Human Pathology (2019) **86**, 129–135

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

Hepatic mast cell concentration directly correlates to stage of fibrosis in NASH☆

Jamie Lombardo MD[a,*], Devin Broadwater MD[a], Ryan Collins MD[a], Katherine Cebe MD[a], Robert Brady MD[a], Stephen Harrison MD[b]

[a]*Department of Pathology and Laboratory Services, San Antonio Military Medical Center, Fort Sam Houston, TX*
[b]*Pinnacle Clinical Research, San Antonio, TX*

Received 6 September 2018; revised 21 November 2018; accepted 30 November 2018

Keywords:
Mast cells;
Hepatic fibrosis;
NASH;
NAFLD;
Tryptase

Summary Mast cells are present throughout the body in low numbers. We know their role in immediate hypersensitivity and the subsequent tissue damage due to release of cytokines, vasoactive amines, and lipid mediators when mast cells are activated. Recent research has found that there is an association between an increased concentration of mast cells in the liver and the severity of hepatic fibrosis in animal models. We currently don't understand the role of mast cells in the liver with regard to fibrosis. This retrospective review study investigated whether there is a correlation between stages of fibrosis and mast cell concentrations. One hundred six tissue slides were collected from a large military hospital of known cases of unremarkable liver, non-alcoholic fatty liver disease (Non-NASH NAFLD), and each stage of NASH (Non-alcoholic steatohepatitis). These were analyzed by staining the slides with tryptase to highlight and quantify the mast cell concentration in each diagnostic category. Three pathologists counted mast cells in five 400× fields (1 square mm) in both the periportal and parenchymal regions of each slide. These numbers were recorded and analyzed with a *t* test, demonstrating an increase in mast cells in NASH stage 3–4 fibrosis compared to unremarkable liver (35.48 versus 18.23, respectively, $P < .001$) and a direct correlation ($r = 0.287$) between the number of mast cells and the stage of fibrosis. Better characterizing the role of mast cells in the development of hepatic fibrosis gives us a greater understanding of the pathophysiology of non-NASH NAFLD and NASH and possibly a pharmaceutical target.
Published by Elsevier Inc.

1. Introduction

Obesity and metabolic syndrome are increasing worldwide, leading to an increase in obesity related illnesses including fatty liver disease. Fat deposition in the liver secondary to obesity can manifest as non-alcoholic fatty liver disease (NAFLD) [1]. Histologic categories of NAFLD include simple steatosis, steatosis with inflammation, and steatohepatitis with or without fibrosis. Nonalcoholic steatohepatitis (NASH) is characterized by hepatocyte injury in the form of ballooning

☆ Disclosures: The authors declare no financial interest or conflict of interest. The view(s) expressed herein are those of the author(s) and do not reflect the official policy or position of Brooke Army Medical Center, the US Army Medical Department, the US Army Office of the Surgeon General, the Department of the Air Force, the Department of the Army, the Department of Defense or the US Government.

* Corresponding author at: 3551 Roger Brooke Drive, Fort Sam Houston, TX 78108.
E-mail address: Jamie.l.lombardo.mil@mail.mil (J. Lombardo).

https://doi.org/10.1016/j.humpath.2018.11.029
0046-8177/Published by Elsevier Inc.

degeneration with or without Mallory-Denk bodies. This hepatocyte injury can eventually lead to progressive stages of fibrosis and, ultimately, cirrhosis [2]. Early hepatic fibrosis is a reactive scarring response to liver damage that impairs liver function. Cirrhosis is the most advanced stage of fibrosis in which the liver parenchyma becomes fragmented into nodules that are separated from each other by bands of scarring fibrosis [3].

Guidelines published in 2012 by the World Gastroenterology Organization suggested that the world prevalence of NAFLD had doubled over the previous 20 years; at that time, up to 34% of Americans had some form of NAFLD [4]. More recent sources estimate the current number of NAFLD cases in the United States to be closer to 40% [5].

NAFLD is a condition in which excess fat is stored in the liver, not related to alcohol use. NASH is a form of NAFLD in which inflammation and hepatocyte damage is present alongside the fat deposition in the liver. Inflammation and liver cell damage then causes fibrosis, which may advance to cirrhosis [5]. It's not well understood why steatosis becomes steatohepatitis but there is a 2-hit theory that the first hit is due to insulin resistance which causes deposition of adipose and the second hit causes hepatocyte injury, inflammation, and fibrosis [6,7]. The multiparallel hypothesis proposed more recently suggests that NASH is the result of numerous conditions acting in parallel, including genetic predisposition, abnormal lipid metabolism, oxidative stress, lipotoxicity, mitochondrial dysfunction, altered production of cytokines and adipokines, gut dysbiosis, and endoplasmic reticulum stress [8,9].

