Published in final edited form as:

Hum Pathol. 2016 June ; 52: 28–37. doi:10.1016/j.humpath.2016.01.001.

Acidophil Bodies in Nonalcoholic Steatohepatitis

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Abstract

The significance of the quantity of acidophil bodies (AB) in nonalcoholic steatohepatitis (NASH) is not certain. We quantified AB in liver biopsies and examined the association with the diagnosis of NASH and other histologic features. We reviewed 157 liver biopsies from the NASH CRN Database collected in 2006. 127 biopsies were from adult patients. Diagnoses were 94 definite NASH, 40 borderline NASH, and 23 definitely not NASH. The total length and average width of the core biopsies were measured and the biopsy areas were calculated (mm^2). Total AB were counted and mean AB count per mm^2 was calculated (AB/mm^2) to derive acidophil body index (ABI). ABI was $0.04 (\pm 0.08)$ in definite NASH and $0.02 (\pm 0.05)$ in borderline/definitely not NASH groups combined ($p=0.02$) in all 157 biopsies; similar findings were present in the 127 adult only biopsies (0.04 ± 0.05 and 0.02 ± 0.05 , respectively, $p=0.05$). In all 157 biopsies, increased ABI was associated with greater lobular inflammation ($p=0.01$) and many ballooned hepatocytes ($p=0.048$). There was a positive relationship between ABI and high nonalcoholic fatty liver disease (NAFLD) activity scores (NAS), but this association was not statistically significant. There was no association between ABI and steatosis or fibrosis stage in either the entire cohorts or in the subset of adult patients. In conclusion, the density of AB is associated with lobular inflammation, ballooned hepatocytes, and the diagnosis of NASH in adult and pediatric liver biopsies, suggesting the implication of the apoptotic pathway in NASH-associated liver cell injury.

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All authors have agreed this manuscript and have no conflicts of interest to disclose. All procedures were performed in compliance with institutional guidelines and approval. Brief running title: Acidophil bodies in nonalcoholic steatohepatitis

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Keywords

nonalcoholic steatohepatitis; acidophil bodies; acidophil body index

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) has become a global epidemic in the 21st century, as it is the hepatic manifestation of the metabolic syndrome that is universally associated with the myriad of metabolic aberrations related to insulin resistance, in which type 2 diabetes, truncal obesity, dyslipidemia, hyperlipidemia and arterial hypertension are the best known features [1–3]. It is estimated 20% to 30% of adults in the United States and Western Europe have excessive fat in the liver and 10% of these individuals (2% to 3% of adult population) may also meet current diagnostic criteria for the progressive disease, nonalcoholic steatohepatitis (NASH) [4]. NASH may result in liver fibrosis and progress to cirrhosis, which may lead to liver-related death, hepatocellular carcinoma, or require liver transplantation [5]. The diagnosis of NASH continues to depend on histopathology. The diagnostic features of NASH centers on the histologic constellation of steatosis, inflammation, and hepatocellular injury in the form of ballooning of hepatocytes. The clinical absence of significant alcohol use is required [6–7]. Among the histologic features, inflammation and hepatocyte ballooning reflect various injury cascades [1, 8]. The morphologic identification of ballooned hepatocytes may be difficult by routine stains, and may depend on the pathologists' experience [6]. Due to the known damage to hepatocytes, immunohistochemistry for keratins 8 and 18 has been proposed for demonstrating this form of injury [9]. Additionally, injury to hepatocytes may be present as other forms including spotty necrosis and apoptosis. For example, apoptotic bodies, also known as acidophil bodies (AB) (Figures 1A and 1B), which are hepatocytes undergoing programmed cell death, are commonly seen in viral hepatitis. In fact, Saxena et al. have suggested the density of apoptotic bodies can reliably distinguish early recurrent hepatitis C from acute cellular rejection in the early phase after orthotopic liver transplantation [10]. Recent clinical and animal studies have suggested apoptosis genes and related proteins are upregulated in NASH [11, 12]. Caspase-3 generated fragment of cytokeratin (CK) 18, a marker for apoptosis, is also increased in individuals with NASH, but not in individuals with steatosis only or borderline NASH [12].

While apoptosis may be associated with liver injury in NASH, it is not certain if the quantity and density of AB is associated with the diagnosis of NASH. In this study, we quantified AB in liver biopsies and examined the association with the diagnosis of NASH and related histologic features.

