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Assessment of siderosis and steatosis by MRI in patients with hyperferritinemia

Ana Carolina Oliveira Gomes da Costa

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Dissertação/Artigo original de candidatura a conclusão de Mestrado Integrado em Medicina, submetida ao Instituto de Ciências Biomédicas Abel Salazar, da Universidade do Porto.

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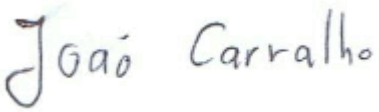

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Resumo:

Objetivo: A hiperferritinemia é um marcador da sobrecarga de ferro, contudo não é específico, sendo também um marcador de atividade inflamatória. A hiperferritinemia é um achado comum na prática clínica sendo importante, durante a sua investigação, avaliar se existe sobrecarga de ferro. A esteatose hepática, por sua vez, é uma manifestação comum a diversas doenças hepáticas difusas e é também uma causa frequente de hiperferritinemia.

Neste estudo, usamos as determinações de R2* por Ressonância Magnética como biomarcador de imagem da deposição de ferro, para avaliar a presença de sobrecarga hepática de ferro em pacientes com hiperferritinemia isolada, e correlacionámos os valores de R2* no fígado, pâncreas, baço e medula óssea. Para além disso, usamos as determinações de Proton Density Fat Fraction (PDFF) por RM para quantificar a esteatose hepática, determinando também a sua relação com a presença de siderose.

Materiais e Métodos: Por meio de ressonância magnética 3 Tesla, entre janeiro de 2014 e dezembro de 2018, avaliou-se a presença de esteatose hepática (PDFF) e deposição de ferro (R2*) no fígado, pâncreas, baço e medula óssea, em pacientes com hiperferritinemia isolada e suspeita de sobrecarga hepática. A esteatose hepática foi definida como um PDFF>4.8%. A deposição de ferro foi definida como R2* hepático superior a $81s^{-1}$ e a sobrecarga hepática de ferro como R2* superior a $200s^{-1}$ (>35 µmol/g).

Resultados: 33 pacientes (59%) apresentaram esteatose hepática (PDFF>4.8%). 52 pacientes (83.9%) apresentaram R2* elevado, sendo que destes, 24 (38,7%) apresentaram sobrecarga hepática de ferro (R2*>$200s^{-1}$). A ferritina sérica não apresentou correlação nem com o R2* hepático nem com a PDFF (p>0.05). De uma maneira geral, PDFF e R2* não mostraram relação entre si (p>0.05). Contudo, a frequência de esteatose em pacientes com elevação ligeira de R2* (81-$200s^{-1}$) foi significativamente superior (25/28) à dos pacientes com sobrecarga hepática de ferro (8/18, tendo-se excluído aqueles com R2*>$487s^{-1}$, já que nestes a medição de esteatose não é possível) (qui-quadrado de Pearson p<0,001). Verificou-se uma correlação significativa entre os valores de R2* hepático e esplénico, mas apenas no grupo de pacientes com R2* normal (0,72, p<0.001) ou ligeiramente aumentado (0,45, p<0.001). Não se verificaram correlações com o pâncreas nem com a medula óssea.

Conclusão: Não se verificou sobrecarga hepática de ferro na maioria dos doentes referenciados por hiperferritinemia isolada. Em pacientes referenciados por hiperferritinemia, particularmente naqueles com apenas uma ligeira elevação do R2*, a esteatose hepática foi o achado dominante.

Palavras Chave: Hiperferritinemia; RM; Sobrecarga de ferro.

Abstract:

Purpose: Hyperferritinemia is common in clinical practice, demanding a differential diagnosis of iron overload. Hepatic steatosis is a frequent cause of hyperferritinemia and a common pathological manifestation of many diffuse liver diseases.

