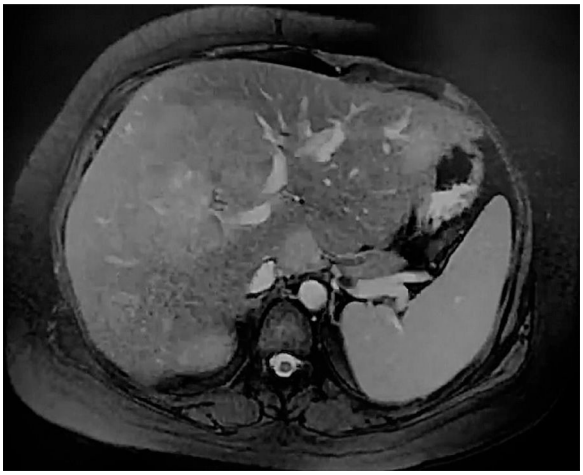

Annex 2. Simple magnetic resonance imaging

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Pirfenidone Prevents Myocarditis by Restoring Metabolic Hormone Levels in a Mouse MASH Model and its Effect on H9c2 Myoblast Viability under Glucolipotoxicity

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Introduction and Objectives: Obesity, global epidemic, can cause metabolic dysfunction-associated steatohepatitis (MASH) and cardiovascular diseases. Pirfenidone (PFD) has anti-inflammatory and antifibrotic properties. We investigated the effects of PFD on metabolic hormones expression and myocarditis in a mouse MASH model and its effect on H9c2 cells viability under glucolipotoxicity.

Materials and Patients: Twenty-week-old male C57BL/6J mice were divided into two groups: one group was fed a normal diet (ND, 3.1 kcal/g plus normal water, n=7), while the other group was fed a high-fat, high-carbohydrate diet (HFHC, 5.1 kcal/g plus water containing 2.31% fructose, 1.89% sucrose; n=14) for 16 weeks. At week 8, seven HFHC mice were administered PFD at a dosage of 300 mg/kg/day by gavage. Insulin tolerance tests (ITT), dry chemistry analysis, ELISA, histological staining (Hematoxylin-Eosin and Masson's Trichrome), and morphometric analyzes of the tissues were evaluated. H9c2 cells were treated with the following concentrations: 100 μM, 200 μM, 400 μM PA (PA), 15 mM, 30 mM glucose, and 0.3 mM, 0.5 mM, 1 mM 1.5 mM PFD. H9c2 cells viability under glucolipotoxicity were evaluated by MTT assay and Oil red O staining. The data were analyzed using one-way ANOVA followed by Tukey's post-hoc test in Graphpad Prism v10.0.

Results: HFHC mice developed MASH, myocarditis and fibrosis ($P \leq 0.05$). Additionally, resistin and AST levels significantly increased ($P \leq 0.05$). PFD prevented elevated parameters in HFHC mice ($P \leq 0.05$), such as body weight, epididymal fat weight, liver weight and heart weight; including body weight/tibia length ratio, heart weight/tibia length ratio and epididymal fat weight/tibia length ratio; hormone levels: insulin, glucagon, leptin, and plasminogen activator inhibitor-1 (PAI-1); lipid profile: total cholesterol, triglycerides, LDL, and VLDL; adipocyte hypertrophy, inflammatory foci, and fibrosis in liver and cardiac tissues. Additionally, PFD reduced ALT expression and tibia length ($P \leq 0.05$). The heart weight/body weight ratio decreased in HFHC mice ($P \leq 0.05$), PFD recovered this ratio ($P \leq 0.05$). H9c2 cells treated with 400 μM PA showed 50% cell viability ($P \leq 0.05$), all other concentrations of the compounds had cell viability > 60% ($P \leq 0.05$), including H9c2 cells treated with 150 μM PA, 15 mM glucosa, and 1 mM PFD ($P \leq 0.05$). H9c2 cells treated with 150 μM and 200 μM PA showed a significant increase in intracellular lipid accumulation ($P \leq 0.001$), and H9c2 cells treated with 150 μM PA and 1.5 mM PDF showed a tendency to reduce intracellular lipid levels.