Previous studies have induced liver steatosis and fibrosis through chemical means in rats and hamsters and incidentally noted increased mast cells [7,10,11]. One study then treated the hamsters that had chemically induced steatosis with a chymase inhibitor, TY-51469. Chymase is a chymotrypsin-like serine protease that is abundant in secretory granules of mast cells [12]. After treatment, these hamsters had a decrease in liver fibrosis, steatosis, and hepatic mast cells, showing that blocking mast cell mediators may alter the course of fatty liver disease [7,11].

Mice genetically deficient in mature mast cells have been found to be resistant to diet induced obesity. They also have a decrease in inflammatory cytokines, chemokines and macrophages in their adipose tissue, indicating that mast cells precede the proinflammatory mediators [13]. A study by Divoux et al discovered that mast cells are activated in human adipose tissue [14]. Upon activation, mast cells secrete an array of biologically active mediators. This release occurs within minutes from pre-formed granules which can be completely or partially emptied through exocytosis. These granules contain numerous biologically active compounds, including histamine, lipid mediators, proteoglycans, and proteases like chymases, tryptases, and carboxypeptidases. Activated mast cells can also generate eicosanoids by enzymatic biosynthesis from arachidonic acid. They can synthesize and release cytokines, chemokines and growth factors to include IL-3, IL-4, IL-5, IL-10, IL-13, GM-CSF, TNF-a, TGF-B, CCL2, CCL5, FGF, and PDGF which occurs over a longer time frame of hours rather than minutes [15]. These factors attract other inflammatory cells and cause collateral damage to surrounding tissue which leads to scar formation and fibrosis.

Although examined in animal models; to date, there have been no studies of mast cell concentrations in human patients with varying levels of fibrosis in fatty liver disease acquired through natural pathologic processes. This is valuable information as important differences exist between animal models and humans, including the brief timeline to developing disease as induced by chemicals and the confounding factors present in humans, such as variable diet and alcohol intake.

There are currently no pharmacotherapies for treatment of non-NASH NAFLD and NASH that have shown long term efficacy [16]. Instead, the only treatments offered to these patients are to make lifestyle changes or seek bariatric surgery. Once the liver fibrosis becomes so severe that the patient is experiencing organ failure, the only option is a liver transplant. Patients often have difficulty with the lifestyle changes, and not everyone is a candidate for bariatric surgery. Moreover, an appropriate liver transplant may or may not become available so identifying a target for pharmacotherapy that could decrease the progression of liver fibrosis in non-NASH NAFLD and NASH is very desirable.

2. Materials and methods

This retrospective review was approved by the institutional review board before any research activities were conducted. This study was a randomized, single blinded, retrospective review of tissue samples collected and stored at a large military hospital from January 1st, 2015 to January 31st, 2017. Cases were identified through the electronic pathology database at our institution (CoPath, by Cerner, Kansas City, MO) using the following terms, "unremarkable liver", "NAFLD", "NASH stage 0-1", "NASH stage 2", and "NASH stage 3-4". The diagnostic categories were determined as follows: non-NASH NAFLD with or without inflammation and without balloon cells and NASH was defined as steatosis with inflammation and ballooning degeneration of hepatocytes. The stages of fibrosis and the grade of steatohepatitis were determined using the Brunt staging criteria [17]. Lobular inflammation, and ballooning degeneration was graded based on the NAFLD Activity Score from the Brunt system [17]. Lobular inflammation was counted in 200× fields averaged over the length of the core biopsy and graded 0 if no foci of inflammation were seen, 1 if <2 foci of lobular inflammation were seen, 2 if 2–4 foci were present, and 3 if >4 foci were present. The unremarkable liver cases had no chronic diseases, normal liver lab studies, normal imaging, and were negative for pathologic changes. The selected cases met the following inclusion criteria; they had to have a diagnosis of unremarkable liver, non-NASH NAFLD, NASH with stage 0–1 fibrosis, NASH with stage 2 fibrosis, or NASH with stage 3–4 fibrosis.