We assessed hepatic iron overload by Magnetic Resonance Imaging-determined R2* (an imaging biomarker of iron) in patients referred with isolated hyperferritinemia, and we correlated R2* values of liver, pancreas, spleen and bone marrow. Furthermore, we used MRI determined Proton Density Fat Fraction (PDFF) for quantifying hepatic steatosis and to interrogate its relationship with siderosis.

Materials & methods: 3-Tesla-MRI determinations of liver steatosis (PDFF) and iron deposition (R2*) in the liver, pancreas, spleen and bone marrow were performed from January 2014 to December 2018 in 62 patients with isolated hyperferritinemia referred with suspicion of iron overload, using a multiecho chemical shift encoded gradient echo sequence. Liver steatosis was defined as PDFF>4.8%. Liver R2* thresholds were defined as $81s^{-1}$ (increased iron deposition) and $200s^{-1}$ (significant iron overload, >35 µmol/g).

Results: Thirty-three patients (59%) had liver steatosis (PDFF >4,8%) and 52 (83.9%) had elevated R2*, 24 (38.7%) having significant hepatic iron overload. Serum ferritin was not correlated with neither liver R2* nor PDFF (p>0.05). PDFF and R2* were in general not correlated (p>0.05) but the frequency of liver steatosis in patients with mildly (81-$200s^{-1}$) increased liver R2* was significantly higher (25/28) than in patients with iron overload (8/18, excluded those with R2*>$487s^{-1}$ in whom steatosis could not be measured) (Pearson Chi-square p<0,001). A significant correlation was observed between liver R2* values and spleen R2* values, only in the group of patients with normal (0,72, p<0.001) or mildly increased liver R2* (0,45, p<0.001). No correlations were found with pancreas or bone marrow R2*.

Conclusion: Significant hepatic iron overload is not found in the majority (>60%) of patients referred with isolated hyperferritinemia. In this group of patients, and particularly those with only mildly elevated R2*, steatosis is the dominant finding.

Keywords: Hyperferritinemia; MRI; Iron Load.

List of Abbreviations:

HF – Hyperferritinemia
HH – Hereditary Hemochromatosis
IO – Iron Overload
LIC – Liver Iron Concentration
MECSE-MR – Multi-Echo Chemical Shift-Encoded Gradient Echo Magnetic Resonance
MRI – Magnetic Resonance Imaging
PDF – Fat Proton Density
PDFF – Proton Density Fat Fraction
PDW – Water Proton Density
RES – Reticuloendothelial System
ROIs – Regions of interest

Index:

List of Tables:

List of Figures:

Introduction:

Elevated serum ferritin (hyperferritinemia, HF) is common in clinical practice and is often discovered during patient's routine evaluation. It is estimated that up to 12% of the normal population has HF. Serum ferritin is a nonspecific marker of iron overload (IO), and its levels can be elevated due to an increase in apoferritin synthesis or secretion; as a result of increased ferritin synthesis due to iron overload (as in hereditary hemochromatosis, HH) and also due to increased release of ferritin from damaged cells. Serum ferritin is also an acute phase protein, and it may be elevated in acute and chronic inflammatory conditions and malignancies.[1-4]

Hereditary hemochromatosis is an autosomal recessive disorder, caused by a mutation in the HFE gene or by other non-HFE mutations. This genetic disorder disrupts the iron body's regulation by greatly increasing its intestinal absorption, with iron accumulating mainly in parenchymal cells of the liver, pancreas, heart and gonads. [5, 6]

HF demands a differential diagnosis of IO. As the diagnosis of HH is a frequent concern in patients with HF, a significant number of patients are referred to tertiary centers for the diagnostic work-up of HH. Nevertheless, HF is more frequently related with inflammation, chronic alcohol consumption, cancer or metabolic disorders, making it essential to rule out this cofactors and comorbidities when first evaluating HF patients. Posterior work-up should focus on documenting hepatic IO because liver iron concentration (LIC) is a surrogate marker of total body iron stores.[7-9] Hepatic IO is the abnormal and excessive accumulation of iron in hepatocytes (parenchymal storage), Kupffer cells (reticuloendothelial system, RES), or in both, and is defined as a LIC greater than 35 μmol/g.[9-11]

