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Mallory-Denk Bodies are Associated with Outcomes and Histologic Features in Patients with Chronic Hepatitis C

Mina O. Rakoski¹, Morton B. Brown², Robert J. Fontana¹, Herbert L. Bonkovsky^{3,4}, Elizabeth M. Brunt⁵, Zachary D. Goodman⁶, Anna S. Lok¹, M. Bishr Omary^{1,7}, and The HALT-C Trial Group

¹Division of Gastroenterology, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI

²Department of Biostatistics, University of Michigan School of Public Health, Ann Arbor, MI

Correspondence: Mina Rakoski, MD, MSc University of Michigan Hospital Department of Internal Medicine Division of Gastroenterology 3912 Taubman Center, SPC 5362 Ann Arbor, MI 48109-5362 minao@med.umich.edu Tel: 734-615-1370 Fax: 734-936-7392.

In addition to the authors of this manuscript, the following individuals were instrumental in the planning, conduct and/or care of patients enrolled in this study at each of the participating institutions as follows: University of Massachusetts Medical Center, Worcester, MA: (Contract N01-DK-9-2326) Gyongyi Szabo, MD, Barbara F. Banner, MD, Maureen Cormier, RN, Donna Giansiracusa, RN

University of Connecticut Health Center, Farmington, CT: (Grant M01RR-06192) Gloria Borders, RN, Michelle Kelley, RN, ANP Saint Louis University School of Medicine, St Louis, MO: (Contract N01-DK-9-2324) Adrian M. Di Bisceglie, MD, Bruce Bacon, MD, Brent Neuschwander-Tetri, MD, Debra King, RN

Massachusetts General Hospital, Boston, MA: (Contract N01-DK-9-2319, Grant M01RR-01066; Grant 1 UL1 RR025758-01, Harvard Clinical and Translational Science Center) Jules L. Dienstag, MD, Raymond T. Chung, MD, Andrea E. Reid, MD, Atul K. Bhan, MD, Wallis A. Molchen, David P. Lundmark

University of Colorado Denver, School of Medicine, Aurora, CO: (Contract N01-DK-9-2327, Grant M01RR-00051, Grant 1 UL1 RR 025780-01), Gregory T. Everson, MD, Thomas Trouillot, MD, Marcelo Kugelman, MD, S. Russell Nash, MD, Jennifer DeSanto, RN, Carol McKinley, RN

University of California - Irvine, Irvine, CA: (Contract N01-DK-9-2320, Grant M01RR-00827) Timothy R. Morgan, MD, John C. Hoefs, MD, John R. Craig, MD, M. Mazen Jamal, MD, MPH, Muhammad Sheikh, MD, Choon Park, RN

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University of Southern California, Los Angeles, CA: (Contract N01-DK-9-2325, Grant M01RR-00043) Karen L. Lindsay, MD, MMM, Sugantha Govindarajan, MD, Carol B. Jones, RN, Susan L. Milstein, RN

University of Michigan Medical Center, Ann Arbor, MI: (Contract N01-DK-9-2323, Grant M01RR-00042, Grant 1 UL1 RR024986, Michigan Center for Clinical and Health Research) Joel K. Greenson, MD, Pamela A. Richtmyer, LPN, CCRC, R. Tess Bonham, BS Virginia Commonwealth University Health System, Richmond, VA: (Contract N01-DK-9-2322, Grant M01RR-00065) Mitchell L. Shiffman, MD, Richard K. Sterling, MD, MSc, Melissa J. Contos, MD, A. Scott Mills, MD, Charlotte Hofmann, RN, Paula Smith, RN Liver Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD: Marc G. Ghany, MD, T. Jake Liang, MD, David Kleiner, MD, PhD, Yoon Park, RN, Elenita Rivera, RN, Vanessa Haynes-Williams, RN

National Institute of Diabetes and Digestive and Kidney Diseases, Division of Digestive Diseases and Nutrition, Bethesda, MD: James E. Everhart, MD, MPH, Leonard B. Seeff, MD, Patricia R. Robuck, PhD, Jay H. Hoofnagle, MD, Elizabeth C. Wright, PhD

University of Washington, Seattle, WA: (Contract N01-DK-9-2318) Chihiro Morishima, MD, David R. Gretch, MD, PhD, Minjun Chung Apodaca, BS, ASCP, Rohit Shankar, BC, ASCP, Natalia Antonov, M. Ed.

