Received: 1 September 2023 | Accepted: 21 October 2023

DOI: 10.1111/eci.14123

ORIGINAL ARTICLE

WILEY

Platelet-associated biomarkers in nonalcoholic steatohepatitis: Insights from a female cohort with obesity

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Funding information
Agència de Gestió d'Ajuts Universitaris de Recerca, Grant/Award Number: AGAUR 2017 SGR 00030-2021; Grup de Recerca en Medicina Aplicada URV, Grant/Award Number: 2016 PFR-URV-B2-72; URV, Grant/Award Number: 2021-10-837-AUGUET

Abstract

Background: There is a lack of noninvasive diagnostic methods for nonalcoholic steatohepatitis (NASH), the severe condition of metabolic dysfunction-associated steatotic liver disease (MASLD). Platelet activation, evaluated through certain related parameters, is associated with liver disease and inflammation, but previous results are inconclusive.

Aim: To investigate the potential utility of platelet-related indices as noninvasive diagnostic markers for the detection and prediction of MASLD, focusing on NASH.

Results: We found that mean platelet volume (MPV), plateletcrit (PCT) and platelet distribution width (PDW) were increased in the severe and morbidly obese (SMO) group compared to the normal weight (NW) group. We found decreased levels of MPV in steatosis and NASH patients. MPV and PCT values were decreased in the presence of mild liver inflammation. Platelet count (PLA) and PCT values were lower in the presence of ballooning. We obtained an area under the ROC curve of 0.84 using MPV and three other variables to predict MASLD.

Conclusions: Some platelet-related indices vary depending on liver condition. Here, we reported decreased MPV in MASLD presence. Moreover, we presented for the first time a predictive model using MPV, ALT levels and the presence of diabetes mellitus and metabolic syndrome to predict MASLD in obese women. Also, MPV is closely related to early liver inflammation in NASH, and PLA and PCT are related to hepatic ballooning. These indices could be widely used for the early detection of NASH since they are usually determined in routine laboratory tests.

KEYWORDS
mean platelet volume, metabolic dysfunction-associated steatotic liver disease, nonalcoholic steatohepatitis, obesity, platelets

Joan Duran-Bertran and Elena Cristina Rusu contributed equally in this work.

1 | INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD), which has recently been renamed metabolic dysfunction-associated steatotic liver disease (MASLD),[1] is a widely prevalent disease originating from excessive accumulation of fat in over 5% of hepatocytes. This multisystemic condition is the leading cause of chronic liver disease on a global scale.[2] The natural history of this disorder begins with simple steatosis (SS), a benign histopathological stage that can progress into nonalcoholic steatohepatitis (NASH). NASH, a more serious condition characterized by severe steatosis, inflammation within the liver and hepatocellular damage, can sometimes lead to hepatic fibrosis and cirrhosis.[3] At present, a pivotal area of research concerning NAFLD/MASLD pertains to the identification of a noninvasive method to diagnose NASH.[4] Therefore, the development of an accurate diagnostic procedure for NASH is needed, particularly in populations at higher risk.[5]

It is important to note that patients with MASLD are generally asymptomatic until advanced stages. At present, the confirmation of NASH and early fibrosis relies exclusively on liver biopsy. However, this method might not be practical due to its associated expenses in medical care and the inherent risks it poses. Noninvasive diagnostic techniques for NASH are currently under intensive investigation. In recent years, molecules linked to the disease's development, like proinflammatory cytokines,[6] as well as certain routine blood test markers, such as lipid profiles and hepatic transaminases (aspartate aminotransferase [AST] and alanine aminotransferase [ALT]), have been subject to study among patients with fatty liver disease.[7] Of the pathways linked to NASH, chronic inflammation takes centre stage. In this context, platelets' role in triggering inflammatory responses is of considerable importance. Suggestions have been made that platelet activation might have connections to processes related to liver disease and inflammation, given their involvement in various pathophysiological conditions like obesity, thrombosis and atherosclerosis.[8]

Platelets are cytoplasmic fragments derived from megakaryocytes and are rich in organelles that contain bioactive proteins involved in inflammation and tissue regeneration. Upon platelet activation, these proteins are secreted from numerous granules, exerting their proinflammatory and haemostatic activities more effectively.[6] Platelet activation triggers morphological changes in platelets, which can be reflected in parameters such as platelet distribution width (PDW) and mean platelet volume (MPV). Consequently, these factors may be considered indicators of platelet activation and function.[7] Moreover, platelet count (PLA) number and its related parameters have proven to be good biomarkers for systemic inflammation.[8]

Recent articles have suggested a duality in platelet activation functions, as it can be both beneficial and detrimental to the liver due to the release of chemokines, cytokines, lipids, serotonin and other factors. These secreted factors are essential for liver regeneration. However, they can also alter the liver and induce inflammation that could exacerbate liver damage.[9] Furthermore, some studies have reported an association between platelet activation and NAFLD. For instance, Oral et al. observed an increase in PLA in NAFLD patients and a positive correlation with the degree of steatosis.[10] In addition, Ozhan et al. reported an increase in MPV levels in subjects with NAFLD and a positive association with insulin resistance (IR).[11] Moreover, PLA has been included in some scoring systems, such as the Fibrosis score[12] and the LiverRisk score, which is a predictive model that includes variables that could be applicable for liver fibrosis detection with a higher accuracy than other fibrosis tests, such FIB-4 or APRI.[13] MPV is not only a biomarker of PLA activation and function but also has been noted for its association with metabolic diseases.[14] Other platelet-related parameters that have also been investigated within the context of MASLD are plateletcrit (PCT) and PDW. While PDW characterizes the range of size differences among platelets, PCT is the volume in percentage occupied by platelets in the blood. An elevated PDW may be a sign of inflammation and platelet activation and seems to convey more information than MPV regarding platelet reactivity.[10] PCT has been reported to be a potential prognostic marker in the early detection of NAFLD/MASLD.[15] All the efforts made in this discipline are to more accurately define the roles that these noninvasive laboratory markers can have in the diagnosis, screening or follow-up of NAFLD/MASLD patients.[16]

Considering the possible dual-functional association between platelet activation and MASLD, the need for a noninvasive diagnosis of NASH, and the availability of platelet-related parameters in routine blood tests, the objective of this study was to analyse the potential utility of PLA, MPV, PDW and PCT, as noninvasive diagnostic markers for the detection and prediction of MASLD, specifically focusing on NASH. We analysed platelet indices in blood samples from a cohort of women with normal weight (NW) and another cohort with severe and morbid obesity (SMO) undergoing bariatric surgery; the latter was classified according to the results of liver histology as normal liver (NL), SS and NASH to evaluate these parameters as possible noninvasive diagnostic biomarkers of NASH. Additionally, our study seeks to explore the potential dual role of platelet activation in MASLD progression.

