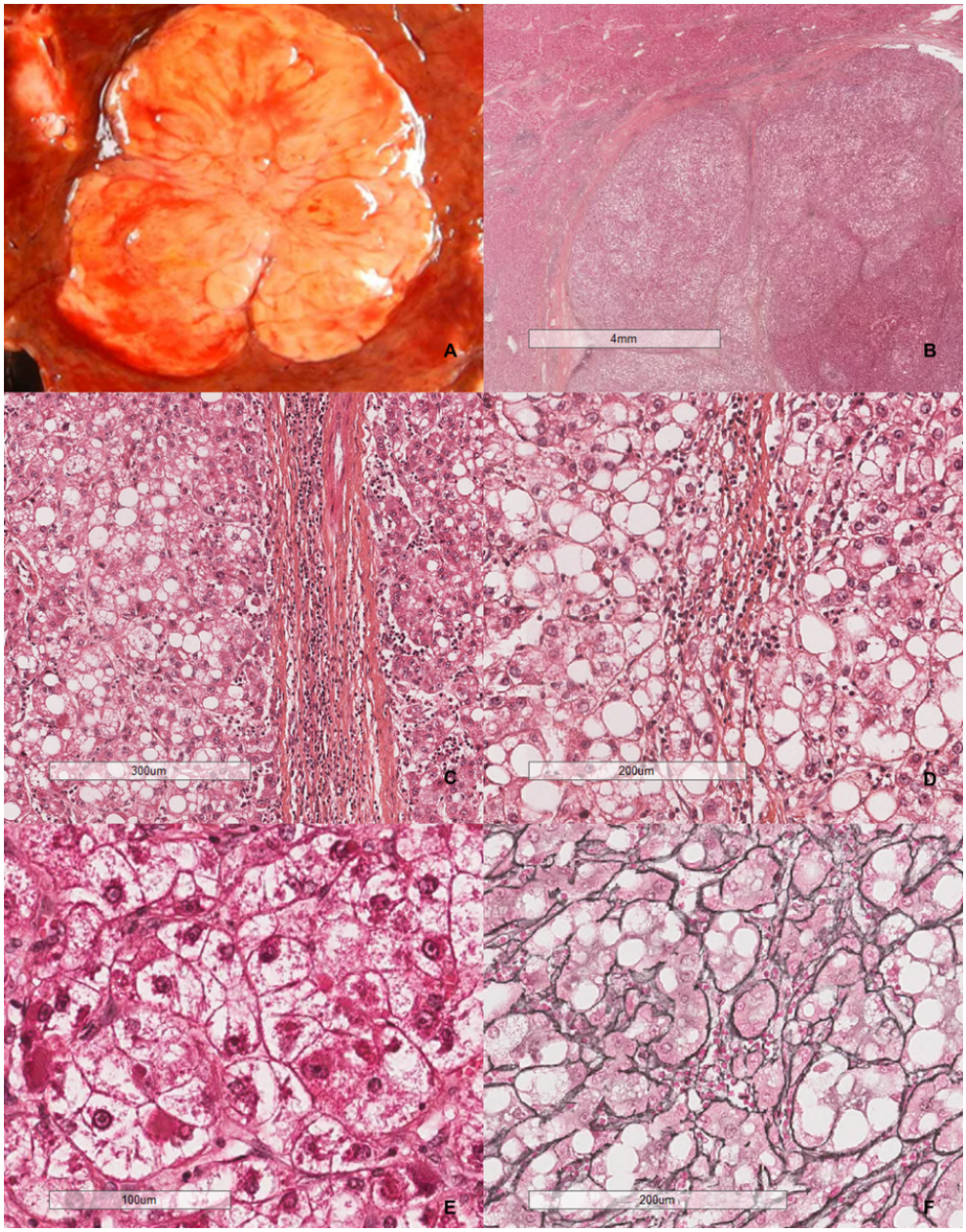


**Figure 3**

**Macroscopic and microscopic features of steatohepatitic hepatocellular carcinoma (SH-HCC)**

A. On gross examination, SH-HCC is well demarcated, unencapsulated, of yellowish color

B. At low magnification, SH-HCC is well demarcated, Steatosis and fibrosis are already apparent

C. High magnification showing steatosis, inflammation and fibrosis (band type)

D. High magnification showing steatosis, inflammation and fibrosis (pericellular type)