MATERIALS AND METHODS

Liver biopsies from the NIDDK sponsored NASH Clinical Research Network (NASH CRN) database collected consecutively from January 1, 2006 through December 31, 2006 were included. These are all needle core biopsies. After those biopsies with length shorter than 2 cm were excluded, 157 cases remained in the study, including 127 adults and 30 subjects

younger than 18 years old. These biopsies were centrally reviewed and given the consensus diagnosis of definite NASH (N=94), borderline NASH (N=40), or definitely not NASH (N=23) according to the NASH CRN criteria [6] by the group of NASH CRN pathologists (see acknowledgement) at a multi-headed microscope. Other histologic features including steatosis, ballooning, acidophil bodies and portal inflammation were also centrally scored as previously described [6]. Acidophil bodies were also semiquantitated by the group of NASH CRN pathologists as none, rare (one acidophil body), or many (at least 2 acidophil bodies) in the biopsy specimen. The total length and average width of the biopsy specimens of each case were measured on hematoxylin and eosin stained slides and the biopsy areas of each specimen were calculated (mm^2). Further, acidophil body counts were obtained upon review by one of the NASH CRN pathologists (MMY) who was blinded to the information of the consensus diagnosis of NASH of all cases. Acidophil bodies were defined as well demarcated, eosinophilic cytoplasmic globules, either anuclear or possessing nuclear fragments. Total acidophil bodies were counted and mean acidophil body count per mm^2 was calculated (AB/mm^2) as acidophil body index (ABI).

Appropriate statistical methods were used. Briefly, differences in baseline characteristics were compared for those biopsies with 0 vs. 1 vs. 2 acidophil bodies in the entire biopsy area. Data are presented as means (SD), medians (IQR), or N (%). P values for baseline characteristics were derived from chi-square tests for categorical variables and from the Kruskal-Wallis test for continuous measures. The mean acidophil body count and acidophil count/area for binary histologic features are also presented, with P values derived from the test for equality of Poisson counts, corrected for over dispersion. $P < 0.05$ was considered to be statistically significant. All analyses were performed using SAS statistical software (version 9.1 of the SAS system for Windows, SAS Institute, Cary, NC, 2002).

RESULTS

Table 1 to 3 show the semiquantitative assessment of acidophil bodies by the NASH CRN Pathology Committee and its association with the patients' demographic, clinical and laboratory data in all 157 patients, adults, and children, respectively. None of the parameters including age, gender, race, liver tests, blood chemistry, and the size of liver biopsy specimens had significant associations with the semiquantitative assessment of acidophil bodies, except higher BMI was observed in the group that had rare acidophil bodies and those children who did not have acidophil bodies observed were all boys.

Our data showed significant association between the absolute acidophil body count and the semiquantitative assessment of lobular inflammation, ballooning, and diagnosis of steatohepatitis, but not steatosis in 157 biopsies. In addition, as is shown in Table 4, in all 157 biopsies (including adults and children), ABI was 0.04 (± 0.08) in the group with diagnosis of definite NASH and 0.02 (± 0.05) in the groups with diagnosis of suspicious or definitely not NASH combined ($p = 0.02$). In all 157 biopsies, ABI in those with lobular inflammation score less than 2 was 0.02 (± 0.04), whereas ABI in those with lobular inflammation scores of 2 or 3 was 0.04 (± 0.08). Therefore, higher ABI correlated with greater lobular inflammation ($p = 0.01$), as is illustrated in a representative case (Figures 1C and 1D). There was a trend of increased ABI when ballooned hepatocytes were many

compared to when they were none or few (0.05 ± 0.08 and 0.03 ± 0.05 , respectively) ($p=0.048$). There was no significant difference in ABI between those biopsies with steatosis grade of 0 or 1 and those with steatosis grade of 2 or 3 (0.04 ± 0.06 and 0.03 ± 0.07 , respectively, $p=0.89$). ABI did not differ significantly in the presence or semi-quantification of other forms of inflammation or features of hepatocellular injuries such as microgranulomas, pigmented macrophages, or Mallory-Denk bodies. There was no significant difference in ABI between early and advanced fibrosis stages (0.04 ± 0.08 and 0.03 ± 0.05 , respectively, $p=0.61$). ABI tended to be higher when NAFLD activity score (Cimbanassi, #50) was greater than 3, but was not statistically significant ($p=0.09$).