Liver biopsy is the gold standard to quantify LIC. However, it suffers from several limitations, such as invasiveness, high variability and sampling biases. Magnetic Resonance Imaging (MRI) has proved to be an accurate alternative to quantify LIC, using transverse relaxation rates (R2*), with hepatic R2* values being directly related to LIC. [12-14] Furthermore, MRI-derived R2* measurements can also be used to assess iron deposition in other organs, such as the pancreas, spleen and bone marrow. This might provide us with valuable insights into the underlying mechanisms of iron accumulation in different IO disorders. For example, parenchymal iron deposition is usually the result of an increased absorption of iron, as in HH. In this case, iron accumulates mostly in the liver and pancreas, whereas extrahepatic reticuloendothelial organs are usually spared. On the other hand, iron deposition in the RES is more frequent in chronic hemolysis and blood transfusions, due to oversupply of exogenous or endogenous iron, and iron accumulates in the liver, spleen and bone marrow. Finally, hepatic iron deposition may also occur in patients with chronic liver disease and dysmetabolic syndrome. [1, 12-14] In this scenario, isolated hepatic iron deposition can be seen, but it is now increasingly recognized that iron can also accumulate in the RES.[12]

Hepatic steatosis is a common pathological manifestation of many diffuse liver diseases, including viral hepatitis, alcoholic liver disease, nonalcoholic fatty liver disease, and dysmetabolic iron overload syndrome. Dysmetabolic iron overload syndrome, also known as insulin resistance-associated hepatic iron overload, is recognized as a common cause of HF, being defined as mild hepatic iron excess in association with one or more metabolic abnormality related with insulin resistance, such as obesity, dyslipidemia or abnormal glucose metabolism. Histologically, these patients may have iron in the Kupffer cells, hepatocytes, or both, and it frequently coexists with liver steatosis.[15, 16] Moreover, the presence of both siderosis and steatosis in diffuse liver diseases has been associated with disease severity and fibrosis development.[14, 17]

New MRI sequences have been recently developed, allowing the simultaneous quantification of hepatic fat and iron deposits, within a single breath-hold acquisition. In 2017, França *et al*, concluded that a 3 Tesla multi-echo chemical shift-encoded gradient echo MR (MECSE-MR) sequence simultaneously quantifies liver steatosis and siderosis with high accuracy in a wide spectrum of diffuse liver disorders, regardless of liver inflammation or fibrosis.[17]

As HF can be related with both hepatic steatosis and siderosis, and because both can be easy, fast and non-invasively assessed by MRI, we aimed to evaluate by MRI a series of patients with HF referred to Oporto Universitary Hospital Center.

The aims of this study were to determine hepatic IO using MRI-R2*, in a series of patients referred for the investigation of HF; to investigate iron deposition in other abdominal tissues, by measuring R2* in the pancreas, spleen and bone marrow; to interrogate the relation between hepatic, pancreatic, splenic and bone marrow R2* measurements; and to quantify hepatic steatosis and its relationship with siderosis.

Materials and Methods:

The study population included patients with isolated HF and suspicion of IO, who were referred to the "Hemochromatosis outpatient clinic" of Oporto Universitary Hospital Center, from January 2014 to December 2018. Exclusion criteria were clinical suspicion of HH (familiar history, elevated transferrin saturation) or any contra-indication for MRI. A final population of 62 patients were evaluated with a 3-Tesla-MRI abdominal examination for determining liver steatosis (Proton Density Fat Fraction measurements, PDFF) and iron deposition (R2* measurements) using a MECSE sequence.

MRI:

The MRI studies were performed with 3 T MRI equipment (TX Achieva, Philips Healthcare, the Netherlands), using a sixteen-channel phased-array surface coil.