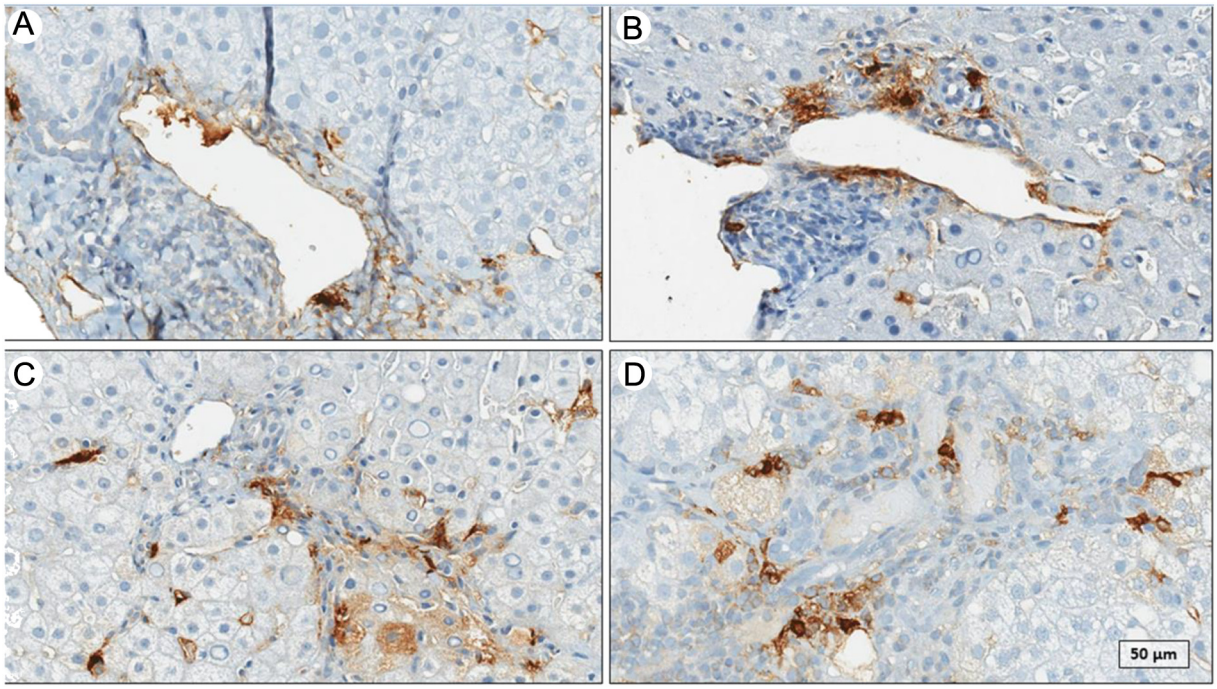

Fig. 1 Representative photomicrograph of tryptase immunohistochemical staining at 400× in liver portal tracts. A, Tryptase IHC at 400×. Portal tract in unremarkable liver. B, Tryptase IHC at 400×. Portal tract in NASH stage 0–1. C, Tryptase IHC at 400×. Portal tract in NASH stage 2. D, Tryptase IHC at 400×. Portal tract in NASH stage 3–4.

Patients were all between ages 18 and 80 and did not have any immunodeficiency, whether it was disease related or drug related. Cases were excluded if the patient had other coexisting liver disease or chronic viral hepatitis, if the remaining paraffin embedded tissue was non-diagnostic or less than 1 square millimeter was present on biopsy, and if the original blocks did