New England Research Institutes, Watertown, MA: (Contract N01-DK-9-2328) Kristin K. Snow, MSc, ScD, Teresa M. Curto, MSW, MPH, May Yang, MPH

Inova Fairfax Hospital, Falls Church, VA: Fanny Monge, Michelle Parks

Data and Safety Monitoring Board Members: (Chair) Gary L. Davis, MD, Guadalupe Garcia-Tsao, MD, Michael Kutner, PhD, Stanley M. Lemon, MD, Robert P. Perrillo, MD

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³Department of Medicine, University of Connecticut Health Center, Farmington, CT

⁴Carolinas Medical Center, Charlotte, NC

⁵Department of Pathology and Immunology, Washington University, St. Louis, MO

⁶Center for Liver Diseases, Inova Fairfax Hospital, Falls Church, VA

⁷Department of Molecular & Integrative Physiology, University of Michigan Medical School, Ann Arbor, MI

Abstract

Background & Aims—Mallory-Denk bodies (MDBs) are inclusions found in hepatocytes of patients with chronic liver diseases. Their clinical significance and prognostic value are not understood.

Methods—We performed cross-sectional and longitudinal analyses of patients with chronic hepatitis C (CHC) enrolled in the HALT-C trial to identify clinical features associated with MDBs and changes in MDBs over time. Biopsies were obtained at baseline and 1.5 and 3.5 years after patients were assigned to groups for the HALT-C trial; and patients were followed to assess clinical and histologic outcomes.

Results—Of biopsy samples collected from 1050 patients, MDBs were present in 15%. They were associated with insulin resistance and laboratory and histological markers of advanced liver disease (higher levels of periportal fibrosis, pericellular fibrosis, steatosis, and inflammation). After adjusting for disease severity (the ratio of aspartate aminotransferase to alanine aminotransferase, albumin, platelets, fibrosis, steatosis), the presence of MDBs was associated with histological progression (odds ratio [OR]=1.97; P=.04). Of the 844 patients from whom serial biopsy samples were collected, 61 (7.2%) developed MDBs (MDB gain) and 101 (12.0%) lost MDBs (MDB loss). The presence or absence of diabetes mellitus was associated with MDB gain (P=.006) or loss (P=.024), respectively. Development of MDBs was associated with decompensation (adjusted HR=2.81, P<.001) and histologic signs of progression (adjusted OR=4.02, P=.004).

Conclusions—The presence of MDBs in liver biopsy samples from patients with CHC is independently associated with fibrosis progression. Gain of MDBs over time is associated with decompensation and progression to cirrhosis; and occurs most frequently among diabetics. MDBs might be used as prognostic factors for patients with CHC.

Keywords

keratins; liver disease; intermediate filaments; cytoplasmic inclusions

INTRODUCTION

Mallory-Denk bodies (MDBs), formerly known as Mallory bodies, are hepatocyte cytoplasmic inclusions found in several chronic liver diseases, including chronic hepatitis C (CHC).^{1,2} Keratin polypeptides 8 and 18 (K8/K18) are the major constituents of MDBs¹ and likely play an essential cytoprotective role in the liver.² Recent studies in mice indicate that the genetic background³ and gender⁴ play critical roles in MDB formation. In humans, keratin genes *KRT8*, *KRT18* and *KRT19* have been associated with increased susceptibility to end-stage liver disease,^{6,7} increased fibrosis in CHC,⁸ and disease severity in primary biliary cirrhosis.⁹

The Hepatitis C Antiviral Long-Term Treatment against Cirrhosis (HALT-C) Trial was a multi-center prospective, randomized, controlled study to determine if long-term treatment with pegylated interferon could slow disease progression in CHC patients with advanced fibrosis or cirrhosis who had previously failed interferon treatment.¹⁰ Patients underwent a baseline liver biopsy, as well as repeat biopsies 1.5 and 3.5 years after randomization and were followed for up to 8 years. The presence or absence of MDBs was recorded by a panel of expert pathologists, thus making this an ideal dataset for the study of risk factors and prognostic value of MDBs while accounting for possible confounders such as diabetes, alcohol, and steatosis. The aim of our study was to identify clinical features associated with MDBs and to evaluate their role in clinical and histological disease progression in a well-characterized cohort of CHC patients that were prospectively followed.