The two-dimensional (2D) multi-echo chemical shift encoded sequence used 12 echoes (echo times [TEs]=0.99 to 8.69, short echo spacing=0.7 ms; repetition time [TR]= 10 ms) with a 10º flip angle. The whole liver was covered under end-expiratory phase single breath-hold acquisition (34 slices; voxel dimensions=3 x 3 mm; slice thickness=7 mm; 0.3 mm gap; reconstruction voxel size=2 x 2 mm; field of view=375 X 302 mm; parallel imaging effective acceleration factor=1.8; bandwidth=2433 hertz per pixel). The total acquisition time for imaging the upper abdomen under end-expiratory phase single breathhold ranged between 12 and 15 seconds.

Quantification of PDFF and iron related R2* measurements were performed using QLiver software (QUIBIM, Valencia, Spain), which is based on least squares analysis by Levenberge Marcquardt algorithm, being used for the simultaneous quantification of R2* and PDFF.

Liver iron-related R2* values (s^{-1}) were calculated as the average measurements of four circular regions of interest (ROIs) of 8 mm diameter, manually placed in the right hepatic segments, avoiding vessels and lesions. Pancreatic R2* values were estimated as the average of 6 mm diameter ROIs, placed within the parenchyma at the head, body, and tail of the pancreas. Splenic R2* values were calculated as the average of three measurements obtained with 8 mm circular ROIs located in the splenic parenchyma. Vertebral bone marrow R2* values were estimated as the average values of three 8 mm ROIs, manually placed in the body of the 12th dorsal to the second lumbar vertebras.

The pixel PDFF was the ratio between the normalized fat proton density and the total (fat and water) proton density (PDFF = PDF/[PDF + PDW]). Liver steatosis was defined as PDFF > 4.8%. The calculated R2* was used to estimate iron content. Based on previous works, normal hepatic R2* measurements were under 81 s^{-1}; IO was defined as higher than 200 s^{-1} (corresponding to a LIC of 35 µmol/g).[18]

Statistical analysis:

Descriptive statistics were used to summarize patients' characteristics. Group data are presented as mean and standard deviation, or median and interquartile range.

The 62 patients were divided in three groups based on their liver R2* values: patients with normal R2* values (R2* < 81 s^{-1}), patients with mildly elevated R2* values (R2* between 81 and 200s^{-1}), patients with significantly elevated R2* (R2* > 200 s^{-1}).

Pearson chi-square test was used to compare the proportion of patients with steatosis in the group of mild iron deposition and in patients with IO.

Spearman's correlation coefficient was used to correlate liver, pancreas, spleen, and vertebral bone marrow R2* values. Spearman's correlation coefficient was also used to correlate ferritin with liver R2* and PDFF measurements, and to calculate the degree of association between liver R2* and PDFF measurements.

As PDFF measurements are corrupted by severe IO and PDFF quantitation is hampered if R2* measurements are higher than 487 s^{-1}, patients with R2* levels higher than 487 s^{-1} were not taken in consideration for PDFF quantification.[17]

SPSS (version 25; SPSS, IBM, Chicago, IL, USA) was used to perform the analysis. A $p<0.05$ was considered statistically significant for all tests.

Results:

The final population of 62 patients included 51 (82,3%) males, and 11 (17,7%) females. The mean age was 54 years (range: 12-81).

Liver R2* and PDFF distributions were considered non-symmetrical and therefore non-parametric tests were used. The mean liver R2* values were 216 s^{-1} (median 168 IQR 297-105, range from 45 to 746 s^{-1}). Mean, median, interquartile range, and range for MECSE-MR-derived PDFF were 7,9%, 7,4%, 11,6%–3.8%, and 0.9%–22.7%, respectively. Mean ferritin levels were 969 ng/dL (standard deviation: 474 ng/dL) and mean transferrin saturation was 37,8% (standard deviation: 15,7%) (Table I).