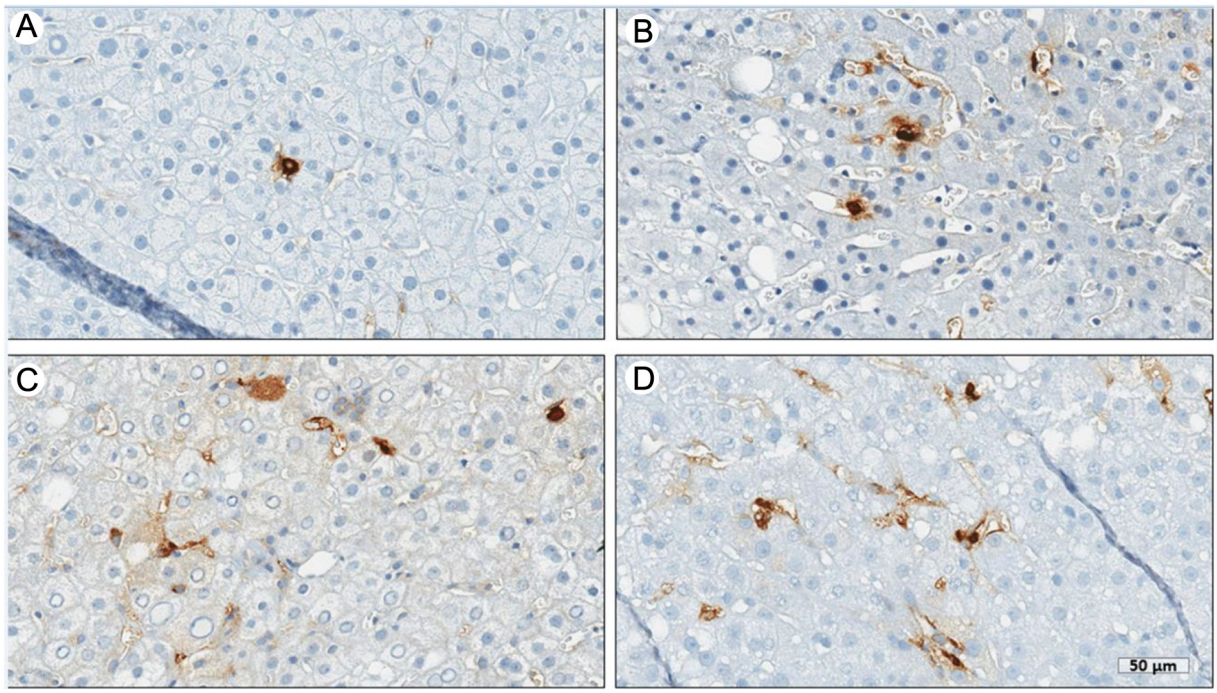

Fig. 2 Representative photomicrograph of tryptase immunohistochemical staining at 400× in liver parenchyma. A, Tryptase IHC, 400×. Unremarkable liver parenchyma. B, Tryptase IHC, 400×. NASH stage 0–1, liver parenchyma. C, Tryptase IHC, 400×. NASH stage 2, liver parenchyma. D, Tryptase IHC, 400×. NASH stage 3–4, liver parenchyma.

Table 1 Patient demographics for cases reviewed

	Mean age	Age Range	Males	Females
Overall	55.72	19–74	54	52
Unremarkable	51.04	19–74	5	5
Non-NASH NAFLD	55.87	34–65	15	10
NASH (Stage 0–1)	51.95	39–67	13	10
NASH (Stage 2)	57.92	23–67	13	11
NASH (Stage 3–4)	58.92	51–71	8	16

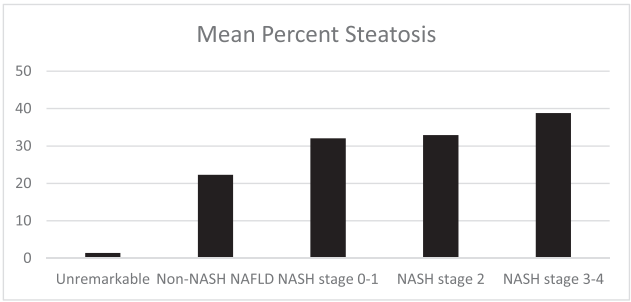

Fig. 3 Mean number of mast cells counted by 3 pathologists in various stages of fibrosis in parenchymal and periportal regions of the liver. Error bars represent ±1 standard deviation.

not have enough tissue for an additional stain to be performed for the study. Each case from the pathology database was cross referenced through the institution's medical record system (AHLTA, by DHA, Falls Church, VA) to determine whether they met inclusion and exclusion criteria. Each case was also reviewed by the same gastrointestinal/hepatic pathologist to confirm the diagnostic category and ensure consistency throughout the study groups.

The associated formalin fixed paraffin embedded tissue blocks were gathered from the on-site tissue storage facility. A slide was cut from each sample and a tryptase immunohistochemical (IHC) stain for mast cells was performed. The tryptase stain was manufactured by Cell Marque from clone G3. Representative photomicrographs of IHC staining were captured on Aperio ImageScope at 400× and are demonstrated in Figs. 1 and 2. All photomicrographs were captured using the Aperio ScanScope AT Turbo (Leica Biosystems, location, CA) at 400× magnification using a 20× FN 26.5 lens with a 2× doubler. Images were processed using the Aperio ImageScope software, version 12.

Three pathologists counted the mast cells in 5 high-power fields involving portal tracts and 5 high-power fields involving the parenchyma on each slide. Counting in 5 high-power (400×) fields gives the total number of mast cells in approximately one square mm of tissue. The total number of mast cells found in the 5 high-power fields from the portal tracts and the parenchyma were recorded. Each pathologist was blinded to the diagnosis and stage of fibrosis for each biopsy and the results of the other counting pathologists. The pathologists were only given the tryptase IHC slide so the amount of fibrosis would not be as apparent as on a hematoxylin and eosin stained slide to avoid bias. At the conclusion of the study, patients who received repeat biopsy had their results reviewed for any changes to the original stage diagnosis.