PATIENTS AND METHODS

The HALT-C Trial

The design and main results of the HALT-C Trial have been described.^{10,11} Briefly, patients with compensated CHC infection and advanced fibrosis (Ishak stage 3) and previous non-response to interferon therapy were treated with pegylated interferon alfa-2a (180 mcg weekly) and ribavirin (1–1.2 gm/day) (Lead-in group). If hepatitis C virus (HCV) RNA was undetectable after 20 weeks, treatment was continued for 48 weeks. If serum HCV RNA remained detectable after 20 weeks of treatment, patients were randomized to receive maintenance pegylated interferon alfa-2a alone (90 mcg weekly) or no therapy (Lead-in Nonresponders). If HCV RNA reappeared during continued treatment (Lead-in Breakthrough) or after completing treatment (Lead-in Relapsers), these patients were also eligible for randomization. Patients who underwent comparable treatment with pegylated interferon and ribavirin outside of the HALT-C trial and who met eligibility criteria were also enrolled in the maintenance phase (Express). Because maintenance interferon treatment had no impact on histological or clinical outcomes, treated and untreated patients were combined, totaling 1050 randomized patients. Informed consent was obtained from each patient in the study. Study protocol was approved by the HALT-C Ancillary Studies Committee. A de-identified dataset was provided by the HALT-C Data Coordinating Center (New England Research Institutes, Watertown, MA) under a data use agreement.

Clinical and Laboratory Evaluation

Baseline information included demographics, body mass index (BMI), history of diabetes, alcohol and tobacco use. Laboratory tests included blood counts, liver panel, HCV genotype, basic metabolic panel, glucose, insulin, serum alpha-fetoprotein (AFP), and ferritin. Insulin resistance was assessed by homeostasis model assessment (HOMA-IR).¹² Patients were followed for a median of 6.1 years (up to 8.7 years) for clinical outcomes. Clinical assessment and blood tests were performed at each visit, and surveillance for HCC was performed per protocol.¹³

All Lead-in and Express patients had a baseline liver biopsy performed within 12 and 18 months prior to enrollment, respectively, and at least 2 months following the last course of interferon.¹⁰ All biopsies were read by a committee of 12 expert pathologists, and findings for all major histological categories were determined by consensus. Consensus was not required for MDBs; however, presence of MDBs was generally obvious and scoring was recorded as either 'present' or 'absent'. If disparity arose, decision was made by majority vote. In all biopsies, MDBs were not associated with surrounding neutrophils (satellitosis), as commonly seen with alcoholic hepatitis. Biopsies were scored for inflammation (0–12), fibrosis (0–6), zone 3 pericellular fibrosis (0–2), steatosis (0–4), and iron (0–3).¹⁴ Hepatocellular ballooning, a common feature associated with nonalcoholic steatohepatitis,

was not recorded. Biopsy length and presence of fragmentation were recorded. Patients with steatosis alone or steatosis with mild to moderate steatohepatitis were enrolled, however those with severe steatohepatitis (defined by the presence of marked steatosis, many MDBs, and extensive zone 3 pericellular fibrosis) were excluded. Additionally, patients were excluded if they had active alcohol abuse within the 12 months prior to enrollment as determined by the principal investigator at each site.¹⁰

Clinical and Histological Outcomes

Two primary outcomes were assessed. A clinical outcome was defined as an increase in Child-Turcotte-Pugh (CTP) score to ≥ 7 on two consecutive occasions, variceal bleeding, ascites, spontaneous bacterial peritonitis, hepatic encephalopathy, HCC, liver transplantation, or liver-related death. A secondary analysis was performed to assess non-HCC clinical outcomes. A histological outcome was defined as an increase in Ishak fibrosis score by ≥ 2 points on follow-up liver biopsies. This analysis included only patients with Ishak fibrosis score ≤ 4 at baseline.