2 | RESULTS

2.1 | Baseline characteristics of the subjects

The clinical and biochemical characteristics of the study population are detailed in Table 1. Initially, patients were categorized based on their body mass index (BMI) into two groups: those with normal weight (NW, $n = 82$) and those with severe and morbid obesity (SMO, $n = 471$), using a threshold value of 35 kg/m^2. These groups were comparable in terms of age, diastolic blood pressure (DBP) and low-density lipoprotein cholesterol (LDL-C). However, the SMO participants presented a higher weight, BMI and systolic blood pressure (SBP) in comparison to the NW group. Distinct alterations in glucose metabolism were evident in the SMO group, as indicated by elevated levels of fasting glucose, insulin, homeostatic model assessment method of insulin resistance (HOMA2-IR) and glycosylated haemoglobin (HbA1c). Notably, five of the 88 NW patients had diabetes mellitus (less than 6%), in contrast to the 36.5% of SMO patients. Additionally, the SMO group displayed lower levels of high-density lipoprotein cholesterol (HDL-C) and significantly higher triacylglyceride (TG) levels. Alterations in liver biomarkers were also observed in the SMO group, marked by elevated levels of AST, ALT, gamma-glutamyltransferase (GGT) and alkaline phosphatase (ALP).

Subsequently, the patients with SMO were subclassified based on their hepatic histology as NL ($n = 115$), SS ($n = 222$) and NASH ($n = 134$). These groups were comparable in terms of age, weight, DBP, SBP, cholesterol, LDL-C and ALP, as shown in Table 2. In this sense, we observed an increment in the BMI when comparing SS and NASH to NL patients, but no difference was observed between SS and NASH. Glucose metabolism was also altered when comparing NL to SS and NL to NASH, regarding fasting glucose, insulin, HOMA2-IR and HbA1c levels, but only insulin was significantly lower in SS compared to NASH. HDL-c was decreased in NASH patients compared to NL, while TG were increased; these latter was also higher in NASH group when compared to SS. Lastly, there was a significant increase in AST, ALT and GGT levels between NL and SS and between NL and NASH, but only AST was significantly different between SS and NASH.

TABLE 1 Clinical and biochemical variables of NW and SMO patients of this study.

Variables	NW ($n = 82$)	SMO ($N = 471$)	*p*-value
Age (years)	43.22 (37.36–54.84)	48.54 (39.79–55.87)	.135
Weight (kg)	58.75 (53.25–63.75)	117.00 (106.50–128.00)	<.001
BMI (kg/m^2)	22.63 (21.15–24.20)	44.98 (41.68–48.89)	<.001
DBP (mmHg)	70.00 (66.00–77.00)	70.00 (60.00–80.00)	.551
SBP (mmHg)	120.00 (110.00–130.00)	125.00 (114.00–140.00)	.009
Glucose (mg/dL)	84.00 (74.00–91.00)	102.00 (87.00–122.75)	<.001
Insulin (mUI/L)	6.60 (4.84–9.50)	13.17 (7.65–21.76)	<.001
HOMA2-IR	0.80 (0.60–1.30)	1.70 (1.20–2.85)	<.001
HbA1c (%)	5.10 (4.80–5.40)	5.60 (5.10–6.40)	<.001
Cholesterol (mg/dL)	185.00 (166.75–203.50)	178.10 (153.20–201.00)	.025
HDL-C (mg/dL)	60.65 (51.00–68.85)	42.00 (35.00–49.00)	<.001
LDL-C (mg/dL)	111.40 (93.88–125.25)	102.00 (82.20–125.00)	.055
TG (mg/dL)	78.00 (57.75–97.25)	136.00 (100.00–185.00)	<.001
AST (UI/L)	20.00 (16.00–24.00)	24.00 (18.00–36.00)	.001
ALT (UI/L)	17.00 (13.00–25.00)	26.00 (18.75–39.00)	<.001
GGT (UI/L)	13.00 (10.25–20.75)	21.00 (15.00–34.50)	<.001
ALP (Ul/L)	64.00 (47.00–78.00)	69.00 (57.00–81.00)	.015

Note: Data are expressed as the median (interquartile range). *p*-values were considered statistically significant when <.05.

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; GGT, gamma-glutamyltransferase; HbA1c, glycosylated haemoglobin; HDL-C, high-density lipoprotein cholesterol; HOMA2-IR, homeostatic model assessment method of insulin resistance; LDL-C, low-density lipoprotein cholesterol; NW, normal weight; SBP, systolic blood pressure; SMO, severe and morbid obesity; TG, triglycerides.

TABLE 2 Clinical and biochemical variables of SMO subjects in accordance to their liver histology.

