

Original article

Non-alcoholic fatty liver disease and thrombocytopenia IV: its association with granulocytopenia

Montserrat Rivera-Álvarez^a, Alejandra Carmina Córdova-Ramírez^a,
Gilberto David Elías-De-La-Cruz^a, Iván Murrieta-Álvarez^{a,b},
Andrés Aurelio León-Peña^a, Yahveth Cantero-Fortiz^{c,d},
Juan Carlos Olivares-Gazca^d, Guillermo José Ruiz-Delgado^{a,b,c},
Guillermo José Ruiz-Argüelles^d

^a Universidad Popular Autónoma del Estado de Puebla, Puebla, México

^b Centro de Hematología y Medicina Interna de Puebla, Puebla, México

^c Laboratorios Clínicos de Puebla, Puebla, México

^d Universidad de las Américas Puebla, Puebla, México

ARTICLE INFO

Article history:

Received 1 March 2021

Accepted 13 June 2021

Available online 21 July 2021

Keywords:

Nafld

Platelets

Granulocytopenia

Thrombocytopenia

Liver

ABSTRACT

Introduction: We have previously shown that some patients present thrombocytopenia (less than $100 \times 10^9/L$ platelets) in non-alcoholic fatty liver disease (NAFLD). To further explore the nature of this association, we have now analyzed the association of thrombocytopenia with neutropenia (less than $0.5 \times 10^9/L$ granulocytes) in NAFLD.

Material and methods: Persons with NAFLD were prospectively accrued in the study after February 2018. The presence of NAFLD was defined by both serologic determinations (Fibro-max[®]) and liver transient elastography (TE/Fibroscan[®]).

Results: In 123 consecutive patients with NAFLD without cirrhosis, thrombocytopenia was identified in 20 (16%), whereas neutropenia was identified in 9 (7%). In the subset of 20 patients with NAFLD and thrombocytopenia, granulocytopenia was identified in 5 (25%), whereas in the subset of 9 patients with granulocytopenia, thrombocytopenia was identified in 5 (55%). We found a significant association between thrombocytopenia and both leukopenia and granulocytopenia (OR 8.25, 95% CI 1.9–34.2, $p = 0.004$).

Conclusions: Both thrombocytopenia and neutropenia were identified in persons with NAFLD and, as there is a significant relationship between these two variables, we speculate that this finding may support the possibility of hypersplenism being involved in the cytopenias found in NAFLD without cirrhosis.

© 2021 Associação Brasileira de Hematologia, Hemoterapia e Terapia Celular. Published by Elsevier España, S.L.U. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

* Corresponding author at: Centro de Hematología y Medicina Interna de Puebla, 8B Sur 3710, 72530 Puebla, PUE, Mexico.

E-mail address: gruiz1@hsctmexico.com (G.J. Ruiz-Argüelles).

<https://doi.org/10.1016/j.htct.2021.06.004>

2531-1379/© 2021 Associação Brasileira de Hematologia, Hemoterapia e Terapia Celular. Published by Elsevier España, S.L.U. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver injury worldwide¹; it encompasses a wide spectrum of liver disorders ranging from steatosis, through steatohepatitis and overt liver cirrhosis. Thrombocytopenia (less than $100 \times 10^9/\text{L}$ platelets) has been described in some cases of NAFLD.^{2–8} The definite diagnosis of NAFLD is ideally performed by means of a liver biopsy; however, several non-invasive diagnostic strategies have been employed as diagnostic alternatives, each with different sensitivities and accuracies.^{9–13} Several studies have demonstrated the predictive value and a better benefit-to-risk ratio than biopsy of combinations of simple serum biochemical markers (Fibromax[®])⁹ and/or liver transient elastography (Fibroscan[®]).^{10–13} In previous studies, we have shown that NAFLD, as defined by Fibromax[®]⁵ and/or Fibroscan[®]^{6,7} is associated with thrombocytopenia, in the absence of overt liver cirrhosis. The cause of thrombocytopenia in NAFLD is strictly unknown; several explanations have been offered, such as a certain degree of hypersplenism, bone marrow hypoplasia, reduced peripheral blood cell survival, thrombopoietin deficiency and others.^{5–7} As hypersplenism may be associated with granulocytopenia, we analyzed if granulocytopenia is associated with NAFLD and/or thrombocytopenia.