Statistical Analysis

To determine factors associated with MDBs, the HALT-C dataset was analyzed in two ways: (i) cross-sectional analysis of MDBs on baseline biopsy, and (ii) a longitudinal analysis to evaluate change in MDB presence or absence from baseline to follow-up biopsy. Of the 1050 randomized patients, 677 (64.4%) had all three biopsies (baseline, Y1.5, and Y3.5). In order to maximize sample size for the longitudinal analysis, patients who had at least one follow-up biopsy, either Y1.5 or Y3.5, were included ($n=894$). For the 677 who had both follow-up biopsies, the findings in the latter biopsy (Y3.5) were used. Follow-up laboratory values were extracted from the clinical visit closest to the follow-up biopsy date. Variables with skewed distributions were transformed using log or square root transformations. For univariate analyses, chi square and t-tests were used for categorical and continuous variables, respectively.

Cox proportional hazard regression was used for assessment of clinical outcomes and adjusted for baseline factors known to be associated with clinical decompensation (platelet count, fibrosis stage, steatosis grade, albumin, AST/ALT ratio),^{15–18} with steatosis entered as an interaction term with fibrosis. Survival analysis for clinical outcomes was based on Kaplan–Meier estimates and comparisons between groups made using log-rank-test. Because MDB gain and loss were determined at the time of follow-up biopsy, time to event was calculated as time from follow-up biopsy to first clinical event, and patients who had clinical outcomes prior to the follow-up biopsy were excluded from this analysis. Logistic regression (adjusted in a similar manner as for clinical outcomes) was used to assess histological outcome.

For the longitudinal analysis of change in MDBs over time, two groups (MDB gain and MDB loss) were compared using four classifications of change in MDB status over time. *MDB gain* compared subjects with: 1) Absent MDBs in baseline biopsy changing to MDB presence in follow-up biopsy (Gain), to 2) Absent MDBs in both baseline and follow-up biopsies. *MDB loss* compared subjects with: 3) MDB presence in baseline biopsy changing to MDB absence in follow-up biopsy (Loss), to 4) MDB presence in both baseline and follow-up biopsies.

Variables that were significantly associated in univariate analysis were included in logistic regressions to predict MDB status. P-values are presented without corrections for multiple comparisons. All analyses were performed at the University of Michigan using STATA 11.0 (College Station, Texas).

RESULTS

MDBs and Patient Characteristics

Cross-Sectional Analysis—Of 1050 randomized patients (Fig.1), 15% (n=158) had MDBs present on baseline biopsy. In univariate analysis, MDB presence (an example of an MDB is shown in Fig.2A) was associated with laboratory markers of disease severity: low platelets, low albumin, high AFP, (each $p<0.001$), and high AST/ALT ratio ($p=0.006$) (Table 1) as well as histological markers of disease severity: higher periportal fibrosis, pericellular fibrosis, steatosis, and inflammation scores (each $p<0.001$) (Fig.2B). Presence of MDBs was also highly correlated with factors associated with obesity and insulin resistance: presence of diabetes ($p=0.018$), high BMI ($p<0.001$), and high HOMA-IR ($p<0.001$). Adjusted multivariate analysis showed that baseline MDBs were associated with increased periportal fibrosis, zone 3 pericellular fibrosis, steatosis, inflammation, and AFP (Supplemental Table 1).

Longitudinal Analysis—Of 1050 randomized patients, 156 (14.9%) had only baseline biopsies and were therefore excluded from the longitudinal analysis (Fig.1). Additionally, 50 (4.8%) subjects had indeterminate classification of MDB gain (MDB absent on baseline, present on Y1.5, and absent on Y3.5 biopsy) and were also excluded. Therefore, 844 patients were included in the longitudinal analysis (Fig.1). Age, smoking, and alcohol consumption at baseline were not associated with change in MDBs over time (Supplemental Table 2). No association was found between MDBs and alcohol consumption at the Year 1.5 and Year 3.5 biopsies, treatment status (Lead in Nonresponder, Relapse/Breakthrough, or Express), and treatment assignment (peginterferon versus control) (data not shown).