Variables	NL ($n=115$)	SS ($n=222$)	NASH ($n=134$)
Age (years)	45.44 (37.44–55.65)	49.74 (41.03–55.85)	47.72 (39.70–56.83)
Weight (kg)	115.00 (101.50–127.25)	118.40 (110.00–128.00)	116.65 (107.00–127.75)
BMI (kg/m^2)	43.14 (39.80–49.33)[a]	45.19 (42.48–48.65)	45.27 (43.13–49.56)[c]
DBP (mmHg)	70.00 (60.00–78.50)	70.00 (61.00–80.00)	70.00 (60.00–80.00)
SBP (mmHg)	125.00 (112.00–137.00)	125.00 (114.75–140.00)	125.00 (115.00–140.00)
Glucose (mg/dL)	88.00 (79.00–97.75)[a]	107.00 (93.00–133.00)	105.00 (90.00–128.00)[c]
Insulin (mUI/L)	8.50 (6.30–13.07)[a]	14.30 (7.24–21.42)[b]	16.50 (9.84–25.06)[c]
HOMA2-IR	1.20 (0.90–1.70)[a]	2.20 (1.20–3.25)	2.05 (1.40–3.10)[c]
HbA1c (%)	5.40 (5.10–5.75)[a]	5.80 (5.15–6.70)	5.70 (5.10–6.60)[c]
Cholesterol (mg/dL)	172.00 (146.25–201.75)	181.50 (155.02–200.50)	179.30 (154.25–197.80)
HDL-C (mg/dL)	44.50 (38.00–55.00)	41.50 (35.00–50.00)	40.00 (35.00–46.00)[c]
LDL-C (mg/dL)	105.00 (81.00–125.00)	105.20 (83.00–126.95)	98.00 (83.50–120.40)
TG (mg/dL)	103.50 (79.25–134.75)[a]	142.90 (104.25–185.75)	153.00 (120.00–207.00)[c]
AST (UI/L)	20.50 (16.00–26.75)[a]	24.00 (19.00–33.75)[b]	29.50 (19.00–46.00)[c]
ALT (UI/L)	20.00 (15.00–26.50)[a]	28.00 (20.00–38.00)	33.00 (22.00–48.00)[c]
GGT (UI/L)	17.00 (12.00–24.50)[a]	23.00 (16.75–36.00)	22.00 (15.00–34.00)[c]
ALP (Ul/L)	66.00 (53.00–75.00)	73.00 (59.00–85.25)	67.00 (56.50–79.00)

Note: Data are expressed as the median (interquartile range).

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; GGT, gamma-glutamyltransferase; HbA1c, glycosylated haemoglobin; HDL-C, high density lipoprotein cholesterol; HOMA2-IR, homeostatic model assessment method of insulin resistance; LDL-C, low density lipoprotein cholesterol; NASH, nonalcoholic steatohepatitis; NL, normal liver; SBP, systolic blood pressure; SS, simple steatosis; TG, triglycerides.

[a]Significant differences between the NL cohort and SS (adjusted $p<.05$).

[b]Significant differences between SS and NASH (adjusted $p<.05$).

[c]Significant differences between NL and NASH (adjusted $p<.05$).

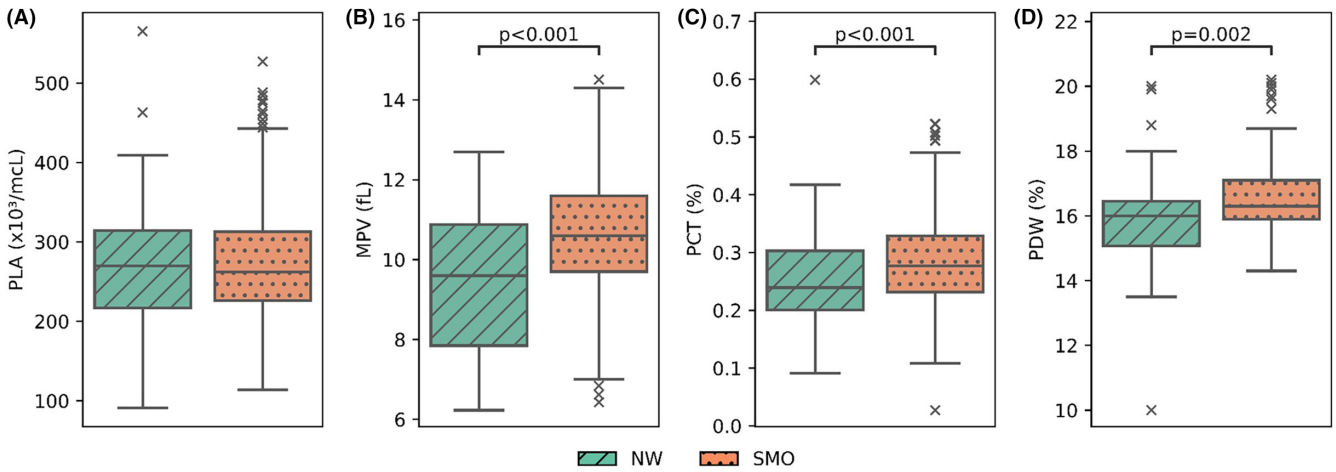

FIGURE 1 Platelet indices between normal weight (NW) and severe and morbidly obese (SMO) patients. (A) Platelet count (PLA), (B) Mean platelet volume (MPV), (C) Plateletcrit (PCT) and (D) Platelet distribution width (PDW). *p*-values were considered statistically significant when <.05. Means and standard deviation are listed in Table S1.

2.2 | Platelets and related parameters according to BMI

Several platelet indices have been reported to be altered in the presence of obesity elsewhere.[17] In this context, we compared PLA and platelet-related parameters between the NW and SMO groups (Figure 1). In this regard, we observed a significant increase in terms of MPV, PCT and PDW levels in the SMO group in comparison to NW subjects. However, PLA presented no significant differences between groups.

2.3 | Platelets and related parameters according to liver histology

Subsequently, our focus shifted to the analysis of platelet indices (PLA, MPV, PCT and PDW) within the SMO cohort based on the presence or absence of MASLD (Figure 2.1). In this analysis, we identified a significant reduction in MPV associated to MASLD compared to NL subjects. Concerning the other platelet-related parameters, no significant differences were observed between the two groups.

Later, we wanted to assess whether there existed significant differences in terms of platelet indices depending on the subjects present NL, SS or NASH (Figure 2.2). In this analysis, we found a significant decrease in MPV when comparing both NL to NASH and SS to NASH. However, we did not find significant differences between the groups regarding PLA, PCT or PDW.

2.4 | Prognostic values of platelets and related parameters in MASLD

Then, we evaluated the diagnostic efficacy of a biomarker panel comprising MPV—the most associated platelet-related parameter based on our previous results—along with other biochemical and clinical parameters involved in MASLD diagnosis such as ALT levels and the presence of DM and MS as covariates in a group of patients with liver histology indicative of MASLD. We built a decision tree (Figure S2) to classify patients between NL and MASLD, which returned the confusion matrix shown in Table 3. To further evaluate the extent to which these metabolites can predict liver status, in Figure 3, we computed the area under the receiver operating characteristic (ROC) curve and obtained an area under the curve of 0.84.