Continuous data are presented as mean and standard deviation, and independent 2-sample *t* tests were performed to compare the mean between unremarkable liver, non-NASH NAFLD, and NASH with each stage of fibrosis as well as between one stage and the next consecutive stage of fibrosis. A linear regression model was performed to determine correlation between increasing stages of fibrosis and increasing concentration of mast cells. Interobserver reliability was calculated using intraclass correlation coefficient to determine the level of agreement between the 3 pathologists who counted mast cells. Statistical analysis was performed using Microsoft

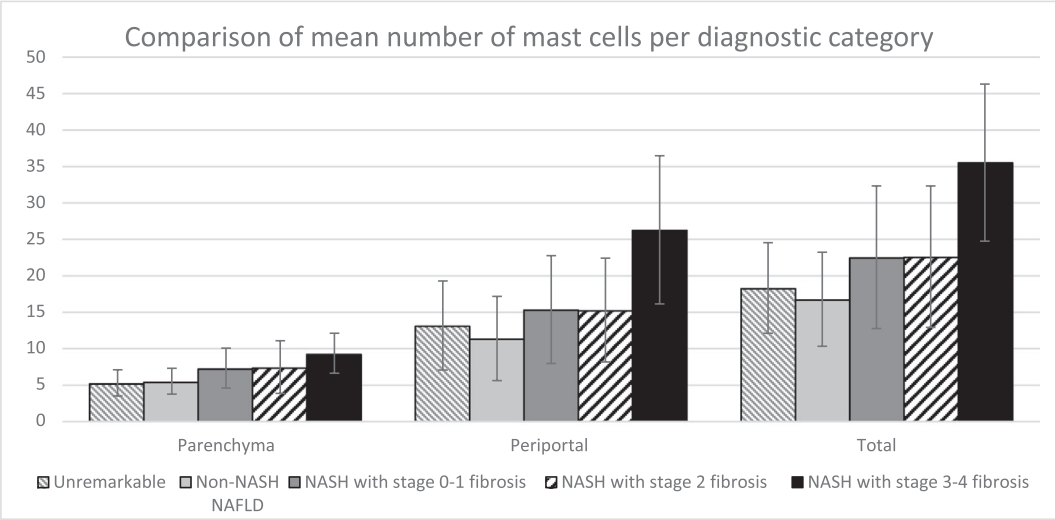

Fig. 4 Mean number of mast cells counted for each diagnosis and region of liver and the associated standard deviation.

Table 2 Mean number of mast cells counted in each region and the statistical significance between the respective region and diagnosis versus NASH stage 3–4

	Unremarkable liver	Non-NASH NAFLD	NASH stage 0–1	NASH stage 2	NASH stage 3–4
Parenchymal	5.17, $P < .001$	5.4, $P < .001$	7.2, $P = .01$	7.35, $P < .05$	9.25
Periportal	13.06, $P < .001$	11.28, $P < .001$	15.26, $P < .001$	15.2, $P < .001$	26.24
Total	18.23, $P < .001$	16.68, $P < .001$	22.46, $P < .001$	22.54, $P < .001$	35.48

Excel 2013 (Redmond, Washington) and SPSS version 22 statistics software.

3. Results

Record search revealed 10 unremarkable controls, 25 non-NASH NAFLD, 23 NASH stage 0–1, 24 NASH Stage 2, and 24 NASH stage 3–4 cases after inclusion and exclusion criteria were met. The mean overall age of the study population was 55.72 years old (range, 19–74 years) with a male to female ratio of 1.04:1. The specific age and male to female ratio for each group is broken down by diagnosis in Table 1.

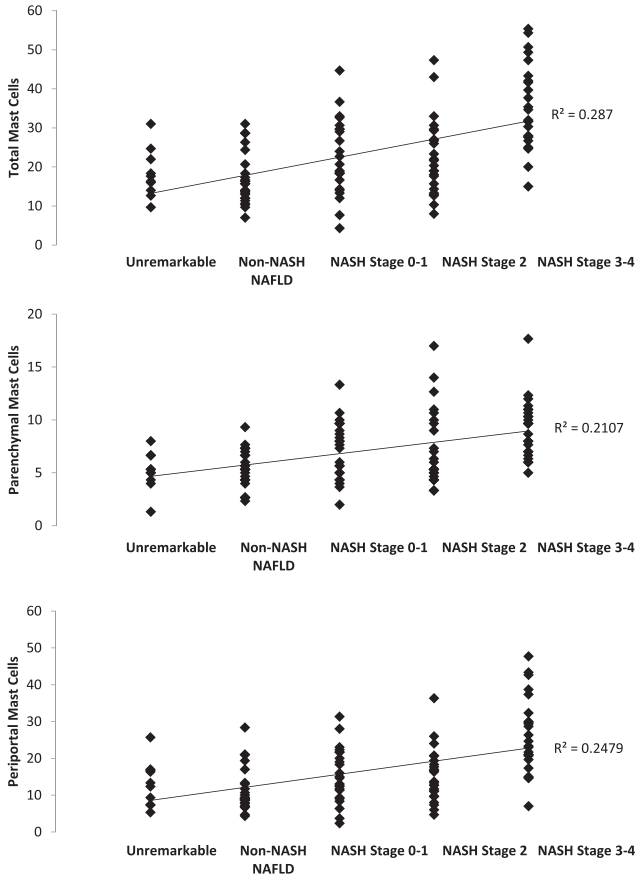

Fig. 5 Linear regression model of the number of mast cells counted compared to the progressive stages of fibrosis and the associated R-squared correlation between data sets.