MDB Gain: Of 719 patients with MDBs absent on baseline biopsy, 61 (8.5%) developed MDBs on follow-up biopsy (MDB gain). Fragmentation of biopsy specimen (baseline and follow-up) did not influence MDB gain, however there was an association between mean *baseline* biopsy size and MDB gain (MDB gain: 1.6 ± 1.7 cm; no MDB gain: 1.8 ± 0.8 cm, $p=0.008$). No association was seen between follow-up biopsy size and MDB gain (MDB gain: 1.7 ± 0.6 cm; no MDB gain: 1.9 ± 2.7 cm, $p=0.58$). Using logistic regression, MDB gain was significantly associated with higher fibrosis and steatosis scores on follow-up biopsies as well as presence of diabetes, female gender, and Hispanic ethnicity (Table 2).

MDB Loss: Of 125 patients who had MDBs present on baseline biopsy, 101 (81%) did not have MDBs on the follow-up biopsy (MDB loss). On univariate analysis, these patients had lower BMI and baseline fibrosis stage and were less likely to have diabetes or to smoke (Supplemental Table 2). Fragmentation of biopsy specimen (baseline and follow-up) did not influence MDB loss, however there was an association between mean *follow-up* biopsy size and MDB loss (MDB loss: 1.6 ± 0.8 cm; no MDB loss: 2.0 ± 1.0 cm, $p=0.021$). No association was seen between biopsy size on baseline biopsy and MDB loss (MDB loss: 1.8 ± 0.7 cm; no MDB gain: 1.7 ± 0.6 cm, $p=0.81$). Using logistic regression, MDB loss was associated with absence of diabetes ($p=0.024$) and being a non-smoker ($p=0.003$) (Table 2).

MDBs and Clinical Outcomes

Cross-Sectional Analysis—Of 1050 randomized patients, 291 (28%) developed a clinical outcome, including 59 patients (20%) whose first clinical outcome was HCC. Clinical outcomes were highly associated with presence of MDBs on baseline biopsy ($p=0.001$), however these associations were not significant ($p=0.79$) after adjustment for baseline variables associated with disease severity (Table 3).

Longitudinal Analysis

MDB Gain: Of the 61 patients with MDB gain and 658 patients without MDB gain, 3 (4.9%) and 62 (9.4%) patients, respectively, had a first clinical event that occurred prior to follow up biopsy (time at which MBD gain was assessed), and were therefore excluded from analysis. Of the remaining 58 patients with MDB gain, 29 (50%) developed a clinical outcome whereas 92 (15%) of 596 subjects who did not have MDB gain developed a clinical outcome ($p<0.001$) (Fig.3). Results were similar when the 15 subjects with HCC were excluded ($p<0.001$). Clinical outcomes remained significantly associated with MDB gain after adjusting for baseline disease severity (Table 3, Fig.3).

MDB Loss: Of the 101 subjects with MDB loss and 24 subjects without MDB loss, 13 (13%) and 2 (8.3%) patients, respectively, had a first clinical event that occurred prior to follow up biopsy, and were excluded from analysis. Of the remaining 88 patients, 20 (23%) developed a clinical outcome, whereas 7 (32%) of 22 subjects who did not have MDB loss developed a clinical outcome ($p=0.49$). Results were similar when the 2 subjects with HCC were excluded ($p=0.89$). Adjusted cox regression showed no association between MDB loss and clinical outcomes (Table 3).

MDBs and Histological Outcomes

Cross-Sectional Analysis—After exclusion of subjects with cirrhosis on baseline biopsy (Ishak score = 5), 468 subjects were included in the cross-sectional analysis of histological outcomes. Of these, 154 (33%) developed a histological outcome. Twenty-nine (55%) of 53 patients with MDB present on baseline biopsy, compared to 125 (30%) of 415 subjects who lacked MDBs on baseline biopsy, developed a histological outcome ($p<0.001$). This association remained significant after adjusting for disease severity (Table 3).