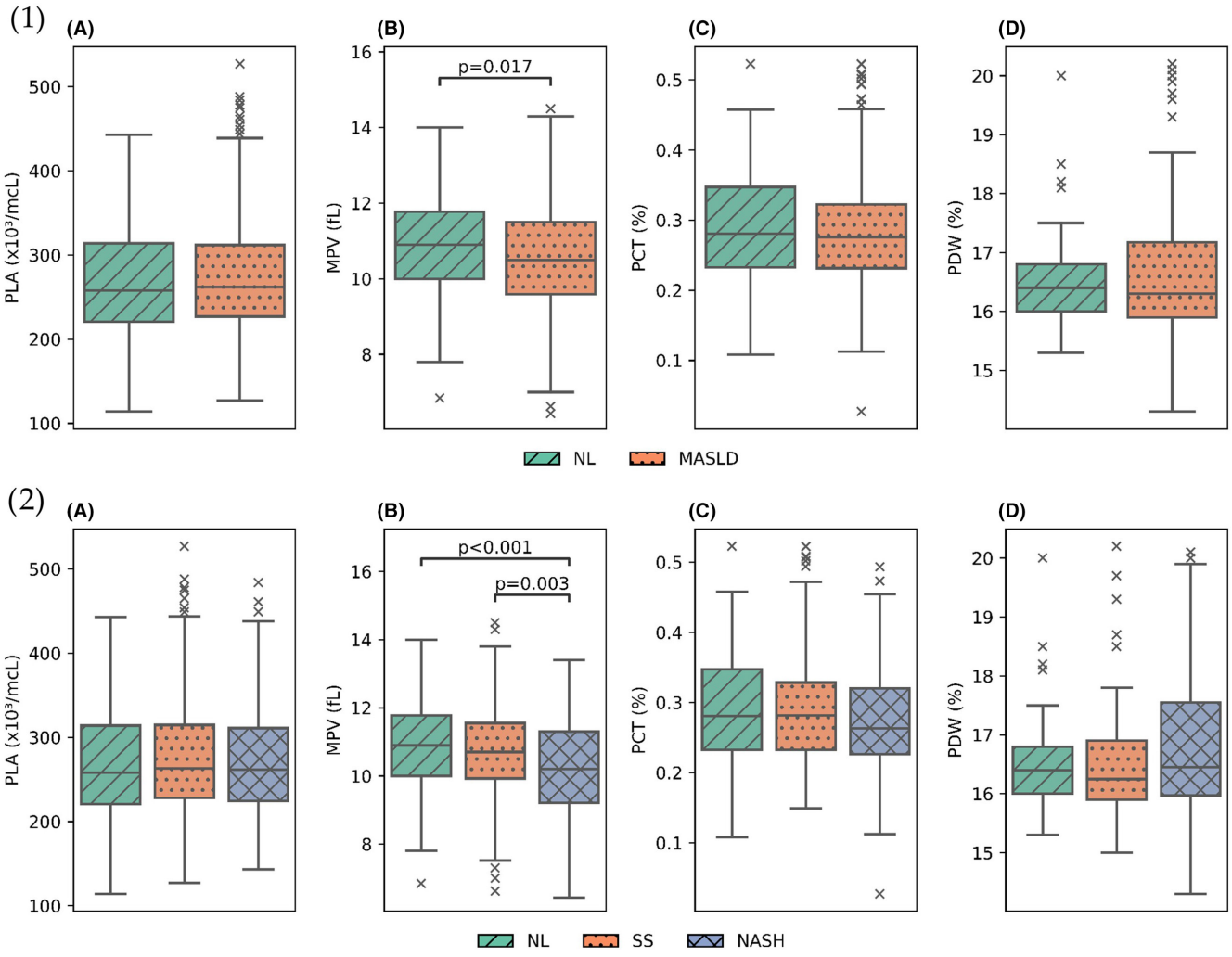

FIGURE 2 Platelet indices between normal liver (NL) and metabolic dysfunction-associated steatotic liver disease (MASLD) and MASLD stages in the severe and morbidly obese (SMO) cohort: (1) NL and MASLD patients, (2) NL, simple steatosis (SS) and nonalcoholic steatohepatitis (NASH) patients. (A) Platelet count (PLA), (B) Mean platelet volume (MPV), (C) Plateletcrit (PCT), (D) Platelet distribution width (PDW). *p*-values were considered statistically significant when <.05. Means and standard deviation are listed in Table S1.

TABLE 3 Confusion matrix and metrics quantifying the quality of the predictive model.

		Predicted values		
		NL	MASLD	
Actual values	NL	49	31	Specificity = 0.61
	MASLD	32	222	Recall = 0.87
		Negative predictive value = 0.6	Precision = 0.88	Accuracy = 0.81

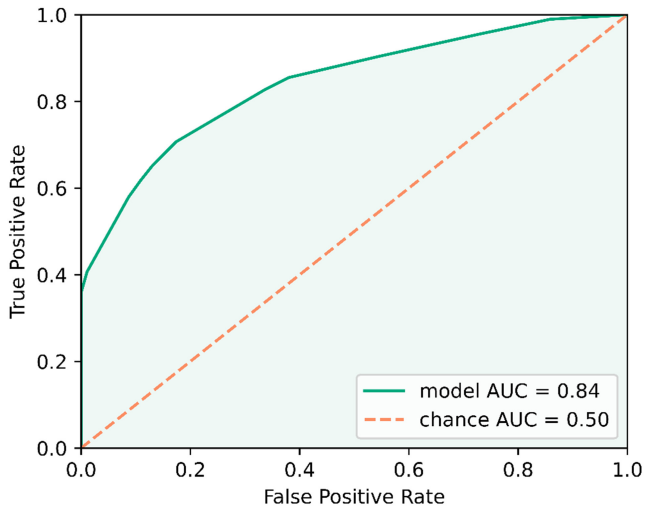

FIGURE 3 Area under the receiver operating characteristic curve for the prediction of MASLD using a decision tree model with mean platelet volume (MPV), alanine aminotransferase (ALT), metabolic syndrome (MS) and diabetes mellitus (DM).

