More on the thrombocytopenia of the non-alcoholic fatty liver disease

Juan Carlos Olivares-Gazca a,b, Ana Karen Nuñez-Cortes a,c, Mariana Alicia Mendez-Huerta b,d, Yahveth Cantero-Fortiz a,e, Juan Gerardo Orea-Martinez a and Guillermo J. Ruiz-Argüelles a,b,d,e

aCentro de Hematología y Medicina Interna de Puebla, Puebla, Mexico; bUniversidad Popular Autónoma del Estado de Puebla, Puebla, Mexico; cBenemérita Universidad Autónoma de Puebla, Puebla, Mexico; dLaboratorios Clínicos de Puebla, Puebla, Mexico; eUniversidad de las Américas Puebla, Puebla, Mexico

ABSTRACT

Background: Using only serologic determinations, we have previously found that thrombocytopenia presents in less than one half of patients with non-alcoholic fatty liver disease (NAFLD).

Material and methods: Employing a more accurate method to define the presence of NAFLD, serologic determinations (Fibromax®) coupled with liver transient elastography (TE/Fibroscan®), we have prospectively studied a group of 211 individuals with a suspicion of a liver disease.

Results: NAFLD was identified in 81 individuals. In 48 persons another causes of liver damage were identified and discarded from further analysis. A subset of 33 patients with NAFLD without liver fibrosis or cirrhosis was analyzed. In eight of them (24%), thrombocytopenia (less than 150 × 10 9/l platelets) was identified. The presence of thrombocytopenia in this subset of persons was associated with overweight, was usually mild, above 50 × 10 9/l, was not associated to mucocutaneous bleeding and did not require treatment.

Conclusions: NAFLD should be considered as a cause of mild thrombocytopenia. Our initial observation has been confirmed and defined more precisely. Additional studies are needed to further define more features of the thrombocytopenia of NAFLD, as well as its mechanisms.

KEYWORDS

NAFLD; platelets; thrombocytopenia; liver

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver injury worldwide [1]. It covers a wide spectrum of hepatic disorders ranging from simple steatosis, through steatohepatitis to liver cirrhosis. Thrombocytopenia has been described in some cases of NAFLD [2–5], but its prevalence and salient features are largely unknown.

The diagnosis of NAFLD requires ideally a liver biopsy, an invasive procedure not free from potential complications; however, several non-invasive diagnostic strategies have been proposed as potential diagnostic alternatives, each with different sensitivities and accuracies [6–8]. Among the non-invasive alternatives to liver biopsy, several studies have demonstrated the predictive value and a better benefit-to-risk ratio than biopsy, such as combinations of simple serum biochemical markers (Fibromax®) [6], or liver transient elastography (TE) (Fibroscan®) [7–9].

In a previous study [5], we showed that NAFLD, as defined by Fibromax®, is associated with thrombocytopenia, in the absence of liver cirrhosis. In an effort to further analyze the so called ‘thrombocytopenia of the NAFLD’, we have now used prospectively two non-invasive methods (Fibromax® and Fibroscan®) to define the presence of NAFLD and its association with thrombocytopenia in a cohort of Mexican mestizo patients.

Material and methods

(a) Patients: Individuals with NAFLD defined by non-invasive methods (Fibroscan® and/or Fibromax®) were prospectively accrued in the study, after October 2015. The study was approved by the Ethics Committee of the Clinica Ruiz and informed consent was obtained from all the patients. Individuals with hepatitis B, hepatitis C, chronic cholestatic diseases, overt liver cirrhosis or alcoholism were excluded from the analysis.

(b) Fibroscan®: To assess TE, a Fibroscan 502 Touch (Echosens, France) apparatus was employed, with the XL probe [10]. The ultrasonic controlled attenuation parameter (CAP) defines steatosis when the measurement is above 200 decibels per milliwatts (dB/m), whereas liver stiffness measurement (LSM) defines fibrosis when being above 7.5 kilopascals (kPa) [10]. In this study, patients with a CAP value above 200 dB/m coupled with a LSM below 7.5 kPa were defined as individuals with liver steatosis [10].
(c) Fibromax®: Alpha 2 macroglobulin, haptoglobin, apolipoprotein A, bilirubin, gamma glutamyl transpeptidase, alamine aminotransferase, aspartate aminotransferase, glucose, cholesterol and triglycerides were measured in all patients; these biochemical markers were analyzed in various ways to define: FibroTest for the quantitative assessment of fibrosis, SteatoTest for the quantitative assessment of steatosis; ActiTest for the quantitative assessment of necroinflammatory activity in chronic viral hepatitis and NashTest for the categorical diagnosis of non-alcoholic steatohepatitis [5–8]. Patients with a score above 50% in either SteatoTest or NashTest, coupled with a score below 50% in the Fibrotest were defined as individuals with NAFLD [5].