Material and methods

a) Patients: Individuals were prospectively included in the study after February 2018. The presence of NAFLD was defined by both Fibroscan[®] and Fibromax[®].^{9–13} The study was approved by the Ethics Committee of the Clinica Ruiz and informed consent was obtained from all the patients. Individuals with autoimmune thrombocytopenia, autoimmune diseases, hepatitis B, hepatitis C, chronic cholestatic diseases, overt liver cirrhosis or alcoholism were excluded from the analysis. Persons with anti-granulocyte antibodies, anti-platelet antibodies, overt viral or bacterial infections, spleen enlargement or clinical data of portal hypertension were also excluded from the analysis. The

exclusion criteria were chosen to eliminate other possible causes of cytopenias.

- b) Fibroscan[®]: To assess transient elastography (TE), a Fibroscan 502 Touch (Echosens, France) instrument was used, with the XL probe.^{10–13} The ultrasonic controlled attenuation parameter (CAP) defines steatosis when its value is above 200 decibels per milliwatts (dB/m) (S1–S3), whereas liver stiffness measurement (LSM) defines fibrosis when above 7.5 kgpascals (kPa) (F2–F4).¹⁰ Patients with a CAP value over 200 dB/m coupled with an LSM below 7.5 kPa were defined as individuals with liver steatosis.^{10–13}
- c) Fibromax[®]: Alpha 2 macroglobulin, haptoglobin, apolipoprotein A, bilirubin, gamma glutamyl transpeptidase, alanine aminotransferase, aspartate aminotransferase, glucose, cholesterol and triglycerides were measured in all patients; these biochemical markers were analyzed in various manners to define: the quantitative assessment of fibrosis (FibroTest), steatosis (SteatoTest) and necroinflammatory activity in chronic viral hepatitis (ActiTest) and the categorical diagnosis of nonalcoholic steatohepatitis (NashTest).^{9–11} Patients with a score above 50% in either the SteatoTest or NashTest, coupled with a score below 50% in the FibroTest, were defined as having NAFLD.^{9–11}

Results

In 123 consecutive patients with NAFLD, as defined by both the Fibroscan and Fibromax, thrombocytopenia (less than $100 \times 10^9/\text{L}$ platelets) was identified in 20 (16%), whereas neutropenia (less than $0.5 \times 10^9/\text{L}$ granulocytes) was identified in 9 (7%). In the subset of 20 patients with NAFLD and thrombocytopenia, granulocytopenia was identified in 5 (25%), whereas in the subset of 9 patients with granulocytopenia, thrombocytopenia was identified in 5 (55%), as shown in Table 1. No patient had splenomegaly. There was a statistically significant association between thrombocytopenia and both leukopenia and granulocytopenia (OR 8.25, 95% CI 1.9–34.2, $p = 0.004$) in patients with NAFLD, as shown in Figures 2 and 3. A trend was found for an inverse relationship between the degree of fatty infiltration of the liver, as defined by the

Table 1 – Salient features of the patients with non-alcoholic fatty liver disease (NAFLD) and thrombocytopenia, neutropenia or both. SD = standard deviation, BMI = body mass index.

	NAFLD and thrombocytopenia $n = 20$	NAFLD and neutropenia $n = 9$	NAFLD, thrombocytopenia and neutropenia, $n = 5$	p
Age in years (SD)	42.5 (17.3)	49.3 (7.3)	61.8 (18.2)	0.3
BMI (SD)	31.3 (4.3)	32.5 (5.4)	30.4 (3.1)	0.5
FibroScan score (SD)	1.9 (0.5)	2.2 (0.4)	2.4 (0.8)	0.1
FibroMax score (SD)	1.3 (0.2)	1.4 (0.4)	1.4 (0.3)	0.7
Platelet count, $\times 10^3/\text{L}$ (SD)	79.1 (41)	123 (32)	68.2 (45)	0.01
Neutrophil count, $\times 10^3/\text{L}$ (SD)	2.6 (1.8)	0.7 (0.9)	0.89 (0.5)	0.02
Hemoglobin, g/dl (SD)	13.4 (0.9)	13.1 (1.2)	14.1 (1.1)	0.3
Splenomegaly	0	0	0	–