2.5 | Platelets and related parameters according to NASH parameters

Since we previously found a significant decrease in MPV in NASH subjects compared to NL and SS subjects, and in order to find a noninvasive diagnostic test for NASH patients, we wanted to further evaluate platelet indices in accordance to NASH presence. First, we compared platelet indices between NASH and non-NASH (comprising both NL and SS) patients in the SMO cohort. In this analysis, shown in Figure 4.1, we found a significant decrease in MPV. In terms of PLA, PCT and PDW, no significant differences were observed.

Secondly, we compared platelet indices according to the degree of liver inflammation (absence, mild or severe) as shown in Figure 4.2. This analysis reveals a significant decrease in MPV among patients with mild inflammation in contrast to those without inflammation. However, this trend was not present when comparing severe to mild liver inflammation. Moreover, no significant differences were observed in terms of PLA, PCT or PDW.

Thirdly, we wanted to analyse platelet indices based on to the presence or absence of hepatocellular ballooning, another most important NASH-related parameters, as shown in Figure 4.3. In this context, our analysis indicated that both PLA and PCT were lower in patients with liver ballooning compared to those without it. In terms of MPV and PDW, we did not find any significant differences.

2.6 | Platelets and related parameters according to NAFLD co-morbidities

Lastly, we wanted to evaluate platelet levels and platelet-related parameters concerning the presence of other MASLD-co-morbidities, such as type 2 diabetes mellitus (DM), dyslipidaemia (DL), hypertension (HTA) or metabolic syndrome (MS). In this context, we found a significantly lower MPV in patients with DM (Figure 5.1), a smaller PCT in patients with DL (Figure 5.3), and both a decreased MPV and PCT in patients with MS (Figure 5.2). However, no significant differences were found neither in other platelet indices, nor regarding hypertension. Regarding treatment, we found no significant differences in MPV levels between patients taking statins or undergoing hypertension treatment (data not shown).

3 | DISCUSSION

In this study, our objective was to study the potential utility of PLA, MPV, PDW and PCT, as noninvasive diagnostic markers for the detection and prediction of MASLD, specifically focusing on NASH, analysing these parameters in blood samples from a cohort of SMO women, using a group of NW women as a control.

First, we analysed platelet associated-parameters regarding obesity and our findings indicated an increase in MPV, PCT and PDW in the SMO group compared to the NW group. Similar results were obtained by Furuncuoglu et al.[18] observing a positive correlation between BMI and PDW and PCT.[18] Moreover, Coban et al. observed an increase in MPV in obese women compared to women with NW.[14] Ozkan et al. reported the same results in a cohort of 108 children.[19] Beavers et al. reported that platelets showed increased activation and aggregability in obesity,[20] so we can hypothesize that platelet indices, including MPV, PCT, and PDW, are elevated in obese individuals, indicating heightened platelet activation perhaps in relation to chronic low inflammation associated with obesity.[21,22]

Then, evaluating platelet-related parameters in accordance with MASLD associated with obesity, we found a decrease in MPV in women with MASLD. In the literature, there are controversial results regarding MPV in