When the 127 adult patients were analyzed separately (Table 5), ABI tended to be greater in the group with diagnosis of definite NASH than in the groups with diagnosis of suspicious or definitely not NASH combined (0.04 ± 0.05 and 0.02 ± 0.05 , respectively), however, it did not reach statistical significance ($p=0.05$). ABI was 0.04 ± 0.05 when ballooned hepatocytes were many, and was 0.03 ± 0.06 when they were absent/few ($p=0.15$). ABI was 0.04 ± 0.1 when NAS was 4–8 and was 0.02 ± 0.1 when NAS was 0–3 ($p=0.15$). As was seen in the entire group, there was no correlation seen with the degree of steatosis or stage of fibrosis in adult only group.

In the 30 pediatric biopsies (Table 6), ABI was 0.12 ± 0.26 when ballooned hepatocytes were many, and was 0.03 ± 0.05 when they were absent/few ($p=0.02$). There was a trend of higher ABI in the group with a diagnosis of definite NASH: ABI was $0.09 (\pm 0.19)$ in the group with diagnosis of definite NASH and $0.02 (\pm 0.03)$ in the groups with diagnosis of suspicious or definitely not NASH combined ($p=0.05$). There was also a trend of higher ABI in the biopsies with lobular inflammatory foci of 2 or greater than that in the biopsies with lobular inflammatory foci less than 2 (0.07 ± 0.14 and 0.006 ± 0.01), but it did not reach statistical significance ($p=0.09$). ABI did not correlate with the degree of steatosis or stage of fibrosis (data not shown).

DISCUSSION

Our cohort consisted of 157 patients with biopsies from eight NASH CRN participant centers across the United States. The results of our study showed there were significant associations between the density of lobular acidophil bodies and lobular inflammation, many ballooned hepatocytes, and the diagnosis of definite NASH in adult and pediatric biopsies. While other studies have documented apoptosis as a feature of NASH, this is the first histopathological study that specifically examines acidophil bodies in the diagnosis and histologic features of NASH using semi-quantitative and quantitative methods.

Two of the interesting results are the lack of association with either the amounts of steatosis or the stage of fibrosis. Both of these can be explained by current concepts of fatty liver disease. The first is that steatosis itself is not necessarily the “offending” agent in the progressive liver disease known as NASH and that subjects with steatosis only have very little risk of progression. The second is more complex. On the one hand, once fibrosis is advanced, the lesions of activity may be gone, thus resulting in the lesion referred to as “cryptogenic cirrhosis”. On the other hand, during early stages of fibrosis, the association of

ABI with only lobular inflammation may be indicative that activity is multifactorial and includes not only hepatocytes necrosis and inflammatory pathway activation, but also apoptotic pathway activation.

The concept of diagnostic criteria for NASH continues to evolve, but steatosis, ballooning, and lobular inflammation are considered the common constellation of minimal criteria for the diagnosis of NASH[4]. The “NASH CRN” or NAFLD activity score[6], modified from the Brunt scheme [7], maintain the above features, and is widely utilized in clinical trials that considered reduction in NAS as an important marker of improvement in outcome after therapeutic modalities [13, 14]. Acidophil bodies were not included as minimal diagnostic criteria at the outset of the NASH CRN study[6]. It has to be noted acidophil bodies were evaluated in a qualitative method in this study, i.e., they were assessed as “none to rare” and “many,” but not in a quantitative manner. Such categorization may only cover a small portion of the range of the acidophil bodies (at the bottom of the scale). Given the growing evidence demonstrating that hepatocytic cell death is a key process involved in NASH pathogenesis, and both the extrinsic and the intrinsic apoptosis pathways have been implicated [11, 12, 15, 16] we investigated whether the density of acidophil bodies may serve as ancillary evidence of such.