Longitudinal Analysis—Of 468 subjects included in cross-sectional analysis of histological outcome, 21 were excluded due to lack of follow-up biopsy. Therefore, 447 subjects were included in the longitudinal analysis for histological outcome (394 evaluated for MDB gain and 53 for MDB loss, Fig.1).

MDB Gain: Of 394 subjects with MDBs absent on baseline biopsy, 24 (6.1%) developed MDBs. Sixteen (67%) of these 24 patients, compared to 103 (28%) of 370 patients without MDB gain, developed a histological outcome. This association remained highly significant after adjusting for baseline disease severity (Table 3).

MDB Loss: Of 53 subjects with MDBs present on baseline biopsy, 48 (91%) demonstrated MDB loss. Of these 48 patients, 26 (54%) developed a histological outcome, whereas 3 (60%) of 5 subjects without MDB loss developed a histological outcome ($p=1.0$).

DISCUSSION

Although the human liver diseases that are associated with MDB formation are clearly defined,^{1,19} the significance of MDBs and whether they represent a benign epiphenomenon of hepatocyte injury or a modifier of liver disease progression is not known. Animal models indicate that MDB formation has a strong genetic component and is associated with oxidative stress, resulting in sequestration of misfolded proteins.¹⁻⁴ Several human studies have suggested that MDBs relate to disease severity and poor outcomes in other chronic liver diseases such as alcoholic liver disease and nonalcoholic steatohepatitis.²⁰⁻²² In evaluation of >1000 patients with chronic hepatitis C, this study furthers our knowledge by demonstrating that MDBs are independently associated with fibrosis progression and that

development of MDBs over time is highly associated with clinical decompensation and progression to cirrhosis.

Interestingly, insulin resistance was significantly associated with presence of MDBs and development of MDBs over time. Specifically, MDBs on baseline biopsy were associated with the presence of diabetes as well as higher BMI, HOMA-IR, steatosis grade, and zone-3 pericellular fibrosis stage. Moreover, the presence of diabetes was associated with MDB gain (OR 2.28, $p=0.006$), whilst the absence of diabetes was associated with MDB loss (OR 5.21, $p=0.024$). In light of these associations, CHC patients with MDBs may have also had concurrent nonalcoholic steatohepatitis (NASH). However, after controlling for several of the histologic features of NASH such as steatosis and fibrosis, the presence of MDBs remained associated with histological progression, suggesting that MDBs may offer additional prognostic information independent of its association with NASH.

The prevalence of NASH in patients with CHC is reported to be 9–18%,^{23,24} which corresponds to the 15% prevalence of MDBs on baseline biopsy found in our study. Similar to our findings, CHC with concurrent NASH has been associated with higher fibrosis stages, suggesting that NASH may hasten fibrosis progression in patients with CHC.²⁴ Using the HALT-C cohort, Everhart et al. showed that factors associated with insulin resistance were associated with clinical and histological outcomes.¹⁶ Our study expands upon this with a longer duration of follow-up (from 3.8 to 8.7 years), thereby allowing a greater number of clinical outcomes. Additionally, our analysis assessed clinical and histological outcomes as separate primary endpoints, with clinical outcomes further evaluated with and without HCC. Taken together, our findings further support the growing evidence that CHC with concomitant NASH features is associated with worse outcomes compared to CHC alone.

In longitudinal analysis, MDB gain was associated with clinical and histological outcomes. The converse, however, was not found to be true (i.e. those with MDB loss did not have improved outcomes). This may be due to smaller numbers or may reflect that the MDBs seen on initial biopsy heralded the coexistence of NASH in these patients. Although MDBs were no longer present on follow-up biopsy, it is unlikely that NASH had regressed and therefore may explain the lack of improved outcomes seen in this group. Other potential explanations include a liver biopsy sampling error or hitherto unknown environmental/genetic factors that might lead to MDB resolution.

Though not significantly associated with MDB presence on baseline biopsy, female gender was associated with MDB gain, suggesting that factors such as genetics⁴ are likely important. Multivariate analysis showed that Hispanics are more likely to demonstrate MDB gain (Table 2), though the small number of Hispanics and the known association between Hispanic ethnicity and metabolic factors warrants independent verification.