Liver steatosis (PDFF > 4,8%) was present in 33 (59%) patients, while the remaining 23 (41,1%) patients had normal PDFF values (< 4,8%) (Table II). Liver steatosis could not be assessed in 6 (9,7%) patients because R2* measurements were higher than 487 s^{-1}.

Ten patients (16,1%) had normal hepatic R2* values (R2* < 81s^{-1}) while 52 patients (83.9%) had elevated hepatic R2*: 28 (45,2%) had mildly increased R2* values (R2* between 81s^{-1} and 200s^{-1}) and 24 (38,7%) had significantly elevated R2* values (R2* > 200s^{-1}) (Table III).

Steatosis was more frequent in the group of patients with mildly increased liver R2* (81s^{-1}- 200s^{-1}) (25/28 patients, 89,3%), than in the group of patients with significantly elevated R2* (200 s^{-1}- 487 s^{-1}) (8/18 patients, 44,4%) (chi-square p-value < 0,001) (Table IV, Figure 1).

Serum ferritin was not correlated with neither liver R2* nor PDFF (p>0.05). However, there was a trend towards higher values of ferritin in the groups of patients with higher R2* (Figure 2).

When the different groups of liver R2* were separately analysed, a significant correlation was observed between liver R2* values and spleen R2* values, only in the group of patients with normal (r = 0,72) or mildly increased liver R2* (r = 0,45) (Table V). No correlation was found with pancreas or bone marrow R2* in any of the groups.

Discussion:

In our study population, liver R2* was elevated in 52 patients (83,9%). However, a significant elevation corresponding to hepatic IO, defined as LIC higher than 35 µmol/g (R2* > 200 s^{-1}), was only observed in 24 patients (38,7%), meaning that most of the patients with isolated HF did not have significant IO. In fact, although raised levels of ferritin often indicate IO, they are not a specific marker, once ferritin elevation can be seen in various conditions. Hepatic IO is found mainly in HH and transfusion related hemosiderosis.[13] As presented in previous literature, during the workup of HF, in about 90% of the patients other causes of HF are found, like chronic alcohol consumption, inflammatory diseases or non-alcoholic fatty liver disease. Consequently, most of the patients with HF will not have clinically relevant IO and will not need to undergo genetic testing.[4, 9]

Mild to moderate hepatic iron accumulation can occur in many liver diseases including chronic viral hepatitis, alcoholic or non-alcoholic fatty liver disease, and dysmetabolic iron overload syndrome. However, the underlying mechanisms to this process still remain unclear.[16] In our study, thirty-three patients (59%) had hepatic steatosis (PDFF>4,8%). In this group, the vast majority had liver R2* values consistent with mild iron deposition (R2* 81-200 s^{-1}), with only 8 having R2* values compatible with hepatic iron overload (>200 s^{-1}).

Because non-alcoholic fatty liver disease is the most prevalent chronic liver disease worldwide, having an estimated prevalence of 20-30% in the western populations, liver steatosis is, comprehensibly, a common finding in the general population. The relationship between serum ferritin levels and steatosis is well established and ferritin levels have even been linked to the severity of steatosis.[19, 20] As non-alcoholic fatty liver disease may be considered the hepatic manifestation of the metabolic syndrome, it is not surprisingly that dysmetabolic iron overload syndrome and HF associated to steatosis may be considered to be two different faces of the same disease spectrum, both related to insulin-resistance.[21, 22] There are many hypotheses linking steatosis to the elevation of serum ferritin levels. The most accepted is the two hits hypothesis. In this model, increased deposition of iron within the liver acts as the “second hit”, an additional insult that leads to increased liver inflammation, leading to tissue injury. The first “hit” is insulin resistance and visceral obesity, which contributes to increased circulating free fatty acids and onset of hepatic steatosis. Serum ferritin is the main iron-storage protein and is also an acute phase protein, so its level are elevated in the presence of liver inflammation.[23] In fatty liver disease, coexisting siderosis may be responsible for the progression of the disease, contributing, together with other insults, to a further increase in liver damage.[20, 24] In our study population, patients did not have any previously known disease apart from HF. Furthermore, particularly in those with only slightly elevated R2*, steatosis was the dominant finding.