Seventy-three patients had demographic data on self-reported alcohol use. Only 18 total patients reported alcohol use. Non-NASH NAFLD had the highest number of alcohol use at 9 patients, whereas NASH stage 0–1 only had 3, NASH stage 2 had 4, and NASH stage 3–4 had 2. There was no difference in BMI between each group with an overall mean of 32.5. Patients with unremarkable liver biopsies had a mean BMI of 27.8, non-NASH NAFLD had a mean BMI of 31.8, NASH stage 0–1 had a mean BMI of 32.9, NASH stage 2 had a mean BMI of 32.7, and NASH stage 3–4 had a mean BMI of 34.5.

All patients that were tested for Hepatitis A, B, and C were negative by serology. The mean steatosis increased with increasing fibrosis with NASH stage 3–4 averaging 38.8%, demonstrated in Fig. 3.

Inflammation varied based on diagnosis; however it did not increase with increasing fibrosis in NASH. Biopsies deemed unremarkable all had a lobular inflammation score of 0. Non-NASH NAFLD's mean score was 0.68 (range, 0–2), NASH stage 0–1 mean score was 1.36 (range, 1–2), NASH stage 2 mean score was 1.53 (range, 1–2), and NASH stage 3–4 mean score was 1.3 (range, 1–2).

The mean number of mast cells counted in unremarkable liver parenchyma was 18.23 (5.17 parenchymal and 13.06 periportal) (Fig. 4). The mean number of mast cells counted in non-NASH NAFLD cases was 16.68 (5.4 parenchymal and 11.28 periportal). The mean number of mast cells counted in NASH with stage 0–1 fibrosis was 22.46 (7.2 parenchymal and 15.26 periportal). The mean number of mast cells counted in NASH with stage 2 fibrosis was 22.54 (7.35 parenchymal and 15.2 periportal). The mean number of mast cells in NASH with stage 3–4 fibrosis was 35.48 (9.25 parenchymal and 26.24 periportal). Statistically significant differences were most prominent in unremarkable liver versus NASH stage 3–4. NASH stage 3–4 had a mean total 17.25 mast cells more than unremarkable liver ($P < .001$) in total. Parenchymal and periportal showed a 4.08 and 13.17 increase in total mean mast cells compared to unremarkable liver, respectively ($P < .001$ for both). Parenchymal mast cells allowed for a statistically significant difference between unremarkable liver and NASH stage 0–1 and NASH stage 2 fibrosis, with 2.04 and 2.2 more mast cells in the fibrotic cases, respectively ($P < .02$ and $P < .03$, respectively). Non-NASH NAFLD cases were not statistically different from the unremarkable control in periportal, parenchymal, and in total mast cell counts. NASH Stage 3–4 was significantly different from all other groups (non-NASH NAFLD, NASH stage 0–1 and NASH stage 2), $P < .05$ for

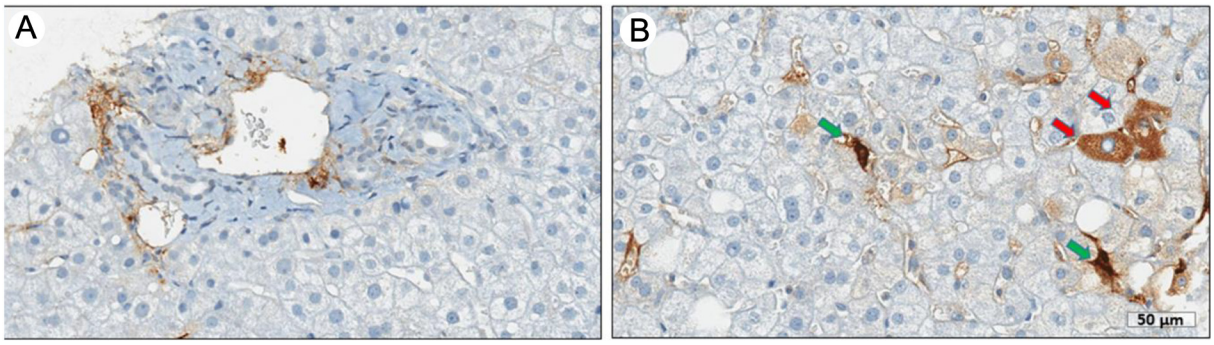

Fig. 6 Representative photomicrograph of tryptase immunohistochemical staining at 400× of non-NASH NAFLD comparing nonspecific staining to definite mast cells illustrating potential pitfalls in stain interpretation. Tryptase IHC of Non-NASH NAFLD liver showing portal tract (A) and Parenchyma (B). Non- specific staining is seen around the portal tract, picked up by endothelial cells, collagen, and hepatocytes. No mast cells were counted in A. The parenchyma (B) shows some mast cells and some nonspecific staining of hepatocytes and sinusoids. Hepatocytes (red arrows) can be distinguished from mast cells by their glycogenated nuclei and polygonal cell shapes. Two mast cells were counted in this field (green arrows).

all comparisons. See Table 2 for a comparison of means and statistical significance of NASH stage 3–4 fibrosis compared to the means of all other categories.