In all 157 patients, ABI was significantly greater in the group with diagnosis of definite NASH than in the groups with diagnosis of borderline or definitely not NASH combined. There was also a trend in both the adult and the pediatric cohorts. These results may have implication in the diagnosis of NASH, as the diagnosis of steatohepatitis relies solely on histology, and pathologists’ observation of ballooned hepatocytes, which are critical element for the diagnosis, may vary depending on thresholds for calling a particular cell ballooned [17]. Hence, in the background of steatosis and lobular inflammation, yet with rare or equivocal ballooned hepatocytes, the observation of increased lobular acidophil bodies may serve as a marker for pathologists to make a more confident diagnosis of NASH, or to search more diligently for ballooned hepatocytes, either by H&E or immunohistochemical stain as mentioned in Introduction. It is important to note that at present the results of this study do not replace the role of ballooned hepatocytes in the diagnostic criteria and scoring system of NASH proposed and validated by NASH CRN [6].

Apoptotic bodies are commonly seen in viral hepatitis, such as CMV hepatitis and hepatitis B and C, particularly in acute hepatitis C or early phase of recurrent hepatitis C after liver transplantation. Several earlier human and animal studies have shown that apoptosis is a crucial response to liver injury in NASH. For example, Wieckowska et al. have demonstrated the detection of cytokeratin-18 cleavage products resulting from caspase 3 activation in the blood is a strong and independent predictor of NASH in human subjects[18]. In Feldstein’s study, the expression of Fas, which is a death receptor member (the so-called extrinsic apoptosis pathway) of the TNFR family, increases in experimental models of NASH and leads to increase sensitivity to Fas-mediated apoptosis[15]. Farrell et al have also demonstrated the extent of apoptosis was significant during evolution of steatosis to steatohepatitis by both TUNEL and active caspase 3 immunohistochemistry assays[11]. Recent studies have also shown cell death is closely regulated by the Bcl-2 protein family and a few of its members have been recently implicated in NASH pathogenesis [19, 20].

These studies all suggest apoptosis is an important form of hepatocytic cell death in both experimental and human NASH. Elucidation of the mechanisms to cell death remains an important research arena in NASH, and may have implications in the development of novel therapeutic modalities.

The association between acidophil bodies and lobular inflammation was significant when adult and pediatric subjects were combined. NASH is an inflammatory disorder[1] in which cytokines have long been suspected of inducing liver injury[21, 22]and lobular inflammation is considered one of the minimal criteria for the histologic diagnosis of NASH [6]. The cellular mechanisms involved in inflammatory cell recruitment and hepatocyte injury in NASH are not well understood, but the roles for oxidant stress, mitochondrial injury, pro-inflammatory cytokines and lipotoxicity have all been suggested [23]. Earlier animal study suggested that liver injuries and inflammatory recruitment in methionine choline diet (MCD)-induced steatohepatitis depends on activation of nuclear factor-kappaB(NF-kB) and c-junN-terminal kinase-1 (JNK-1)[24, 25], which is consistent with activation of cytokine death-receptors. Because all death receptors cause apoptosis by activating NF-kB and JNK-1, it is therefore plausible to deduce acidophil bodies are associated with the lobular inflammation component in NAS. Such mechanistic understanding of hepatocellular injury and inflammatory recruitment in NASH is important, as it may have implication for the development of targeted therapeutic interventions.

The association between apoptosis and ballooned hepatocytes is intriguing. The latter has been considered as a crucial form of liver cell injury for pathologists to recognize in NASH. It is characterized primarily by cytoplasmic alterations, as well as swelling and enlargement of hepatocytes, resulting in loss of its normal hexagonal shape. The cytoplasm is rarefied or finely reticulated; this may be the result of accumulation of intracellular fluid, ultrastructural level of minute vacuoles of steatosis and/or other acute or chronic toxic cell injuries [26]. An earlier study provided the groundwork that has led to the recognition that hepatocytic ballooning is a significant histologicalfeature for distinguishing progressive NAFLD from the less aggressive forms[5]. Nonetheless, definitive histologic identification of ballooned hepatocytes may be challenging, especially when they are borderline. While immunostaining showing loss of the normal hepatocytic keratin 8/18 may serve as a marker of ballooned hepatocytes [27], such immunohistochemical technique may not be routinely performed by pathologists practiced in the general laboratories and the interpretation may also vary among observers. Associations between ballooning and apoptosis through keratin metabolism and depletion, as well as through the endoplasmic reticulum stress response have been suggested [28]. In our study, the acidophil bodies are typically observed in the centrilobular region, similar to the location of ballooned hepatocytes. Whether apoptosis may promote hepatocellular ballooning, or vice versa needs further investigation.