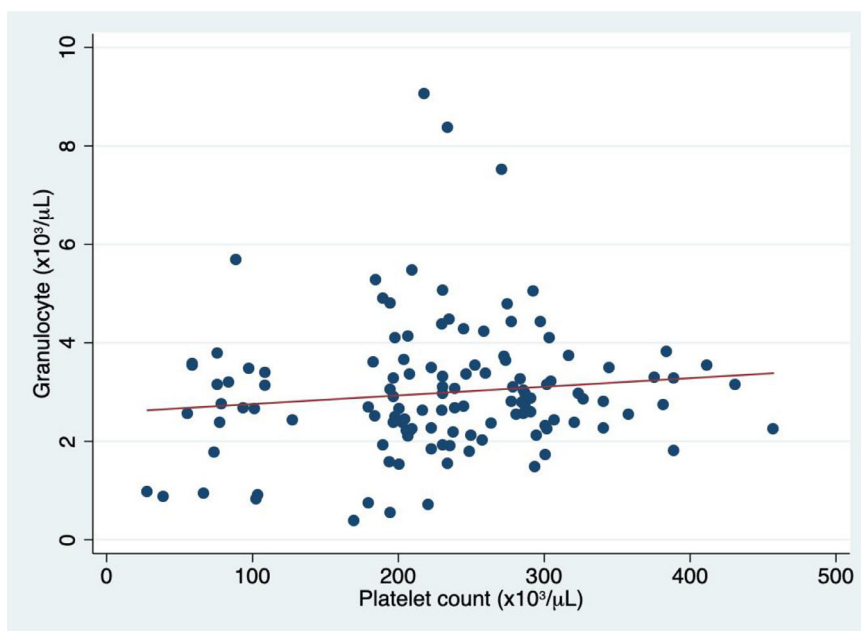


Figure 1 – In patients with non-alcoholic fatty liver disease (NAFLD), there was an association between granulocyte count and platelet count, ($r = 0.22$, $p = 0.04$, 95% CI 0.1–0.5).

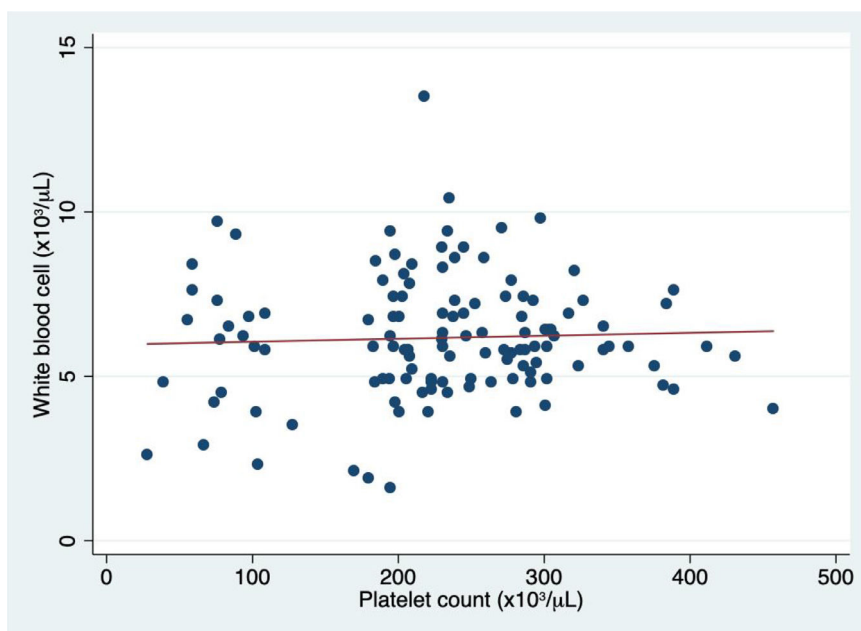


Figure 2 – In patients with non-alcoholic fatty liver disease (NAFLD), there was an association between white blood cell count and platelet count, ($r = 0.2$, $p = 0.04$, 95% CI 0.09–0.3).