Conclusions: PFD restores the expression levels of metabolic hormones, which are involved in lipids and carbohydrates metabolism, improving lipid and aminotransferases levels, thus preventing myocarditis and fibrosis in MASH mice. These findings suggest the potential of PFD for the prevention of myocarditis and fibrosis in obesity-induced MASH mice.

Ethical statement: CUCS Research Committee at the University of Guadalajara approved this study (protocol number: CI-01419, CI-02423).

Declaration of interests: None.

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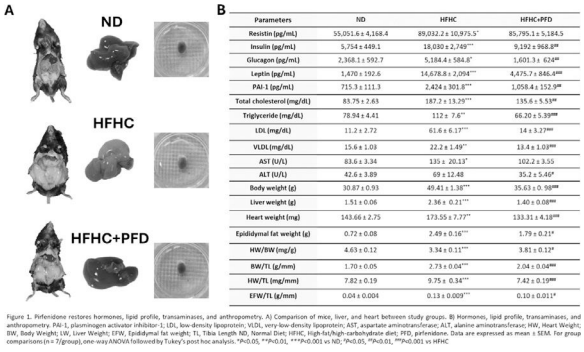

Parameters	ND	HFHC	HFHC+PFD
Resistin (pg/mL)	55,051.6 ± 4,168.4	89,032.2 ± 10,975.5*	85,795.1 ± 5,184.5
Insulin (pg/mL)	5,754 ± 449.1	18,030 ± 2,749****	9,192 ± 968.8##
Glucagon (pg/mL)	2,368.1 ± 592.7	5,184.4 ± 584.8*	1,601.3 ± 624##
Leptin (pg/mL)	1,470 ± 192.6	14,678.8 ± 2,094****	4,475.7 ± 846.4###
PAI-1 (pg/mL)	715.3 ± 111.3	2,424 ± 301.8****	1,058.4 ± 152.9##
Total cholesterol (mg/dL)	83.75 ± 2.63	187.2 ± 13.29****	135.6 ± 5.53##
Triglyceride (mg/dL)	78.94 ± 4.41	112 ± 7.6**	66.20 ± 5.39###
LDL (mg/dL)	11.2 ± 2.72	61.6 ± 6.17****	14 ± 3.27###
VLDL (mg/dL)	15.6 ± 1.03	22.2 ± 1.49**	13.4 ± 1.03###
AST (U/L)	83.6 ± 3.34	135 ± 20.13*	102.2 ± 3.55
ALT (U/L)	42.6 ± 3.89	69 ± 12.48	35.2 ± 5.46#
Body weight (g)	30.87 ± 0.93	49.41 ± 1.38****	35.63 ± 0.98###
Liver weight (g)	1.51 ± 0.06	2.36 ± 0.21****	1.40 ± 0.08###
Heart weight (mg)	143.66 ± 2.75	173.55 ± 7.77****	133.31 ± 4.18###
Epididymal fat weight (g)	0.72 ± 0.08	2.49 ± 0.16****	1.79 ± 0.21#
HW/BW (mg/g)	4.63 ± 0.12	3.34 ± 0.11****	3.81 ± 0.12#
BW/TL (g/mm)	1.70 ± 0.05	2.73 ± 0.04****	2.04 ± 0.04###
HW/TL (mg/mm)	7.82 ± 0.19	9.75 ± 0.34****	7.42 ± 0.19###
EFW/TL (g/mm)	0.04 ± 0.004	0.13 ± 0.009****	0.10 ± 0.011#

Figure 1. Pirfenidone restores hormones, lipid profile, transaminases, and anthropometry. A) Comparison of mice, liver, and heart between study groups. B) Hormones, lipid profile, transaminases, and anthropometry. PAI-1, plasminogen activator inhibitor-1; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HW, Heart Weight; BW, Body Weight; LW, Liver Weight; EFW, Epididymal fat weight; TL, Tiba Length ND, Normal Diet; HFHC, high-fat/high-carbohydrate diet; PFD, pirfenidone. Data are expressed as mean ± SEM. For group comparisons (n = 7/group), one-way ANOVA followed by Tukey's post hoc analysis. *P<0.05, **P<0.01, ***P<0.001 vs ND; #P<0.05, ##P<0.01, ###P<0.001 vs HFHC

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Large volume paracentesis: Is there a limit?