E. High magnification showing tumor cells ballooning and Mallory-Denk bodies

F. Reticulin staining, at high magnification, showing a pericellular pattern (nest of one or few tumor cells)

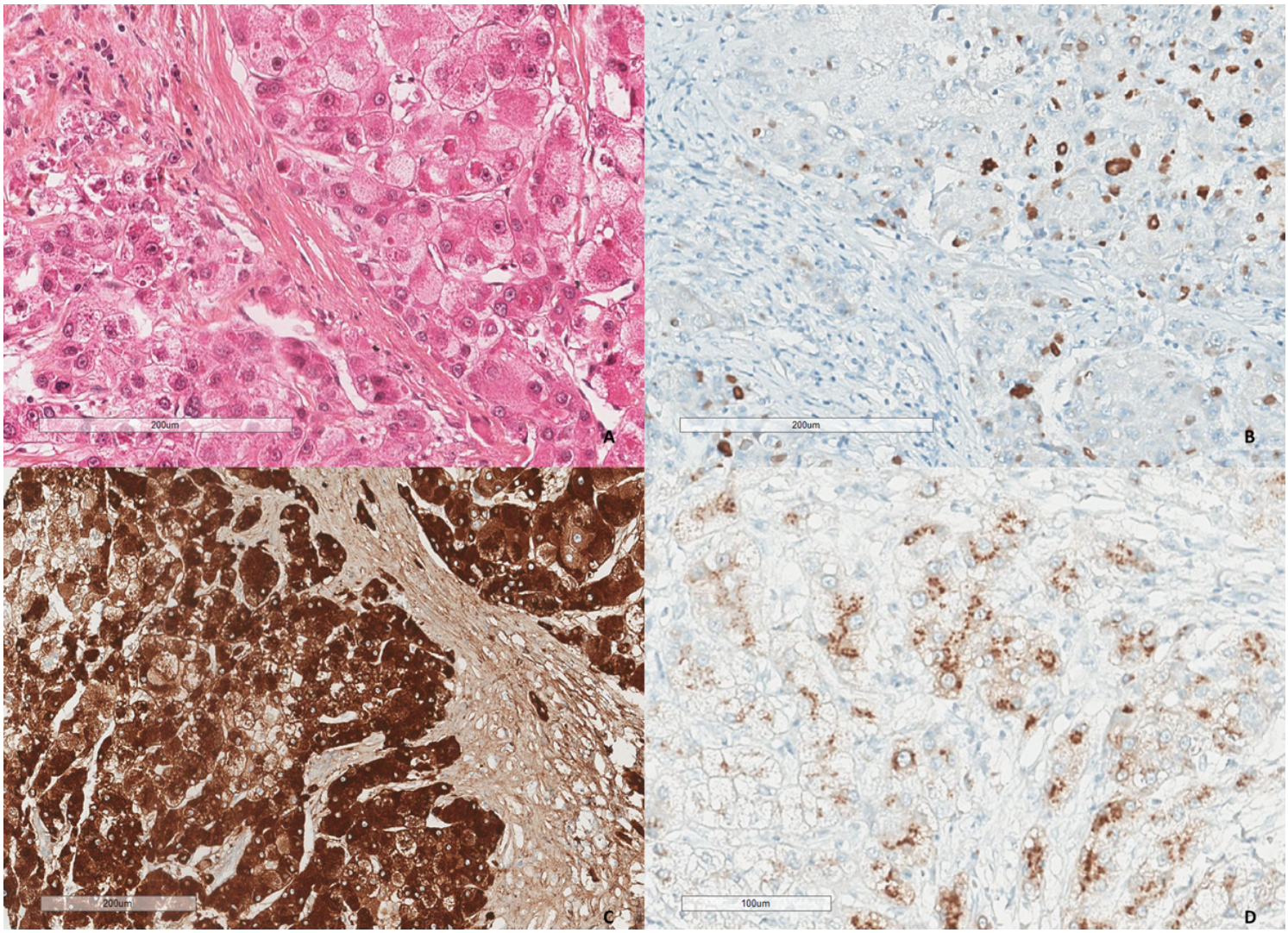


**Figure 4**

**Immunostaining of steatohepatitic hepatocellular carcinoma (SH-HCC)**

A. HES staining showing ballooning with Mallory-Denk bodies, inflammation and fibrosis

B. CK8/18 staining underlining the Mallory-Denk bodies