Fibroscan®, and the platelet count, the white blood cell count and the granulocyte count (Figures 3–5).

Discussion

The NAFLD is the hepatic component of the metabolic syndrome, as its features are similar to those of metabolic disorders, such as obesity, inflammation, IR and type 2 diabetes

mellitus (T2DM).^{14,15} IR is one of the hallmarks of NAFLD, being pivotal in the pathogenesis of the disease, associated with obesity and an important early factor in the development of T2DM, which may be present for years before the emergence of any changes in the glycemic control.¹⁴

We have previously shown that persons with NAFLD, as defined by the Fibromax^{®5} and/or Fibroscan^{®6,7} may have diminished platelet counts in the absence of liver cirrhosis, an association which had been previously suggested^{3,4} and

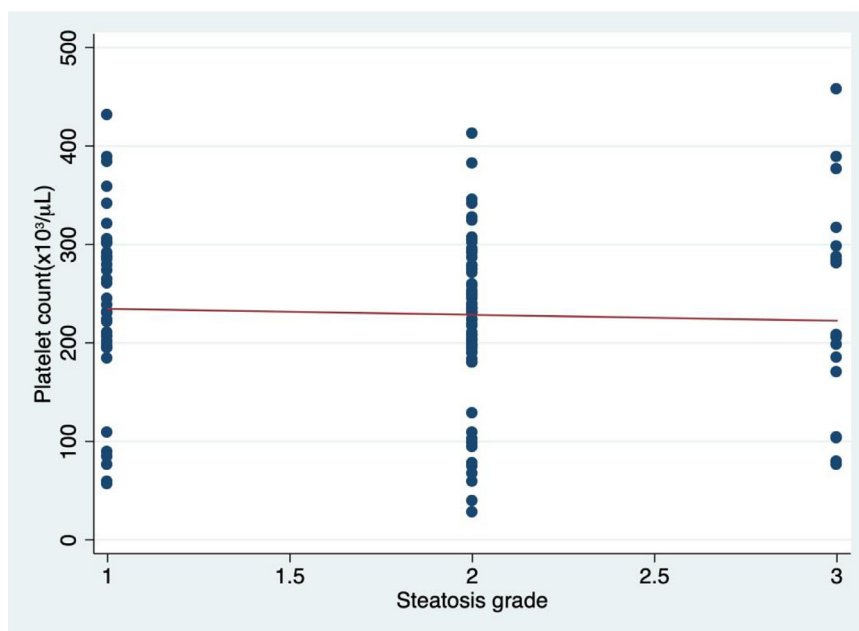


Figure 3 – Correlation between the degree of fatty infiltration of the liver as defined by the Fibromax[®] and the platelet count, expressed in platelets $\times 10^9/L$. ($r = -0.04$, $p = 0.6$, 95% CI $-0.4-0.3$).

subsequently confirmed by other investigators.⁸ The salient features of the NAFLD-associated thrombocytopenia are: a) it presents in 3–24% of the patients;^{5–8} b) it is associated with overweight; c) it is usually mild (above $40 \times 10^9/L$); d) it is not associated with mucocutaneous bleeding, and; e) it does not need treatment. The prevalence of thrombocytopenia in NAFLD is variable: Employing non-invasive methods to define it, we have prospectively found it in 28%,⁶ 24%⁷ and 16%

(these data) of persons with NAFLD. Panke et al.,⁸ in a retrospective study and using liver biopsy, found it in 3.2% of patients; it seems that the prevalence of thrombocytopenia in NAFLD varies according to the method of defining the liver steatosis and also to the nature of the study (prospective or retrospective). In our prospective studies, we have previously shown a significant association between the NAFLD and thrombocytopenia (OR = 13, CI 1.5–162, $p = 0.05$);⁷ in this