In evaluating the prognostic role of MDBs, the HALT-C trial offered several advantages including a prospective follow-up of a large cohort of patients, a range of races/ethnicities, and longitudinal data on alcohol consumption and metabolic factors. Most importantly, serial biopsies were available, all reviewed by expert hepatopathologists. However, the HALT-C cohort does have some limitations with regard to assessing MDBs. First, it is possible that MDBs that were present but not prominent may have been scored as absent. Though immunohistochemical (ubiquitin or K8/18) staining would have improved detection and reproducibility of finding MDBs, such staining was not part of the initial HALT-C analysis due to time and cost constraints. Sampling error of smaller biopsies may have influenced analysis of MDBs over time, as evidenced by smaller *baseline* biopsies in subjects with MDB gain and smaller *follow-up* biopsies in those with MDB loss. However, the overall differences in biopsy size are small despite being statistically different. The

longitudinal analysis of histological outcome in patients with MDB loss may have been limited due to small numbers (n=53), with 5 patients showing no MDB loss (Fig.1) though all other analyses are largely robust with much larger sample sizes. The HALT-C trial enrolled only patients with advanced histological disease (Ishak fibrosis stage 3), therefore our findings may not apply to patients with early-stage fibrosis. Similarly, all patients were previous non-responders to interferon therapy and half of the patients underwent maintenance interferon treatment, which may restrict generalization of the results to all patients with CHC. The large proportion of patients with high BMI (mean=29.9±5.5) and diabetes (24.1%) may also have contributed to our findings, though this trend reflects the known association between non-response to CHC treatment and traits such as obesity and diabetes.

In conclusion, MDBs were highly associated with markers of insulin resistance as well as independently associated with fibrosis progression in patients with CHC, which could be attributed to hastened fibrosis progression from coexistent NASH. In longitudinal analysis, patients with underlying diabetes appear to be at greatest risk of developing MDBs on follow-up biopsy, which was found to be associated with clinical outcomes as well as fibrosis progression. Therefore, assessment of MDBs in liver biopsies of patients with CHC appears to offer prognostic significance. Further studies with a broader spectrum of disease severity and in patients with other chronic liver diseases are warranted to further delineate the potential pathogenic factors and clinical implications of MDB assessment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

MDBs	Mallory-Denk bodies
CHC	chronic hepatitis C
K	keratin
HALT-C	Hepatitis C Antiviral Long-Term Treatment against Cirrhosis
HCV	hepatitis C virus
AFP	alpha-fetoprotein
BMI	body mass index

HCC	hepatocellular carcinoma
HOMA-IR	homeostasis model assessment of insulin resistance
OR	odds ratio
HR	Hazard ratio

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Fig. 1.
Study Design: Cross-sectional analysis (n=1050) and Longitudinal analysis (n=844).

Fig. 2.

(A) Representative hematoxylin and eosin staining of a liver biopsy from a patient with CHC (arrow points to a hepatocyte with a ring-like collection of MDBs). (B) Percentage of patients whose baseline biopsies had MDBs present by levels of fibrosis (3–6), zone 3 pericellular fibrosis (0–2), steatosis (0–4), and inflammation (3–12), $p < 0.001$ for each.

* MDB gain was assessed at time of follow up biopsy. Therefore, patients were excluded from this analysis if first clinical event occurred prior to follow up biopsy, resulting in exclusion of 3 and 62 patients with MDB gain and no MDB gain, respectively.

Fig. 3.
Kaplan Meier analysis: Time from follow-up biopsy to first clinical event in patients with MDB Gain versus no MDB Gain.

Table 1

Baseline characteristics of the HALT-C trial patients.