There was no statistical significant difference between NASH stage 0–1 and NASH stage 2 fibrosis. A total of 12 patients of 106 received repeat biopsy at the conclusion of the study. Stage 3–4 accounted for 7 of the biopsies, while the remaining 5 were originally diagnosed stage 2. Mean follow up time was 482 days from original diagnosis (range, 296–832 days). Only 2 patients were recorded to have a change in their stage of fibrosis. A patient with stage 3–4 fibrosis was stage 2 on subsequent biopsy, and a patient with stage 2 fibrosis was stage 3 on subsequent biopsy. Both cases had lower than average mast cell counts in both periportal and parenchymal regions.

Linear regression models for total, parenchymal, and periportal mast cells counted showed correlations as fibrosis increased (R^2 = 0.287, 0.211, 0.250, respectively) demonstrated in Fig. 5.

Interrater reliability calculated as intraclass correlation coefficient between the 3 pathologists had the highest correlation when comparing total mast cells counted (r = 0.73). Periportal mast cell counts were also strongly correlated (r = 0.67). Parenchymal mast cell counts showed weak correlation (r = 0.28).

4. Discussion

The results of our study demonstrated mast cells increase in number as fibrosis increases. This effect is statistically significant and most prominent in NASH stage 3–4 fibrosis. Although it was not statistically significant, the increase in mast cells between unremarkable liver and early fibrosis (stage 0–2) is notable and could likely show significance if a greater number of cases were reviewed. Non-NASH NAFLD cases demonstrated no difference in number of mast cells counted compared to the unremarkable liver control, suggesting mast cells play little role in non-NASH NAFLD's disease process. Parenchymal mast cells provided the only statistically significant indicator in early to late fibrosis, with an average number of 7 or more mast cells in 1 square millimeter suggesting fibrosis. Regardless of where mast cells were found, they only weakly correlated to increased levels of fibrosis at early stages. This is likely secondary to their presence not being prominent until stage 3–4 fibrosis was present. Further, the correlation was limited as number of mast cells was not statistically different between NASH stage 0–1 and NASH stage 2 in all areas of the liver.

The data also demonstrate the increase in mast cells is not directly related to an overall increase in inflammation, as the average NAFLD activity score for lobular inflammation did not increase linearly with increasing fibrosis. This suggests the mast cells provide a better indicator of the degree of NASH than lobular inflammation, which is typically composed of lymphocytes.

At the conclusion of the study, 2 patients of 14 with repeat biopsy showed a change in stage. Both patients had lower than average mast cell counts in all liver regions. Interclass correlation coefficient demonstrated strong agreement in total and periportal mast cell counts between the 3 pathologists. The parenchymal agreement is likely limited to how each pathologist selected the areas to count. Pathologists were to look at 5 random high-power fields and count the mast cells in the parenchyma. Adequate liver core biopsies have abundant parenchyma in terms of high-power fields, making it likely that the 3 counting pathologists all looked at different high-power parenchymal fields when counting mast cells in the parenchyma, especially on larger biopsies.

There were 2 notable limitations in the study. First, the tryptase IHC stain was often difficult to interpret, which is a known issue with non-specific staining with this antibody. A CD117 immunohistochemical stain is much cleaner; however, it also highlights the hepatic stem cells. As both mast cells and hepatic stem cells look like intact cells with the CD117 stain, there is no way to tell them apart. The tryptase stain highlights

the granules in the mast cells and the nonspecific staining is due to degranulation. Fig. 6 demonstrates the tryptase IHC stain.

In order to reduce this limitation, the 3 pathologists used a multiheaded scope to agree on what cells would be counted as intact mast cells and what staining pattern was just degranulation artifact and would not be counted prior to data collection.

A second limitation involves how fields were selected between each pathologist. Because slides were independently reviewed, it is extremely likely each field used was different between each counting pathologist. Despite this limitation, it allows for a greater generalizability of the study, as the means used for statistical analysis likely represent a larger proportion of each case to a greater degree.