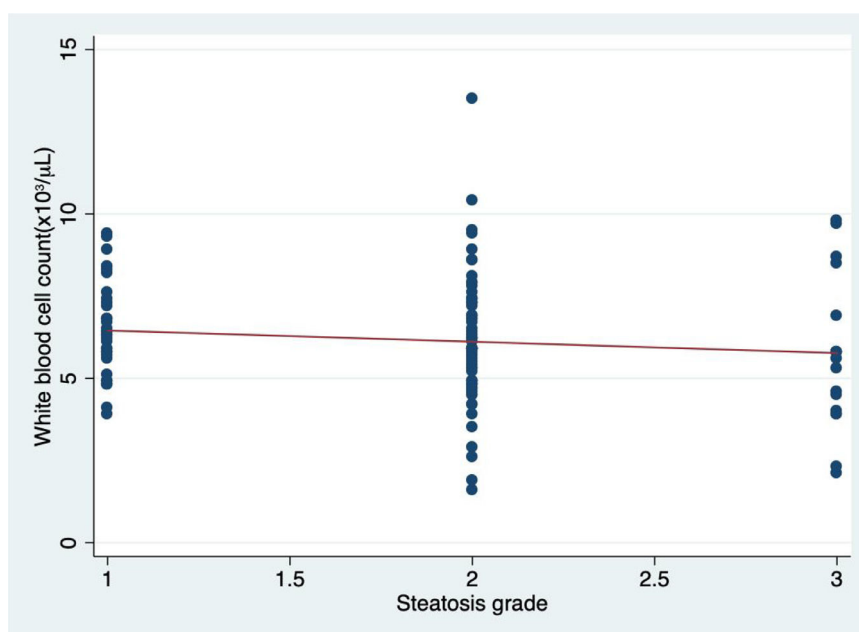


Figure 4 – Correlation between the degree of fatty infiltration of the liver as defined by the Fibromax[®] and the white blood cell (WBC) count, expressed in WBC $\times 10^9/L$. ($r = -0.12$, $p = 0.1$, 95% CI $-0.3-0.4$).

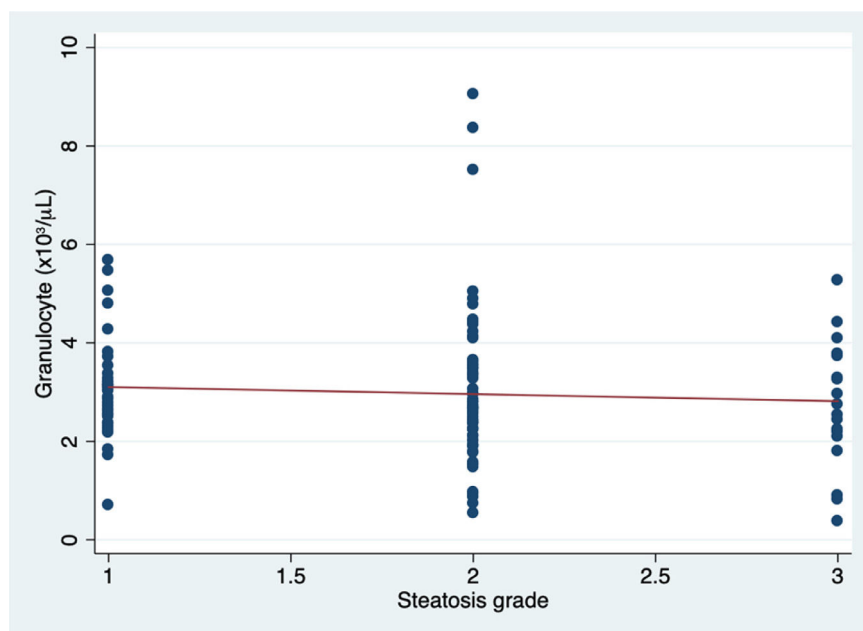


Figure 5 – Correlation between the degree of fatty infiltration of the liver as defined by the Fibromax[®] and the granulocyte count, expressed in granulocytes $\times 10^9/L$. ($r = -0.06$, $p = 0.4$, 95% CI $-0.02-0.3$).

study, we have confirmed this association and furthermore, we have shown a statistically significant relationship between thrombocytopenia and leukopenia/granulocytopenia in persons with fatty infiltration of the liver. Another interesting finding in this study is that a trend between fatty infiltration of the liver and the severity of the cytopenias was observed.