The results of absolute acidophil body counts, acidophil body index and semi-quantitative assessment are consistent, in which they all associated with lobular inflammation, ballooning, and the diagnosis in NASH, but not with steatosis. Steatosis is the morphologic manifestation of triglyceride deposition in the hepatocytes, which has been thought to play the role of the first hit in the pathogenesis of, whereas inflammation, apoptosis, and ballooning are thought to be the phenomenon recruited by the subsequent hits [8], therefore

acidophil bodies and steatosis may reflect morphologic changes at different phases of NASH and hence it is not surprising acidophil bodies may not be necessarily associated with steatosis.

In conclusion, our results show that an increase in its number or density (ABI) of acidophil body, while currently not a prerequisite for diagnosing NASH, may alert the pathologists and elevate their level of suspicion for NASH when other histologic features are equivocally present. Our data also further support and validate the previous experimental and clinical studies demonstrating that the apoptotic pathway is involved in NASH-associated liver injury[11, 12]. Further investigation is warranted to delineate the mechanisms for potential therapeutic intervention in NASH.

Acknowledgments

NIDDK Sponsored NASH Clinical Research Network (NASH-CRN) and NASH-CRN

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Source of funding

The Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN) is supported by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) (grants U01DK061718, U01DK061728, U01DK061731, U01DK061732, U01DK061734, U01DK061737, U01DK061738, U01DK061730, U01DK061713). Additional support is received from the National Center for Advancing Translational Sciences (NCATS) (grants UL1TR000439, UL1TR000077, UL1TR000436, UL1TR000150, UL1TR000424, UL1TR000006, UL1TR000448, UL1TR000040, UL1TR000100, UL1TR000004, UL1TR000423, UL1TR000058, UL1TR000454, UL1TR000040).

Figure 1.

Acidophil bodies (arrows) are present in the background of steatohepatitis (A, 200X magnification). Under higher magnification, acidophil bodies (arrows) are characterized by eosinophilic and shrunken cytoplasm and pyknotic nuclei that may eventually disappear (B, 400X magnification). Acidophil bodies (black arrows) are commonly present with ballooned hepatocytes (C, blank arrows, 400X magnification), and lobular inflammatory infiltrates (D, white arrows, 400X magnification) that are composed of lymphocytes and Kupffer cells.

A

B

C

D **Figure 2.**

Beam plots of absolute acidophil body counts by semiquantitative assessment of (A) steatosis, (B) ballooning, (C) lobular inflammation, and (D) diagnosis of steatohepatitis (N=157). Note: The dots represent individual data points, the line extends from the minimum to maximum of each group, and the red triangle indicates the mean acidophil count.

Table 1

Baseline characteristics by semiquantitative acidophil body assessment in NASH CRN patients (N=157)^a

	Acidophil body assessment in entire biopsy				
	0 (n=94)	1 (n=34)	2 (n=29)	Total (n=157)	P value ^b
Age at biopsy (years)	41.4 ± 17.8	36.3 ± 17.7	45.8 ± 18.4	41.1 ± 18.0	0.10
Adult (≥ 18 years)	76 (80.9)	25 (73.5)	26 (89.7)	127 (80.9)	0.28
Male gender	46 (48.9)	16 (47.1)	10 (34.5)	72 (45.9)	0.40
Race: White vs. non-white	77 (81.9)	30 (88.2)	25 (86.2)	132 (84.1)	0.79
Hispanic/Latino	16 (17.0)	5 (14.7)	8 (27.6)	29 (18.5)	0.37
BMI (kg/m ²)	34.2 ± 6.6	37.2 ± 7.4	32.3 ± 5.0	34.5 ± 6.7	0.02 *
AST (U/L)	45 (33–68)	47 (36–66)	63 (39–76)	47 (34–69)	0.20
ALT (U/L) [‡]	74 (47–101)	79 (55–107)	81 (37–116)	74 (47–104)	0.58
Triglycerides (mg/dL)	134 (101–197)	159 (114–239)	165 (109–234)	149 (107–208)	0.22
Total cholesterol (mg/dL)	192 (170–223)	185 (166–228)	212 (169–222)	193 (169–223)	0.65
Glucose (mg/dL)	91 (82–103)	95 (83–103)	93 (84–102)	92 (83–103)	0.76
Insulin (μU/mL)	17 (11–29)	18 (14–36)	19 (12–30)	17 (12–29)	0.33
HOMA-IR	3.8 (2.5–6.4)	4.8 (3.2–7.5)	4.5 (3.1–7.1)	4.3 (2.9–6.7)	0.29
Area of biopsy (mm ²)	25.3 ± 6.9	24.9 ± 7.5	28.6 ± 9.8	25.8 ± 7.7	0.47

^aValues are N (%), means ± SD, or medians (IQR).