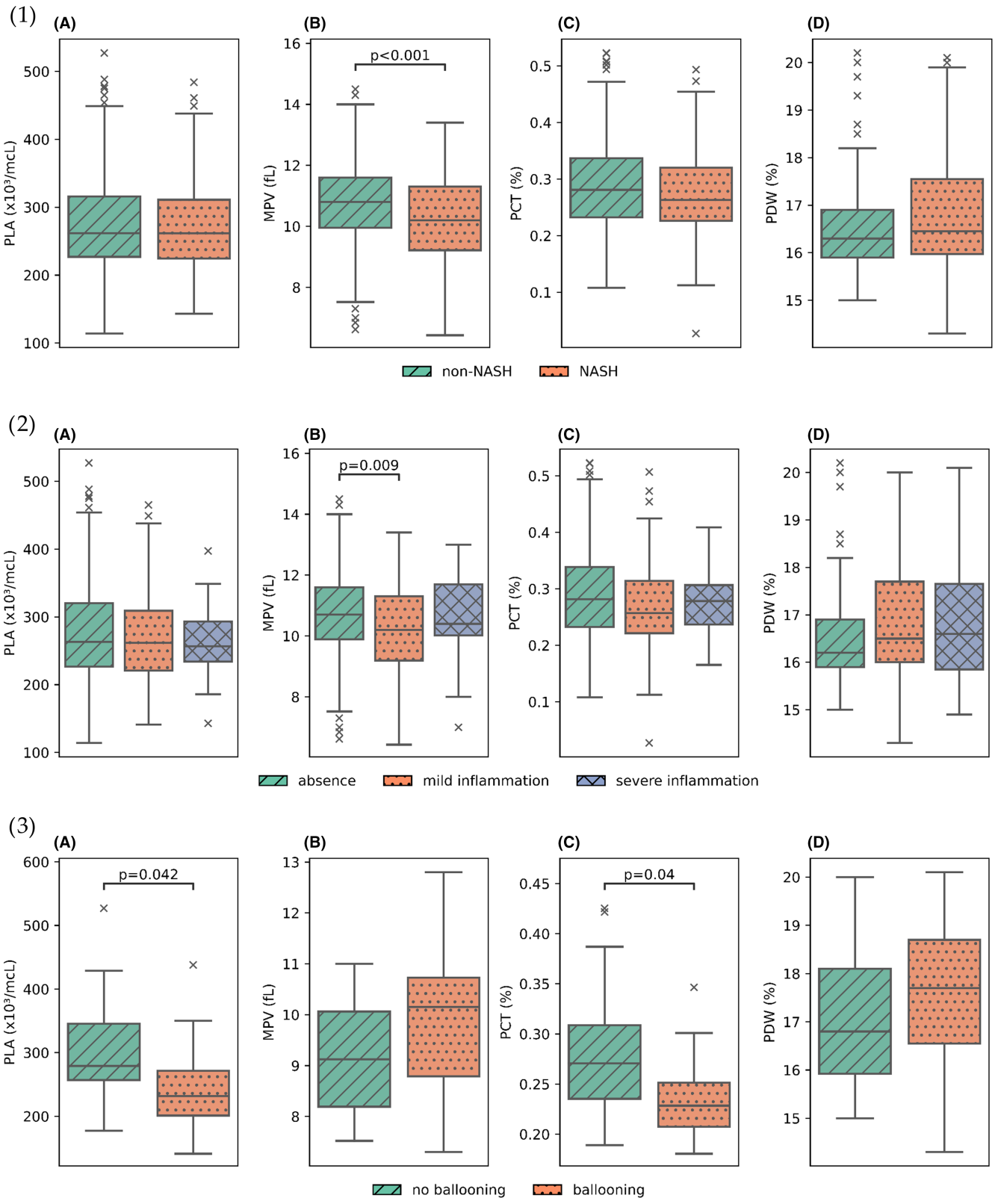

FIGURE 4 Platelet indices in nonalcoholic steatohepatitis and related parameters: (1) platelet indices in the absence and presence of NASH, (2) in different degrees of inflammation and (3) in the absence or presence of ballooning. (A) Platelet count (PLA), (B) Mean platelet volume (MPV), (C) Plateletcrit (PCT) and (D) Platelet distribution width (PDW). *p*-values were considered statistically significant when <.05. Means and standard deviation are listed in Table S1.

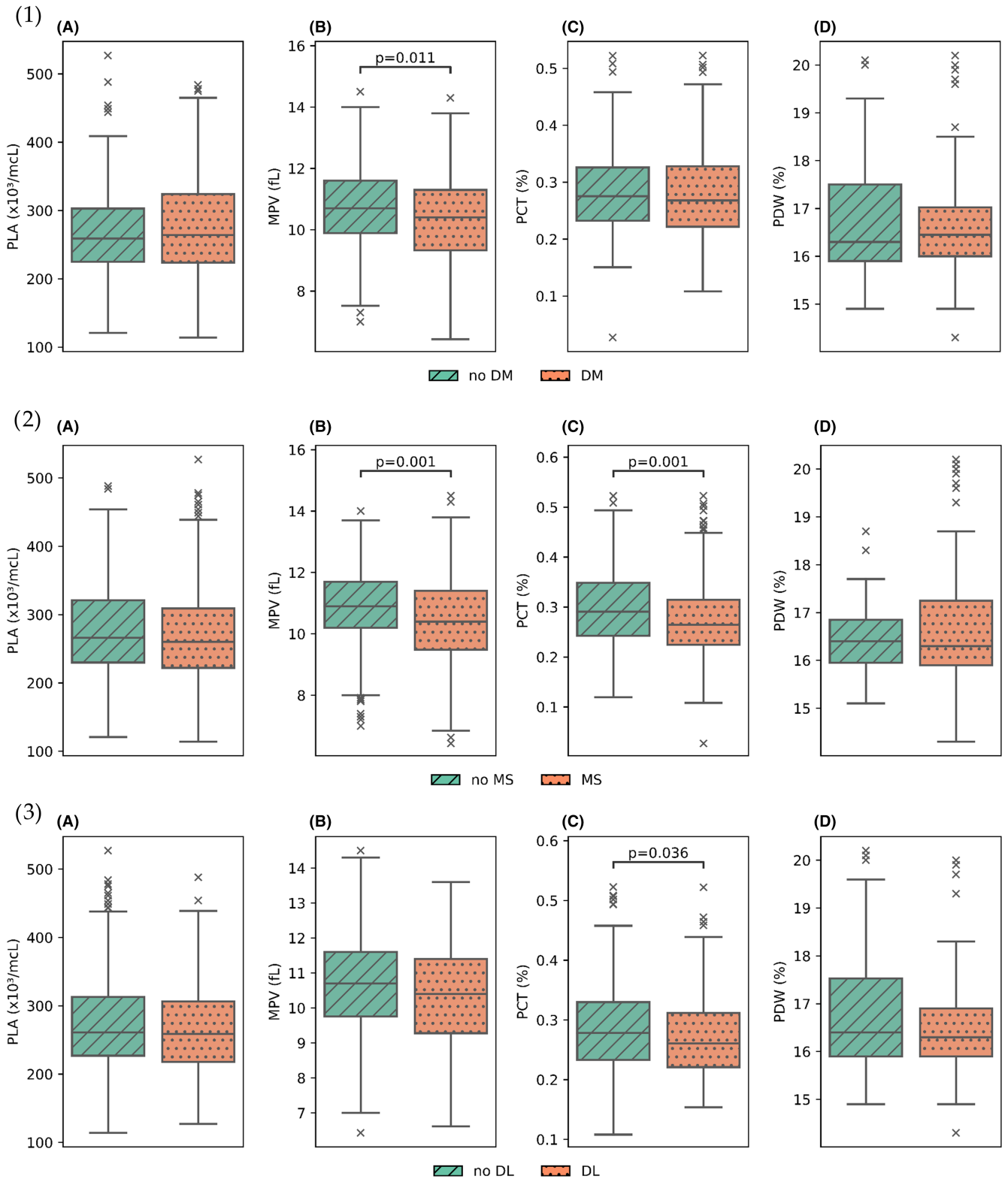

FIGURE 5 Platelet indices in presence and absence of NAFLD co-morbidities: (1) Type 2 diabetes mellitus (DM), (2) metabolic syndrome (MS) and (3) dyslipidaemia (DL). (A) Platelet count (PLA), (B) Mean platelet volume (MPV), (C) Plateletcrit (PCT) and (D) Platelet distribution width (PDW). *p*-values were considered statistically significant when <.05. Means and standard deviation are listed in Table S1.