One of the explanations which has been offered for the thrombocytopenia of the NAFLD is certain degree of hypersplenism stemming from the changes in the liver and portal circulation.^{6,14} The findings in this paper could support this as a possible explanation, but other studies aiming at the assessment of hypersplenism are needed to support this suggestion. Other explanations have been offered for the low platelet count found in some persons with NAFLD, such as deficiency of thrombopoietin,^{3–8} which may also be related to liver damage, decreased lifespan of the peripheral blood cells, vitamin deficiencies, etc.^{5–7}

In summary, in a larger number of patients, we have confirmed our previous observations about thrombocytopenia being present in approximately one-fifth of persons with NAFLD. In addition, we found that the prevalence of granulocytopenia in NAFLD is lower than that of thrombocytopenia and that these two cytopenias in the absence of overt liver cirrhosis may suggest hypersplenism in NAFLD. Additional studies are needed to more clearly explain the presence of peripheral blood cytopenias in the setting of the NAFLD

REFERENCES

1. Ibrahim MA, Kelleni M, Geddawy A. Nonalcoholic fatty liver disease: current and potential therapies. *Life Sci*. 2013;92:114–8.
2. Mawatari H, Yoneda M, Kirikoshi H, Maeda S, Nakajima A, Saito S. Thrombocytopenia is more severe in patients with chronic hepatitis C than in patients with nonalcoholic fatty liver disease. *J Gastroenterol*. 2012;47:606–7.
3. Dasanu CA, Lamana S, Trikudanathan G. Thrombocytopenia, NAFLD, and metabolic syndrome: is there a link? *South Med J*. 2010;103:1071.
4. Dasanu CA, Lamana S, Trikudanathan G. Thrombocytopenia in NAFLD: is thrombopoietin involved? *South Med J*. 2010;103:1278–9.
5. Ruiz-Argüelles GJ, Velázquez-Sánchez-de-Cima S, Zamora-Ortiz G, Hernández-Reyes J, Ruiz-Delgado GJ. Nonalcoholic fatty liver disease may cause thrombocytopenia. *Acta Haematol*. 2014;132:159–62.
6. Olivares-Gazca JC, Núñez-Cortés AK, Méndez-Huerta MA, Cantero-Fortiz Y, Orea-Martínez JG, Ruiz-Argüelles GJ. More on the thrombocytopenia of the non-alcoholic fatty liver disease. *Hematology*. 2017;22:316–9.
7. López-Trujillo MA, Olivares-Gazca JM, Cantero-Fortiz Y, et al. Nonalcoholic fatty liver disease and thrombocytopenia III: its association with insulin resistance. *Clin Appl Thromb Hemost*. 2019;25:1–4.
8. Panke CL, Tovo CV, Villela-Nogueira CA, et al. Evaluation of thrombocytopenia in patients with non-alcoholic fatty liver disease without cirrhosis. *Ann Hepatol*. 2020;19:88–91.
9. Morra R, Munteanu M, Imbert-Bismut F, Messous D, Ratzu V, Poynard T. FibroMAX: towards a new universal biomarker of liver disease? *Expert Rev Mol Diagn*. 2007;7:481–90.
10. Festi D, Schiumerini R, Marzi L, et al. Review Article: the diagnosis of non-alcoholic fatty liver disease. *Aliment Pharmacol Ther*. 2013;37(4):392–400.

Conflicts of interest

The authors declare that they have no conflicts of interest.

11. Kaswala DH, Lai M, Afdhal NH. Fibrosis assessment in nonalcoholic fatty liver disease (NAFLD) in 2016. *Dig Dis Sci*. 2016;61:1356–64.
12. Lupsor-Platon M, Badea R. Noninvasive assessment of alcoholic liver disease using unidimensional transient elastography (Fibroscan®). *World J Gastroenterol*. 2015;21:11914–23.
13. Wong LH. Transient elastography: kill two birds with one stone. *World J Hepatol*. 2013;5:264–74.
14. Cobb J, Gall W, Adam KP, et al. A novel fasting blood test for insulin resistance and prediabetes. 2013;7:100–10.
15. Kitade H, Chen G, Ni Y, Ota T. Nonalcoholic fatty liver disease and insulin resistance: new insights and potential new treatments. *Nutrients*. 2017;9:387–96.