(d) Aspartate aminotransferase to platelet index (APRI) was calculated in all the patients included in the study.

Results

After October 2015, 211 individuals were prospectively studied with the Fibroscan®. In a subset of 81 persons, liver steatosis was defined when the CAP value was found to be above 200 dB/m; of these 48 were excluded from further analysis: Two individuals with hepatitis B, four with hepatitis C, eight with autoimmune diseases, three with chronic alcoholism and 31 with overt liver cirrhosis. Accordingly, a subset of 33 individuals with NAFLD was identified and analyzed; eight were found to have a platelet count below 150 × 10^9/l; accordingly, the prevalence of thrombocytopenia was 8/33, 24%. Table 1 shows the salient data of these eight patients. The median platelet count was 101.5 × 10^9/l, (range 53–126 × 10^9/l), whereas median platelet volume was normal at 9 fl (range 6.7–11.7). All patients had an increased body mass index (BMI), in the range of 25.5–36.3 kg/m² (median 27.5 kg/m²). All patients denied alcohol or other substance abuse. None of the patients was taking any drugs or over-the-counter remedies known to cause thrombocytopenia. The manual and citrated platelet counts confirmed the automated ones in all individuals. No platelet clumping or red cell abnormalities were present. There was no anemia, vitamin B₁₂ or folic acid deficiency, disseminated intravascular coagulation or abnormal kidney function in the analyzed cohort. All patients had normal or negative values for antinuclear antibody titers, hepatitis serologies, human immunodeficiency virus testing, antiphospholipid antibodies, and direct Coombs testing. No patient had splenomegaly. One patient had abnormally high levels of transferases, whereas two had increased gamma glutamyl transpeptidase levels. One patient displayed both decreased haptoglobin levels and increased bilirubin, a finding consonant with a mild degree of hemolysis, the hemoglobin levels being normal in all of them, see Table 1. Thrombopoietin (TPO) levels were not measured. All patients with both NAFLD and thrombocytopenia were followed for periods of 9–150 months, median 9 months; in some patients, the TE/Fibroscan was done after the initial finding of thrombocytopenia.

Table 1. Salient features of the patients with NAFLD and thrombocytopenia.

| Patients | Reference values |
|----------|------------------|
| Sex, M/F |                  |
| Age, years |               |
| BMI |                  |
| Fibroscan steatosis |               |
| CAP (dB/m) |               |
| LSM (kPa) |                  |
| APRI |                  |
| Alpha2M, g/l |               |
| Haptoglobin, g/l |             |
| Apo A1, g/l |               |
| Bilirubin, mg/dl |              |
| Gamma GT, IU |                |
| ALT, IU |                  |
| AST, IU |                  |
| Glucose, mg/dl |              |
| Triglycerides, mg/dl |          |
| Cholesterol, mg/dl |             |
| CBC |                  |
| Hb, g/dl |                  |
| Htc, % |                  |
| WBC, ×10^9/l |              |
| Platelets (1), ×10^9/l |           |
| Platelets (2), ×10^9/l |          |
| MPV, fl |                  |

BMI = Body mass index; CAP = Controlled attenuation parameter; LSM = Liver stiffness measurement; APRI = AST to platelets ratio index; Alpha2M = Alpha-2 macroglobulin; ALT = alamine transaminase; Apo A1 = apolipoprotein A1; AST = aspartate transaminase; CBC = complete blood cell count; Gamma GT = gamma glutamyl transpeptidase; Hb = hemoglobin; Htc = hematocrit; MPV = mean platelet volume; Platelets (1) = platelets at diagnosis; Platelets (2) = last platelet count; WBC = white blood cell count.
Without any specific treatment, the platelet count remained relatively stable and no patient had less than 40 × 10^9/l platelets along this period. Figure 1 depicts the platelet counts in these eight patients. No treatment whatsoever was given to the patients and all of them.