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Introduction and Objectives: Ascites is observed in 5-10% of cirrhotic patients. Large volume paracentesis (LVP), where >5 liters are drained, is safe. Albumin is essential to prevent post-paracentesis circulatory dysfunction (PPCD), with the literature indicating that its incidence increases when draining >8 liters in one session, suggesting draining a smaller amount.

Materials and Patients: An observational, analytical, and retrospective study was conducted, which included the clinical records of patients over 18 years of age admitted to the Gastroenterology service of the General Hospital of Mexico "Dr. Eduardo Liceaga" from January 2020 to March 2024 with a diagnosis of Grade II or III ascites, without criteria for acute kidney injury (AKI) according to the International Ascites Club (ICA) and with baseline creatinine available in

the last 3 months before assessment. The amount of ascites that were drained was evaluated, with no limit of liters in a session, and the occurrence of AKI during the following 7 days after paracentesis as a manifestation of PPCD. The definition of AKI was according to the latest definition by KDIGO / ICA. We excluded patients admitted with a diagnosis of AKI or a history of chronic kidney disease (CKD) of any etiology, and those in whom it was not specified whether albumin was administered after paracentesis. Descriptive statistics were performed with measures of central tendency and dispersion. We used X2, Student's T test, and Mann-Whitney U test to compare the variables. A value of $P < 0.05$ was considered statistically significant.

Results: We included 60 patients with a diagnosis of cirrhosis, administered for grade II and grade III ascites, 53.3% were men, with an overall mean age of 51.1 ±10.5 years. Regarding the etiology, 45% were due to alcohol, 21.7% to Fatty Liver Disease Associated with Metabolic Dysfunction (MASLD), as well as the etiology of no filiation; with MELD-Na 17.5 ± 5.7 points. Regarding ascites, 26.7% were grade II and 73.3% grade III, and up to 10% with refractory ascites. The average of liters of ascites drained per session was 8.5 ± 3.8 liters, with a minimum drainage of 5 liters and a maximum of 19.4 liters per session. Of the total patients evaluated, 5% (3) developed AKI after paracentesis, with an elevation of creatinine > 0.3 mg/dl in 48 hours. When comparing groups regarding the presence of ACLF, Child-Pugh, or MELD-Na; Regarding the DPPC, 41.66% (0%) drained less than 8 liters vs 58.34% (8.57%) more than 8 liters, all with refractory ascites, with no significant difference in the development of AKI (p=>0.05).

Conclusions: LVP is safe as long as the albumin dose is adequately replaced at a dose of 6-8 grams per liter of drained ascites in a single session, with caution in patients with refractory ascites, due to the advanced stage of portal hypertension.

Ethical statement: The patients signed informed consent for the PGV, and their data were protected.

Declaration of interests: None.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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Kasabach-Merritt syndrome in an adult treated by embolization prior to liver transplantation: a case report.

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Introduction and Objectives: Hepatic hemangioma, the most common benign tumor of the liver. Large ones may develop Kasabach Merrit syndrome (KM) if associated with coagulopathy.

Objective: to describe diagnostic approach and treatment of hemangioma with KM syndrome in an adult with complications during pregnancy, treated with embolization and liver transplantation, review of the literature.