5. Conclusion

Previous studies evaluated mast cells in chemically induced steatosis and liver fibrosis in rodents. This study is the first analysis of mast cells and liver fibrosis due to normal pathologic processes occurring in humans. These data support the theory that mast cells play an active role in progression of hepatic fibrosis in humans and identifies a target for pharmacologic therapy. In this study, only 14 patients had received repeat biopsy at the time of this publication and only 2 of these patients changed their stage of fibrosis. One patient increased their stage of fibrosis while the other decreased their stage of fibrosis. No conclusions can be drawn from only 2 cases and they demonstrated no significant correlation to mast cell counts and their change in fibrosis stage. Further research is needed to see if mast cell counts can predict future changes in a patient's fibrosis stage. Additional studies can be performed, evaluating mast cells in other causes of chronic liver disease that lead to fibrosis.

Author Contributions

Jamie Lombardo coordinated IRB approval, performed the study, wrote manuscript, proof-read manuscript, and took photomicrographs. Devin Broadwater performed the study, calculated statistics, wrote results section, built tables and figures, and proof-read manuscript. Ryan Collins performed the study, proof-read manuscript, assisted with Aperio digital imager for collection of photomicrographs, and confirmed statistical analysis with SPSS software. Katherine Cebe assisted with IRB approval, collected and reviewed cases for inclusion in this study, and proof-read manuscript. Robert Brady assisted with IRB approval, collected and reviewed cases for inclusion in this study, and proof-read manuscript. Stephen Harrison assisted with IRB approval, developed the concept for this research, wrote the abstract, and proof-read the manuscript.

Acknowledgements

The authors would like to thank Dr Erica Kao, MD for contributing medical illustrations to the graphical abstract.

No funding or grants were accepted for this research.

References

[1] Juarez-Hernandez E, Chavez-Tapia NC, Uribe M, Barbero-Becerra VJ. Role of bioactive fatty acids in nonalcoholic fatty liver disease. Nutr J 2016;15(1):72.

[2] Rosso N, Chavez-Tapia NC, Tiribelli C, Bellentani S. Translational approaches: from fatty liver to non-alcoholic steatohepatitis. World J Gastroenterol 2014;20(27):9038-49.

[3] Friedman SL. Mechanisms of hepatic fibrogenesis. Gastroenterology 2008;134(6):1655-69.

[4] LeBrecque D, Pakistan ZA, Austria PF, et al. Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis. WGO: World Gastroenterology Organisation; Published June 2012 http://www.worldgastroenterology.org/guidelines/global-guidelines/nafld-nash/nafld-nash-english, Accessed date: 20 November 2018.

[5] Diehl AM, Tetri BA. U.S. Department of Health and Human Services, N. I.o.H. Definition and Facts of NAFLD and NASH. https://www.niddk.nih.gov/health-information/liver-disease/nafld-nash/definition-facts; Published November 2016, Accessed date: 20 November 2018.

[6] James O, Day C. Non-alcoholic steatohepatitis: another disease of affluence. Lancet 1999;353:1634-6.

[7] Jarido V, Kennedy L, Hargrove L, et al. The emerging role of mast cells in liver disease. Am J Physiol Gastrointest Liver Physiol 2017;313(2):G89-G101.

[8] Caligiuri A, Gentilini A, Marra F. Molecular pathogenesis of NASH. Int J Mol Sci 2016;17(9):1575.

[9] Tilg H, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. Hepatology 2010;52(5):1836-46.

[10] Henderson NC, Iredale JP. Liver fibrosis: cellular mechanisms of progression and resolution. Clin Sci (Lond) 2007;112(5):265-80.

[11] Tashiro K, Jin D, Komeda K, et al. Chymase inhibitor prevents the nonalcoholic steatohepatitis in hamsters fed a methionine- and choline-deficient diet. Hepatol Res 2010;40(5):514-23.

[12] Yahiro E, Miura S, Imaizumi S, Uehara Y, Saku K. Chymase inhibitors. Curr Pharm Des 2013;19(17):3065-71.

[13] Ghigliotti G, Barisione C, Garibaldi S, et al. Adipose tissue immune response: novel triggers and consequences for chronic inflammatory conditions. Inflammation 2014;37(4):1337-53.

[14] Divoux A, Moutel S, Poitou C, et al. Mast cells in human adipose tissue: link with morbid obesity, inflammatory status, and diabetes. J Clin Endocrinol Metab 2012;97(9):E1677-85.

[15] Overed-Sayer C, Rapley L, Mustelin T, Clarke DL. Are mast cells instrumental for fibrotic diseases? Front Pharmacol 2013;4:174.

[16] Lam B, Younossi ZM. Treatment options for nonalcoholic fatty liver disease. Therap Adv Gastroenterol 2010;3(2):121-37.

[17] Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis; a proposal for grading and staging the histological lesions. Am J Gastroenterol 1999;94:2467-74.