^bP-values derived from Fisher's exact test for categorical variables, and from Kruskal-Wallis Test for age at biopsy, BMI, and laboratory measures.

Abbreviations: NASH: nonalcoholic steatohepatitis; CRN: Clinical Research Network; BMI: body mass index; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; HOMA-IR: homeostasis model assessment of insulin resistance.

* Statistically significant

Table 2

Baseline characteristics by semiquantitative acidophil body assessment in NASH CRN patients – Adult patient biopsies (N=127) ^a

	Acidophil body assessment in entire biopsy				
	0 (n=76)	1 (n=25)	2 (n=26)	Total (n=127)	P value ^b
Age at biopsy (years)	48.4 ± 11.6	44.6 ± 12.5	49.8 ± 14.7	47.9 ± 12.5	0.25
Male gender	28 (36.8%)	11 (44.0%)	8 (30.8%)	47 (37.0%)	0.64
Race: White vs. non-white	62 (81.6%)	21 (84.0%)	22 (84.6%)	105 (82.7%)	1.00
Hispanic/Latino	5 (6.6%)	1 (4.0%)	6 (23.1%)	12 (9.5%)	0.04 [*]
BMI (kg/m ²)	34.2 ± 6.5	37.3 ± 7.6	33.0 ± 4.8	34.5 ± 6.5	0.08
AST (U/L)	42 (33–67)	46 (40–64)	56 (37–76)	46 (33–69)	0.28
ALT (U/L)	68 (42–99)	65 (55–96)	68 (36–107)	67 (42–100)	0.63
Triglycerides (mg/dL)	146 (113–198)	160 (114–239)	161 (109–234)	158 (111–216)	0.40
Total cholesterol (mg/dL)	195 (175–224)	187 (169–228)	209 (169–223)	196 (170–223)	0.84
Glucose (mg/dL)	93 (84–106)	97 (86–111)	95 (83–102)	94 (84–106)	0.41
Insulin (μU/mL)	15 (11–24)	17 (13–24)	17 (12–25)	16 (11–25)	0.29
HOMA-IR	3.3 (2.3–6.0)	4.3 (3.0–6.6)	4.4 (2.9–6.3)	3.7 (2.5–6.3)	0.25
Area of biopsy (mm ²)	26.7 ± 6.8	24.9 ± 7.9	29.1 ± 10.2	26.5 ± 7.9	0.59

^a Values are N (%), means ± SD, or medians (IQR).

^b P-values derived from Fisher's exact test for categorical variables, and from Kruskal-Wallis Test for age at biopsy, BMI, and laboratory measures.

Abbreviations: NASH: nonalcoholic steatohepatitis; CRN: Clinical Research Network; BMI: body mass index; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; HOMA-IR: homeostasis model assessment of insulin resistance.

^{*} Statistically significant

Table 3

Baseline characteristics by semiquantitative acidophil body assessment in NASH CRN patients – Pediatric patient biopsies (N=30) ^a

	Acidophil body assessment in entire biopsy				
	0 (n=18)	1 (n=9)	2 (n=3)	Total (n=30)	P value ^b
Age at biopsy (years)	12.1 ± 2.7	13.3 ± 3.1	11.0 ± 2.6	12.4 ± 2.8	0.52
Male gender	18 (100.0)	5 (55.6)	2 (66.7)	25 (83.3)	0.008 [*]
Race: White vs. non-white	15 (83.3%)	9 (100.0%)	3 (100.0%)	27 (90.0%)	0.66
Hispanic/Latino	11 (61.1%)	4 (44.4%)	2 (66.7%)	17 (56.7%)	0.76
BMI (kg/m ²)	34.1 ± 7.5	36.8 ± 7.2	26.6 ± 2.7	34.2 ± 7.5	0.11
AST (U/L)	50 (40–73)	65 (35–81)	69 (66–118)	56 (40–81)	0.28
ALT (U/L)	80 (66–114)	87 (69–142)	177 (109–212)	83 (68–132)	0.14
Triglycerides (mg/dL)	150 (112–319)	142 (120–173)	186 (88–248)	128 (97–186)	0.34
Total cholesterol (mg/dL)	187 (157–215)	173 (162–210)	213 (166–215)	185 (162–215)	0.80
Glucose (mg/dL)	85 (80–90)	83 (78–87)	89 (85–95)	85 (80–90)	0.44
Insulin (μU/mL)	29 (23–45)	29 (20–52)	34 (20–95)	29 (22–44)	0.78
HOMA-IR	6.3 (4.8–7.6)	6.0 (3.8–10.0)	7.1 (4.4–22.3)	6.5 (4.4–8.4)	0.78
Area of biopsy (mm ²)	21.7 ± 6.3	24.9 ± 6.6	23.7 ± 3.2	22.8 ± 6.2	0.22