**Discussion**

NAFLD represents an emerging disease of great clinical interest: the condition is the hepatic expression of metabolic syndrome, whose prevalence is growing around the world at an alarming rate [1,11]. The molecular and cellular mechanisms underlying hepatic damage in NAFLD are not fully understood; insulin resistance, oxidative stress, inflammation, and genetic factors interact to initiate the development of NAFLD have been mentioned [11].

Assessment of liver fibrosis and steatosis is crucial in chronic liver diseases in order to determine the prognosis, the need of treatment, to monitor disease progression and the response to treatment. Liver biopsy is limited by its invasiveness and patient acceptability. Transient elastography (TE, Fibroscan®) has proved to be a non-invasive tool with satisfactory accuracy and reproducibility to estimate both liver fibrosis and steatosis. TE has been well validated in non-alcoholic fatty liver disease and other liver diseases [7–10], and a good correlation with liver damage as assessed by means of Fibromax® has been shown [12].

In a previous study, we had shown that some patients with NAFLD have decreased platelet counts [5,13], an association which had been suggested previously [3–4]. In our initial study [5], we defined the presence of NAFLD using biochemical markers (alpha 2 macroglobulin, haptoglobin, apolipoprotein A, bilirubin, gamma glutamyl transpeptidase, alanine aminotransferase, aspartate aminotransferase, glucose, cholesterol and triglycerides) analyzed in various ways (Fibromax®) to define: FibroTest for the quantitative assessment of fibrosis, SteatoTest for the quantitative assessment of steatosis; actiTest for the quantitative assessment of necroinflammatory activity in chronic viral hepatitis and NashTest for the categorical diagnosis of NAFLD [5]. We have now employed a more accurate method to define the presence of NAFLD in the absence of liver fibrosis or cirrhosis, the TE/Fibroscan®. By means of employing both Fibromax® and TE/Fibroscan®, we have identified eight patients with thrombocytopenia in a subset of 33 individuals with NAFLD in whom we have reasonably excluded other causes of thrombocytopenia; accordingly, we have found that the prevalence of thrombocytopenia in patients with NAFLD defined more accurately is 24%. In this new prospective study, we have been able to show that the thrombocytopenia of NAFLD: (a) presents in some patients (b) it is associated with overweight, (c) it is usually mild, above 40 × 10^9/l, (d) it is not associated to mucocutaneous bleeding and (e) it does not require treatment.

A deficiency of TPO has been mentioned as a possible cause of the thrombocytopenia of individuals with NAFLD [3–5] and there is information about the usefulness of TPO mimetics in the treatment of several liver diseases [14]. It is thus possible that TPO agonists could be useful in the treatment of the thrombocytopenia of NAFLD, when necessary.

This study, conducted in a more properly defined population of individuals with NAFLD, has confirmed our previous observations about this condition and added data of the features of this association; additional studies are needed to further define more features of the thrombocytopenia of NAFLD, as well as its mechanisms. NAFLD should be considered as a cause of mild thrombocytopenia and it is possible that all patients with thrombocytopenia need a detailed search for NAFLD even with normal liver function tests.
Disclosure statement
No potential conflict of interest was reported by the authors.

Notes on contributors
Juan Carlos Olivares-Gazca is an undergraduate medical student from the Universidad Popular Autónoma del Estado de Puebla completing the social service period in the Centro de Hematología y Medicina Interna, at the Clínica RUIZ de Puebla.

Ana Karen Nuñez-Cortes is an undergraduate medical student from the Benemérita Universidad Popular Autónoma de Puebla completing the social service period in the Centro de Hematología y Medicina Interna, at the Clínica RUIZ de Puebla.

QC. Mariana Alicia Mendez-Huerta is a first-year resident in Laboratory Medicine in Laboratorios Clínicos de Puebla at the Clínica RUIZ de Puebla.

Yahveth Cantero-Fortiz is an undergraduate medical student from the Universidad de las Américas Puebla.

Dr. Juan Gerardo Orea-Martinez is the medical director of Fibroscan-Puebla, at the Clínica RUIZ de Puebla.

Prof. Guillermo J. Ruiz-Arguelles is the Director General of the Centro de Hematología y Medicina Interna de Puebla at the Clínica RUIZ de Puebla. He is a professor of Hematology at the Universidad Popular Autónoma del Estado de Puebla and the Universidad de las Américas Puebla.

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