The different distribution of iron within parenchymal or RES storage areas indicates different pathogenetic mechanisms of iron accumulation. We found a correlation between liver and spleen R2* in the group of patients with normal and mildly elevated liver R2*.[1, 11] The association between hepatic and splenic R2* in these groups points towards the accumulation of iron in the RES cells. This pattern of deposition is classically associated with chronic hemolysis and blood transfusions, but it was also described in diffuse hepatic diseases.[12] Steatosis is a common pathological manifestation of many diffuse liver diseases and dysmetabolic iron overload syndrome. Among our study population, those with RES iron deposition can potentially have their HF justified by the presence of liver steatosis, which was highly frequent in our sample. This data can be corroborated by the fact that among our patients with mildly elevated liver R2*, a high prevalence of liver steatosis was found.

On the other hand, some authors described that the distribution of iron in dysmetabolic iron overload can happen as hepatocellular iron deposition only, as RES cells only or it can appear as a mixed pattern of both hepatocellular and RES cells accumulation. The mechanisms leading to this different iron deposition patterns in patients with steatosis is not entirely clear, but is likely multi-factorial.[24] Therefore, is not possible to ascertain that the verified correlation is due to the presence of steatosis and larger studies are needed to confirm the relationship between steatosis and RES iron deposition.

In hemochromatosis and iron loading anemias, IO happens mainly in the hepatocytes in the liver and eventually in other organs, predominantly in the pancreas' β cells, usually sparing the spleen and bone marrow. In the group of patients with significantly elevated R2*, no correlations were found between liver R2* and the other abdominal tissues, meaning that iron deposition occurred mostly in the liver. These patients with hepatic IO are those who could benefit from further investigation, namely to exclude HH.[1, 11]

Our study has limitations. First of all, we had a small number of patients. Also, to our best knowledge, normal R2* values in the spleen, pancreas and bone marrow were not previously reported, neither their correlation with iron concentration in those organs. Therefore, iron deposition in these organs remains difficult to ascertain. Furthermore, the subgroups of patients were based on thresholds determined in previous studies, which can be influenced by the respective study populations. These thresholds may vary if other MRI sequences or magnetic fields are used instead.

In conclusion, significant hepatic iron overload is not found in the majority (>60%) of patients referred with isolated HF. In this group of patients, and particularly those with only mildly elevated R2*, steatosis is the dominant finding.

Appendix:

Table I – Liver R2, Liver PDFF and Ferritin descriptive statistics.*

			Liver R2 (s^{-1})*	*Liver PDFF (%)*	*Ferritin (ng/dL)*
N	*Valid*		*62*	*62*	*61*
	Missing		*0*	*0*	*1*
Mean			*216*	*7,9*	*969*
Median			*168*	*7,4*	*951*
Range			*701*	*21,8*	*2048*
Minimum			*45*	*0,9*	*185*
Maximum			*746*	*22,7*	*2233*
Percentiles		*25*	*105*	*3,8*	*650,5*
		50	*168*	*7,4*	*951*
		70	*297*	*11,6*	*1273,5*

Table II - Frequency and percentage of patients with and without steatosis.