Materials and Patients: A 35-year-old woman referred from Durango by angiology to the hepatology department for a failed laparoscopic biopsy attempt due to the presence of unspecified vascular lesions which presented bleeding due to severe coagulation disorders, controlled in her hospital of origin. During the consultation, imaging and biochemical characteristics of thrombocytopenia and anemia were evaluated and KM syndrome was considered, complementing the diagnosis with Leukocytes 5.3 × 103/uL, HB 10.3 g/dL, Hto 29.8%, VCM 99.2 fL, HCM 34.3 pg platelets 111 × 103/uL, Cr 0.61mg/dL, BT 1. 05mg/dl, FA 64 U/L, GGT 55 U/L AST 15 U/L, ALT 20 U/L, albumin 4.82g/dL, fibrinogen 52, dimer D 49.46 ug/dl, AFP 1.21 ng/ml, carcinoembryonic 0.94 ng/ml, Ca 19-9 2. 0 U/ml TP 14.6 INR 1.0, it was decided to perform a biopsy to rule out hemangioepithelioma, presenting severe hemorrhage requiring transarterial embolization on two occasions. Subsequently, she returned to the clinic with a normoevolutive pregnancy and a considerable increase in the size of the lesions, requiring cesarean section due to placenta accrete, again generating hemorrhage and development of ascites. Due to the hepatic deterioration, a protocol for transplantation was established and successfully performed in March 2024, with a total reversal of the coagulation disorders after the procedure and currently with no alterations.

Results: Hepatic hemangiomas are mostly asymptomatic and small; those larger than 10 cm are considered giants and present with non-specific symptoms such as abdominal pain, fatigue, etc. They are diagnosed by tomography (CT) or magnetic resonance imaging (MRI); in CT they are observed as relatively well-defined hypodense nodules, hypoattenuated in relation to parenchyma and centripetal peripheral enhancement with contrast medium, with complete and persistent opacification in late sections. It presents complications such as intralesional hemorrhage, mass effect in adjacent structures, and rupture with intraperitoneal hemorrhage. Some lesions may develop KM syndrome, a vascular disease characterized by thrombocytopenia, microangiopathic hemolytic anemia, coagulopathy and hepatic vascular lesions. The pathogenesis is due to the sequestration of platelets and coagulation factors in the abnormal endothelium of the vascular lesion. It requires biopsy to rule out malignant neoplasms (hemangioepithelioma). Occurs in neonates, rarely in adults. Transarterial embolization and chemoembolization can be used as a treatment for bleeding. Surgical resection is not recommended because of technical difficulty and risk of intraoperative bleeding. When there is severe liver dysfunction or recurrent bleeding, liver transplantation should be considered.

Conclusions: KM syndrome should be suspected in large vascular lesions accompanied by anemia, thrombocytopenia and coagulopathy; it is an uncommon complication that can generate hemorrhage and require management with interventional radiology or liver transplantation as in the case presented. Management should be multidisciplinary.

Ethical statement: The patient's identity was protected. Consentment was obtained directly from the patient.

Declaration of interests: None

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

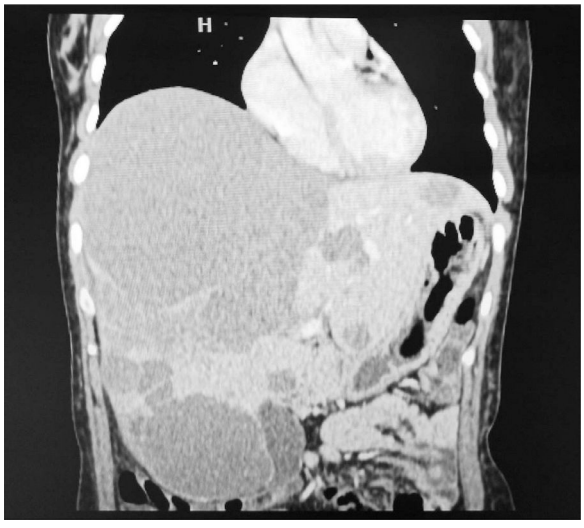

Figure.

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