^a Values are N (%), means ± SD, or medians (IQR).

^b P-values derived from Fisher's exact test for categorical variables, and from Kruskal-Wallis Test for age at biopsy, BMI, and laboratory measures.

Abbreviations: NASH: nonalcoholic steatohepatitis; CRN: Clinical Research Network; BMI: body mass index; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; HOMA-IR: homeostasis model assessment of insulin resistance.

^{*} Statistically significant

Table 4

Acidophil body counts with histologic features of NASH (N=157)

Histologic Feature reviewed by NASH-CRN	Acidophil body Counts			Acidophil body count/area (ABI) ^a		
	Mean	SD	p ^b	Mean	SD	p ^b
Steatosis grade			0.93			0.89
0-1 (n=52)	0.85	1.30		0.04	0.06	
2-3 (n=105)	0.87	1.85		0.03	0.07	
Lobular inflammation			0.01 *			0.01 *
<2 (n=62)	0.52	1.00		0.02	0.04	
2+ (n=95)	1.08	1.98		0.04	0.08	
Ballooning score			0.02 *			0.048 *
None/few (n=94)	0.66	1.26		0.03	0.05	
Many (n=63)	1.16	2.15		0.05	0.08	
Fibrosis stage			0.66			0.61
0 (n=32)	0.78	1.26		0.04	0.06	
1-2 (n=95)	0.94	1.96		0.04	0.08	
3-4 (n=30)	0.70	1.06		0.03	0.05	
Mallory-Denk bodies			0.71			0.71
Rare/absent (n=125)	0.84	1.79		0.03	0.07	
Many (n=32)	0.94	1.19		0.04	0.06	
Microgranulomas			0.14			0.17
No (n=16)	0.38	0.81		0.02	0.04	
Yes (n=141)	0.91	1.75		0.04	0.07	
Pigmented macrophages			0.31			0.41
Rare/absent (n=11)	0.45	0.93		0.02	0.05	
Many (n=146)	0.89	1.73		0.04	0.07	
Nonalcoholic steatohepatitis			0.007 *			0.02 *
No/suspicious (n=63)	0.51	1.09		0.02	0.05	
Definite (n=94)	1.10	1.96		0.04	0.08	
NAFLD Activity Score (NAS)			0.06			0.09

Histologic Feature reviewed by NASH-CRN	Acidophil body Counts			Acidophil body count/area (ABI) ^a		
	Mean	SD	P ^b	Mean	SD	P ^b
	<4 (n=34)	0.47	1.26	0.02	0.06	
4 (n=123)	0.97	1.77		0.04	0.07	

^aAcidophil count/area: Acidophil body count/mm².

^bP-value for equality of Poisson counts, corrected for over dispersion

Abbreviations: NASH: nonalcoholic steatohepatitis; CRN: Clinical Research Network; ABI: acidophil body index; NAFLD: nonalcoholic fatty liver disease.