NAFLD/MASLD. While several studies indicated that MPV seemed to be significantly increased in the population with NAFLD with or without obesity,[11,23–26] other authors reported nonsignificant differences.[27,28] In contrast, Michalak et al. reported a significant decrease in MPV among individuals with NAFLD.[15] This observation

is consistent with our study findings. Overall, the discrepancies may be due to most of these studies used a healthy and nonobese cohort of subjects as a control group,[14,18,26,28,29] while in our study, the control group in relation to liver disease was composed of obese patients with normal liver histology. This should be taken into account due to the influence of obesity on MPV values.[17] Another factor that could affect MPV values and other platelet parameters and that could contribute to the mentioned discrepancies is the method of blood sample collection.[30] In our study, we used EDTA tubes, and we analysed MPV by the sheath flow DC detection method using Sysmex-XN (Roche, Norderstedt, Germany). In this sense, some editorial letters recommended the standardization of the MPV analytic methods before considering this parameter as a marker of NAFLD.[31,32] Moreover, it has been suggested that MPV varies according to age, ethnicity and sex, although no conclusive data are available on this topic.[33] Thus, it is important to highlight the homogeneity of our cohort, a measure taken to mitigate potential biases.

Regarding the association between MPV and MASLD, we build a predictive model that demonstrated a good predictive value. This result suggests that MPV could be a good indicator in an ordinary blood analysis to predict MASLD associated with obesity in a female cohort. Recently, it has been reported that the use of the LiverRisk score, a predictive model that includes variables such as PLA, could be applicable for liver fibrosis detection with a higher accuracy than other fibrosis tests such FIB-4 or APRI.[13] There are predictive scores that include platelet-related parameters to evaluate liver stiffness.[13] However, as of today, there are no studies that have presented a predictive model using MPV to predict NAFLD/MASLD.[11,23–26] In any case, these findings need to be validated in other homogeneous cohorts. The importance of using a comparable control group must be highlighted, as the use of healthy NW control subjects is widespread. Thus, this comparison might not capture the specific trend of MPV regarding liver involvement. Moreover, MPV analytical methods need to be standardized to consider MPV as a possible biomarker for NAFLD.[34,35] In any case, we have presented for the first time a statistically established predictive model using MPV, ALT levels and the presence of DM and MS to predict MASLD. It must be noted that this model is developed for obese subjects with a BMI > 35 kg/m^2 (and more specifically, for women), therefore it could not be applied to overweight subjects, even though their NAFLD prevalence is similar to the one in the obese population.[29]

Moreover, MPV values were lower in SS and NASH patients than in subjects with NL histology. It must be noted that in the early stages of NASH, platelets are among the first nonresident cells to infiltrate the liver, with their involvement correlating with key NASH-associated morphological changes such as steatosis and hepatocyte swelling.[30] A crucial interaction in this context is the binding of platelets to Kupffer cells via the CD44 receptor on platelets and its ligand, hyaluronan.[31] This interaction triggers an inflammatory response that is exacerbated in NASH.[31] In the context of NASH, a lower MPV might reflect a distinct stage or aspect of platelet activation and involvement. While increased MPV generally indicates platelet activation, we hypothesize that a lower MPV could signify a more advanced inflammatory state or a different immune response. Finally, the significant difference in MPV between non-NASH and NASH subjects could lead to the conclusion that it would be an early biomarker of NASH.

Specifically, we also observed that MPV was significantly decreased in the presence of mild liver inflammation. Conversely, Pan et al.[32] found no association between MPV and liver inflammation in a cohort of 677 patients with chronic hepatitis B infection, but reported a negative association between PLA and PDW with liver inflammation. Other authors have reported a significantly higher MPV in patients with a histological activity index score above 10,[33] indicative of liver inflammation. These results are therefore controversial and we acknowledge a lack of studies focusing on the potential mechanisms linking platelet activity and liver inflammatory status. Furthermore, we found that PLA and PCT values were decreased in the presence of ballooning. In contrast, Abdel-Razik et al. reported that MPV was increased in NASH patients compared to non-NASH patients.[36] Additionally, these authors recruited a NASH group with different degrees of fibrosis,[36] while our subjects did not have liver fibrosis. Regarding the discrepancies, the most important aspect to mention is that platelet induction mediated by inflammation modifies the morphology and functional status of platelets. In proinflammatory situations, dichotomous responses can occur: higher platelet production that is usually associated with lower volume or the opposite,[37] as well as dual role situations on the part of platelet activation in the liver. Thus, platelet activation could be beneficial or deleterious to the liver due to the release of different secreted factors, some of which are involved in liver regeneration and other processes, inducing liver inflammation.[9,38] Unfortunately, in our study, we have not analysed the relationship between platelet-related indices and proinflammatory molecules or liver regeneration. Therefore, we suggest that future studies answer these questions. Concerning MASLD co-morbidities, we found that MPV was decreased in SMO patients with DM. In addition, MPV and PCT levels were reduced in subjects presenting MS. Moreover, PCT levels were decreased in women with DL. Regarding DM, in a cohort of

11,730 subjects, Shah et al. showed that MPV values are usually increased in patients with diabetes.[39] This trend was also reported by other authors, but with the addition that an improved glycaemic control comes in hand with a decrease in the MPV.[40] In this sense, 83.6% of the patients with diabetes from our cohort received specific anti-diabetic treatment, at least partially explaining the lower MPV levels that we found when compared to other obese individuals without DM. In relation to MS, several authors reported a nonsignificant association between MS and MPV,[39,41] but one of these studies found that some components of MS were associated with a higher MPV.[39] However, Park et al. found that the MPV was significantly decreased in a cohort of Korean women suffering from MS,[42] and other authors have also reported similar results.[43] In addition, an inverse association between MPV and the presence of MS was found in a study cohort of 31,548 women.[44] Thus, our results are in accordance with the findings of these studies with larger cohorts. Finally, in relation to DL, in the cohort studied by Singh et al., there was a positive association between DL and MPV, but nonsignificant results were found regarding PCT.[45] In contrast, other studies with considerably larger cohorts reported a positive association between LDL/VLDL cholesterol and PCT and PLA, but no association with MPV was reported.[46,47] Given our paradoxical findings, we should note that 59.5% of dyslipidaemic patients in our cohort were treated with statins and, therefore, did not present a significant increase in LDL cholesterol levels. In addition, it has been stated that the use of statins can affect MPV.[48] Overall, it must be noted that these co-morbidities are closely intertwined, as 55.2% of MS patients from our cohort suffer from DM and 49.1% of MS patients also present DL.