C. CRP staining: high cytoplasmic expression of the tumor cells

D. SAA staining: granular and cytoplasmic expression of the tumor cells

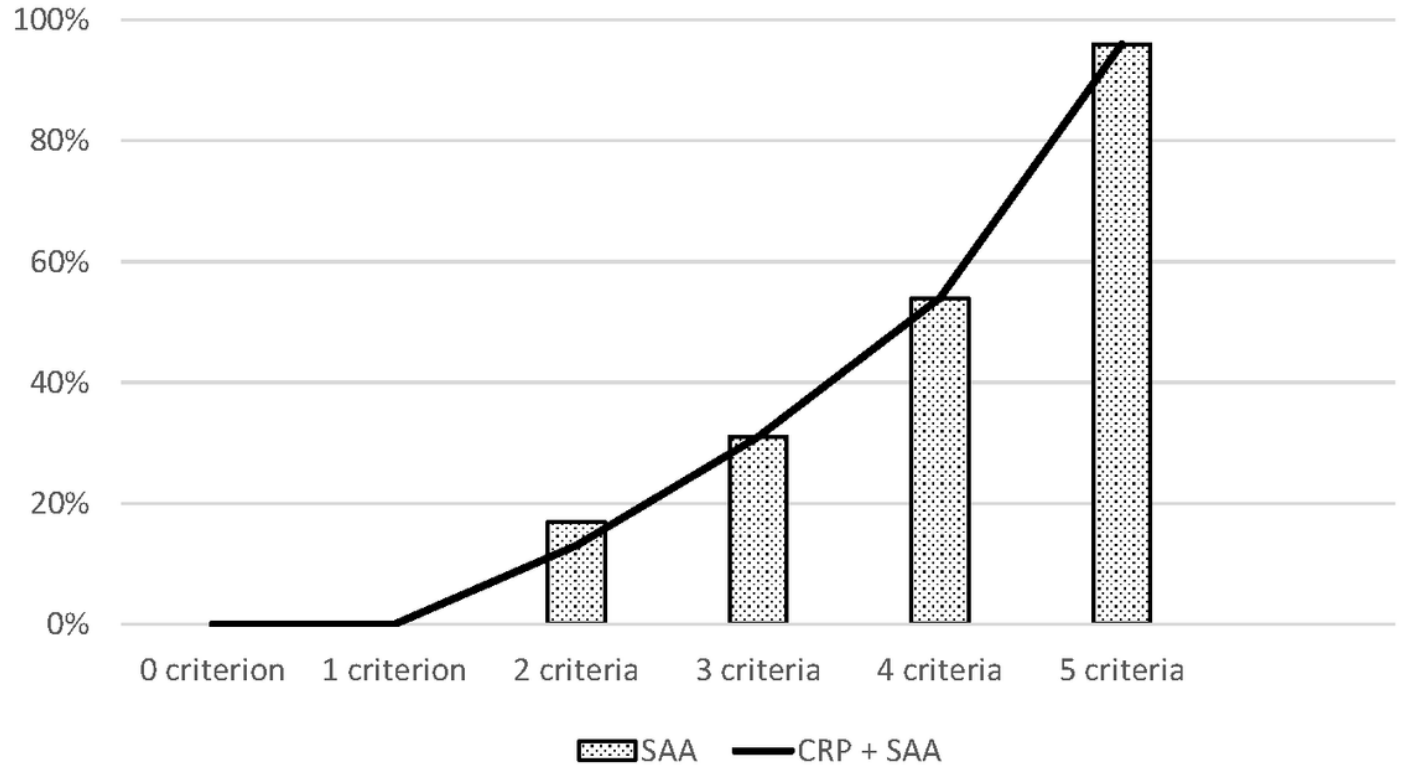


Figure 5

Expression of C-reactive protein (CRP) and serum amyloid A (SAA) by number of SH criteria in 127 HCC cases (including 38 SH-HCC)

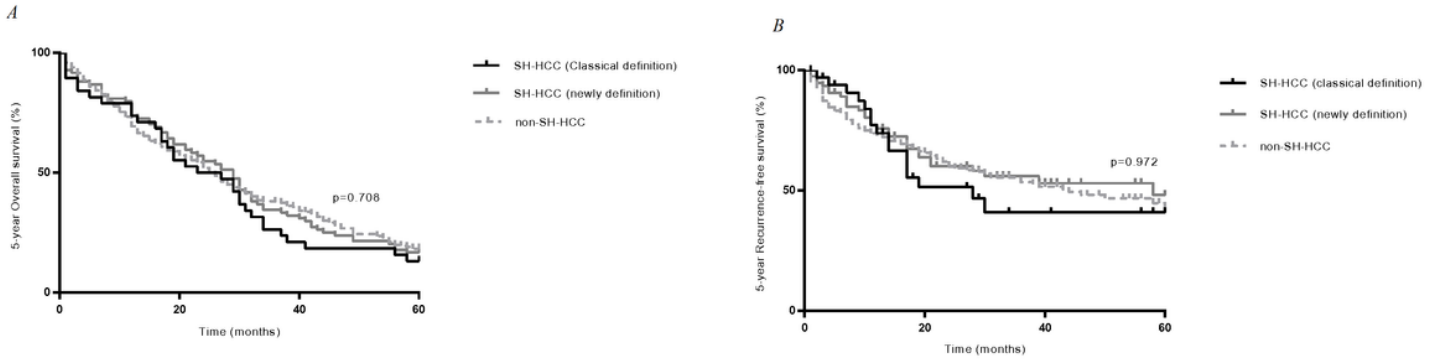


Figure 6

Kaplan-Meier curves for 5-year overall (A) and 5-year recurrence-free (B) survival comparing SH-HCC (classical and newly definition) to non-SH-HCC.