* Statistically significant

Table 5
Acidophil body counts and ABI with histologic features of NASH – Adult patient biopsies (N=127)

Histologic Feature reviewed by NASH-CRN	Acidophil body counts			Acidophil body count/area ^a		
	Mean	SD	p ^b	Mean	SD	p ^b
Steatosis grade			0.50			0.35
0–1 (n=44)	0.91	1.39		0.04	0.06	
2–3 (n=83)	0.76	1.23		0.03	0.05	
Lobular inflammation			0.09			0.10
<2 (n=51)	0.59	1.08		0.03	0.05	
2+ (n=76)	0.96	1.39		0.04	0.06	
Ballooning score			0.10			0.15
None/few (n=69)	0.65	1.30		0.03	0.06	
Many (n=58)	1.00	1.24		0.04	0.05	
Fibrosis stage			0.74			0.78
0 (n=25)	0.68	1.11		0.03	0.06	
1–2 (n=74)	0.88	1.41		0.03	0.06	
3–4 (n=28)	0.75	1.08		0.03	0.05	
Mallory-Denk bodies			0.49			0.41
Rare/absent (n=95)	0.77	1.32		0.03	0.05	
Many (n=32)	0.94	1.19		0.04	0.06	
Microgranulomas			0.13			0.18
No (n=16)	0.38	0.81		0.02	0.04	
Yes (n=111)	0.87	1.33		0.03	0.06	
Pigmented macrophages			0.51			0.59
Rare/absent (n=9)	0.56	1.01		0.03	0.05	
Many (n=118)	0.83	1.30		0.03	0.05	
Nonalcoholic steatohepatitis			0.03 [*]			0.05
No/suspicious (n=43)	0.49	1.22		0.02	0.05	
Definite (n=84)	0.98	1.29		0.04	0.05	
NAFLD Activity Score (NAS)			0.11			0.15

Histologic Feature reviewed by NASH-CRN	Acidophil body counts		Acidophil body count/area ^a	
	Mean	SD	Mean	P ^b
<4 (N=27)	0.48	1.40	0.02	0.06
4 (N=100)	0.90	1.24	0.04	0.05

^aAcidophil count/area: Acidophil body count/mm².

^bP-value for equality of Poisson counts, corrected for over dispersion

Abbreviations: NASH: nonalcoholic steatohepatitis; CRN: Clinical Research Network; ABI: acidophil body index; NAFLD: nonalcoholic fatty liver disease.

* Statistically significant

Table 6
Acidophil body counts and ABI with histologic features of NASH – Pediatric patient biopsies (N=30)

Histologic Feature reviewed by NASH-CRN	Acidophil body counts			Acidophil body count/area ^a		
	Mean	SD	p ^b	Mean	SD	p ^b
Steatosis grade			0.35			0.37
0–1 (n=8)	0.50	0.53		0.02	0.03	
2–3 (n=22)	1.27	3.30		0.05	0.13	
Lobular inflammation			0.09			0.09
<2 (n=11)	0.18	0.40		0.006	0.01	
2+ (n=19)	1.58	3.49		0.07	0.14	
Ballooning score			0.02 *			0.02 *
None/few (n=25)	0.68	1.14		0.03	0.05	
Many (n=5)	3.00	6.71		0.12	0.26	
Fibrosis stage			1.00			1.00
0 (n=7)	1.14	1.77		0.05	0.09	
1–2 (n=21)	1.14	3.26		0.05	0.13	
3–4 (n=2)	0.00	0.00		0.00	0.00	
Mallory-Denk bodies			n/c			n/c
Rare/absent (n=30)	1.07	2.84		0.04	0.11	
Many (n=0)	--	--		--	--	
Microgranulomas			n/c			n/c
No (n=0)	--	--		--	--	
Yes (n=30)	1.07	2.84		0.04	0.11	
Pigmented macrophages			1.00			1.00
Rare/absent (n=2)	0.00	0.00		0.00	0.00	
Many (n=28)	1.14	2.93		0.05	0.12	
Nonalcoholic steatohepatitis			0.04 *			0.05
No/suspicious (n=20)	0.55	0.76		0.02	0.03	
Definite (n=10)	2.10	4.79		0.09	0.19	
NAFLD Activity Score (NAS)			0.34			0.42

Histologic Feature reviewed by NASH-CRN	Acidophil body counts			Acidophil body count/area ^a		
	Mean	SD	P ^b	Mean	SD	P ^b
	<4 (n=7)	0.43	0.53	0.02	0.03	
4 (n=23)	1.26	3.22		0.05	0.13	

^aAcidophil count/area: Acidophil body count/mm².

^bP-value for equality of Poisson counts, corrected for over dispersion

Abbreviations: ABI: acidophil body index; NASH: nonalcoholic steatohepatitis; CRN: Clinical Research Network; NAFLD: nonalcoholic fatty liver disease.

* Statistically significant