Variable [†]	MDB Absent(n=892)	MDB Present(n=158)	P value [‡]
Age (years)	50.3 ±7.3	49.5 ±6.5	0.21
Gender:			
Male	642 (72)	103(65)	0.083
Female	250 (28)	55 (35)	
Race:			
White	645 (72)	107(68)	0.61
Black	159(18)	32 (20)	
Hispanic	70(8)	14(9)	
Other	18(2)	5(3)	
BMI (kg/m ²)	29.6 ±5.4	31.3 ±5.5	<0.001
HOMA-IR	5.5 ±4.9	7.0 ±4.7	<0.001
Diabetes	204 (23)	50 (32)	0.018
Current drinker	140 (16)	33 (21)	0.10
Current smoker	263 (30)	53 (34)	0.29
HCV Genotype-1	840 (94)	140 (87)	0.010
Laboratory values			
Albumin (g/dL)	3.9 ±0.4	3.7 ±0.4	<0.001
Total bilirubin (mg/dL)	0.8 ±0.4	0.8 ±0.4	0.081
Platelet (K/mm ³)	168 ±65	148 ±66	<0.001
INR	1.0 ±0.1	1.1 ±0.1	0.029
AFP (ng/mL)	15.5 ±26.8	28.2 ±39.2	<0.001
AST/ALT ratio	0.9 ±0.3	0.9 ±0.3	0.006
Ferritin (ng/mL)	344 ±398	443 ±446	0.009
Histology			
Biopsy length (cm)	1.8 ± 0.8	1.9 ± 1.3	0.43
Fragmented biopsy	211 (24)	36 (23)	0.81
Fibrosis	4.0 ±1.3	4.6 ±1.2	<0.001
% Bridging fibrosis (3–4)	62.5	41.1	
% Cirrhosis (5–6)	37.5	58.9	

Variable [†]	MDB Absent(n=892)	MDB Present(n=158)	P value [‡]
Steatosis (0–4)	1.3 ±0.9	2.0 ±0.9	<0.001
Inflammation (0–12)	7.4 ±2.1	8.4 ±1.9	<0.001
Zone-3 pericellular fibrosis (0–2)	0.37 ±0.6	0.66 ±0.7	<0.001
Iron grade (0–3)	0.51 ±0.70	0.35 ±0.61	0.008

[†]Data reported as mean ±standard deviation (mean ±S.D.) for continuous variables and n (%) for categorical variables.

[‡]t-test used for continuous variables; chi-square test for categorical variable.

Table 2

Multivariate analysis of variables associated with MDB gain and MDB loss.

Variable	OR	95% CI	P value
<i>MDB Gain (n=719)[†]</i>			
Female	1.88	1.06–3.34	0.031
Hispanic	2.94	1.28–6.77	0.011
Presence of diabetes	2.28	1.26–4.13	0.006
Fibrosis, baseline	1.03	0.78–1.35	0.84
Fibrosis, follow-up	1.84	1.39–2.43	<0.001
Steatosis, baseline	0.93	0.63–1.37	0.71
Steatosis, follow-up	2.14	1.33–3.44	0.002
<i>MDB Loss (n=125)[†]</i>			
Absence of diabetes	5.21	1.24–21.9	0.024
Non-smoker	8.96	2.20–38.7	0.003
Fibrosis, baseline	0.59	0.32–1.10	0.097
Fibrosis, follow up	0.93	0.54–1.60	0.80
BMI, baseline (kg/m ²)	1.00	0.86–1.18	0.92
Weight change	0.98	0.94–1.01	0.37

[†]Model includes variables significant in univariate analysis.

Table 3

Effect of baseline MDB, MDB gain, and MDB loss on clinical and histological outcomes

Outcome	Unadjusted			Adjusted [‡]	
	Ratio [†]	95% CI	P value	Ratio [†]	P value
<i>MDBs Present on Baseline Biopsy</i>					
Clinical	1.62	1.21–2.15	0.001	0.96	0.79
Histological	2.80	1.57–5.01	<0.001	1.97	0.04
<i>MDB Gain</i>					
Clinical	3.93	2.58–5.97	<0.001	2.81	<0.001
Histological	5.18	2.15–12.48	<0.001	4.02	0.004
<i>MDB Loss</i>					
Clinical	0.73	0.31–1.76	0.49	1.09	0.85
Histological	0.79	0.12–5.15	0.80	0.41	0.43

[†] Hazard Ratio for Clinical and non-HCC clinical outcome; Odds Ratio for Histological outcome

[‡] Adjusted for baseline albumin, platelet, AST/ALT ratio, fibrosis, steatosis, and interaction between fibrosis and steatosis.