The main strength of our study is that we worked with a well-characterized and homogeneous cohort of women with SMO, using a healthy and NW control group to put into context our findings, but then focusing on biopsy-proven SMO subjects to study platelet-related parameters in liver involvement. The main limitations of this study are that it was difficult to compare our results with previously published data, given the heterogeneity of the different cohorts and methods. Related to this, it must be noted that most of the SMO individuals from our cohort receive some kind of treatment, so excluding all medicated patients is impractical in this scenario. The treatment may also hinder the association between some co-morbidities and platelet indices. Finally, since our cohort included only women, these results could not be extrapolated to men. In addition, the predictive model developed in this study was carried out with women with severe or morbid obesity, so it cannot be extrapolated to people with mild obesity or who are normal weight.

4 | MATERIALS AND METHODS

4.1 | Subjects

First of all, we want to emphasize that we have an initial cohort of 645 patients, comprising of both men and women. This cohort is made up of 518 patients who have undergone bariatric surgery as well as a control group of 127 subjects, comparable in age and sex, with biological samples stored in a biobank. To achieve the objective of this study, a cohort made up of 553 Caucasian women was included. Initially, they were divided according to their BMI into women with NW (BMI 19–25 kg/m^2) as a control group ($n=82$) and women with SMO (BMI ≥35 kg/m^2, $n=471$). The decision not to include men in this study was based on the very small number of male patients ($n=92$) compared to female patients. Additionally, there are gender-dependent differences in platelet indices (Appendix S1). In this sense, the PLA in women has been reported to be higher than in men, which seems to reflect different hormonal profiles or a compensatory mechanism associated with menstrual blood loss.[49] For the purpose of this study, metabolic syndrome was defined according to the definition given by the International Diabetes Federation 2006.[50]

All participants signed a written informed consent. This study was approved by the institutional review board (Institut Investigació Sanitària Pere Virgili (IISPV) CEIm; 23c/2015). All SMO subjects underwent planned laparoscopic bariatric surgery in which a liver biopsy was obtained if hepatic impairment was suspected and a blood sample was collected preoperatively.

The exclusion criteria of this study were as follows: (1) male patients, (2) an intake of ethanol greater than 10 g/day or exposure to other toxins, (3) women in menopause, (4) or taking contraceptives (to avoid interference from hormones), (5) infectious disease, neoplastic disease or acute or chronic liver disease other than MASLD, (6) any type of antiplatelet medication; (7) subjects with any acute prothrombotic process and (8) treatment with antibiotics in the previous 4 weeks.

4.2 | Liver pathology

Mason's trichrome and haematoxylin–eosin stain were assessed in liver samples from the SMO group, collected during bariatric surgery. These samples were categorized by a skilled pathologist who evaluated steatosis, portal and lobular inflammation, hepatocellular ballooning and fibrosis using the Kleiner criteria.[51] The liver histology classification based on the cohort's samples resulted in the following distribution: 115 patients had NL, 222 had

SS and 134 had NASH. In our cohort of NASH patients, no fibrosis nor cirrhosis was present.

4.3 | Anthropometric evaluation and biochemical analyses

Physical, anthropometric and biochemical evaluations were performed on the entire cohort. Specialized nurses used a BD Vacutainer® to collect blood samples prior to bariatric surgery. Empty and ethylenediaminetetraacetic acid (EDTA) tubes were used to subsequently obtain aliquots of serum and plasma by centrifugation (3500 rpm, 4°C, 15 min). We used EDTA tubes for blood collection to determine biochemical and platelets-related parameters. Citrate tubes were only used when required by laboratory for the presence of platelet aggregation. Biochemical parameters were evaluated with a conventional automated analyser and insulin resistance was estimated by HOMA2-IR. PLA was analysed by PLT-O method, MPV, PDW and PCT were evaluated through sheath flow DC detection using a Sysmex-XN analyser (Roche, Norderstedt, Germany).

4.4 | Statistical analysis

The data obtained in this study were analysed using python (v.3.11.3) with the aid of the statistical package SciPy (v.1.10.1). Comparative analyses were performed using the nonparametric Mann–Whitney *U* test and the *p*-values were adjusted for multiple comparisons with the Benjamini–Hochberg method. Values of $p < .05$ were statistically significant and variables were reported as the median and interquartile range (25th–75th). Plots were generated with matplotlib and seaborn packages. The decision tree classifier was computed with the sklearn package. We controlled overfitting by allowing a maximum depth of 5 and a minimum of 12 samples per leaf. All the variables used were assessed for multicollinearity.

5 | CONCLUSIONS

MPV is decreased in women with MASLD associated with obesity and closely related to early liver inflammation in NASH. Specifically, PLA and PCT are related to hepatic ballooning. These indices could be widely used for the early detection of NASH since they are usually determined in routine laboratory tests. Moreover, we presented for the first time a statistically established predictive model using MPV, ALT levels and the presence of DM and MS to predict MASLD in obese women.

FUNDING INFORMATION

This study has been funded by funds from Agència de Gestió d'Ajuts Universitaris de Recerca (AGAUR 2017 SGR 00030-2021 Teresa Auguet), the Grup de Recerca en Medicina Aplicada URV (2016 PFR-URV-B2-72 to Cristóbal Richart), the Investigador actiu Program from the URV (2021-10-837-AUGUET to Teresa Auguet) and own financing.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All relevant data supporting the findings of this study are included within this article and the accompanying supplementary material. Any additional information or data can be obtained upon reasonable request to the corresponding author.

INSTITUTIONAL REVIEW BOARD STATEMENT

This study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of Institut Investigació Sanitària Pere Virgili (IISPV) (CEIm; 23c/2015; 11 May 2015).

INFORMED CONSENT STATEMENT

Informed consent was obtained from all subjects involved in this study.

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How to cite this article: Duran-Bertran J, Rusu EC, Barrientos-Riosalido A, et al. Platelet-associated biomarkers in nonalcoholic steatohepatitis: Insights from a female cohort with obesity. *Eur J Clin Invest*. 2023;00:e14123. doi:10.1111/eci.14123