Steatosis	*Frequency*	*Percent (%)*
PDFF < 4,8%	*23*	*41*
PDFF > 4,8%	*33*	*59*
Total	*56*	*100*

Table III – Frequency and percentage of patients in each of liver R2 values categories.*

	Frequency	*Percent (%)*
Liver R2 < 81 s^{-1}*	*10*	*16,1*
Liver R2 81 – 200 s^{-1}*	*28*	*45,2*
Liver R2 > 200 s^{-1}*	*24*	*38,7*
Total	*62*	*100*
Liver R2 > 487 s^{-1}*	*6*	*9,7*

Table IV – The frequency of liver steatosis in patients with mildly increased R2 was significantly higher (25/28) than in patients with significantly elevated R2* (8/18) (Chi-square p-value = 0,001).*

		Liver R2 81-200 s^{-1}*	*Liver R2* 201-487 s^{-1}*	*Total*
Steatosis	*PDFF < 4,8%*	*3*	*10*	*13*
	PDFF > 4,8%	*25*	*8*	*33*
Total		*28*	*18*	*46*

Table V – Spearman correlation coefficients between liver R2 groups and spleen, pancreas and bone marrow R2*.*

Liver R2 (s^{-1})*	*Spleen R2* (s^{-1})*	*Pancreas R2* (s^{-1})*	*Bone marrow R2* (s^{-1})*
Normal	***0,72 (p = 0,02)****	*-0,08 (p = 0,83)*	*0,44 (p = 0,2)*
Mildly elevated	***0,45 (p = 0,02)****	*0,13 (p = 0,51)*	*0,03 (p = 0,88)*
Significantly elevated	*-0,42 (p = 0,08)*	*-0,13 (p = 0,62)*	*-0,29 (p = 0,25)*

* Correlation is significant at the 0.01 level (2-tailed).

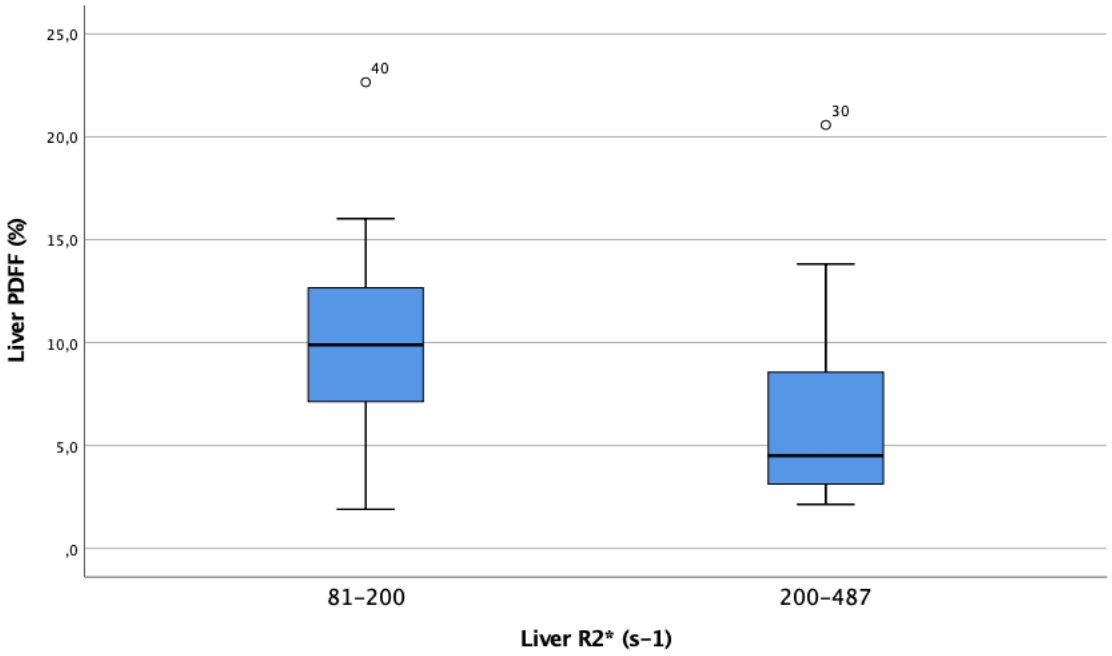

Figure 1 - Boxplot of liver PDFF in patients with mildly (81-200 s^{-1}) and significantly (200-487 s^{-1}) elevated liver R2 values.*

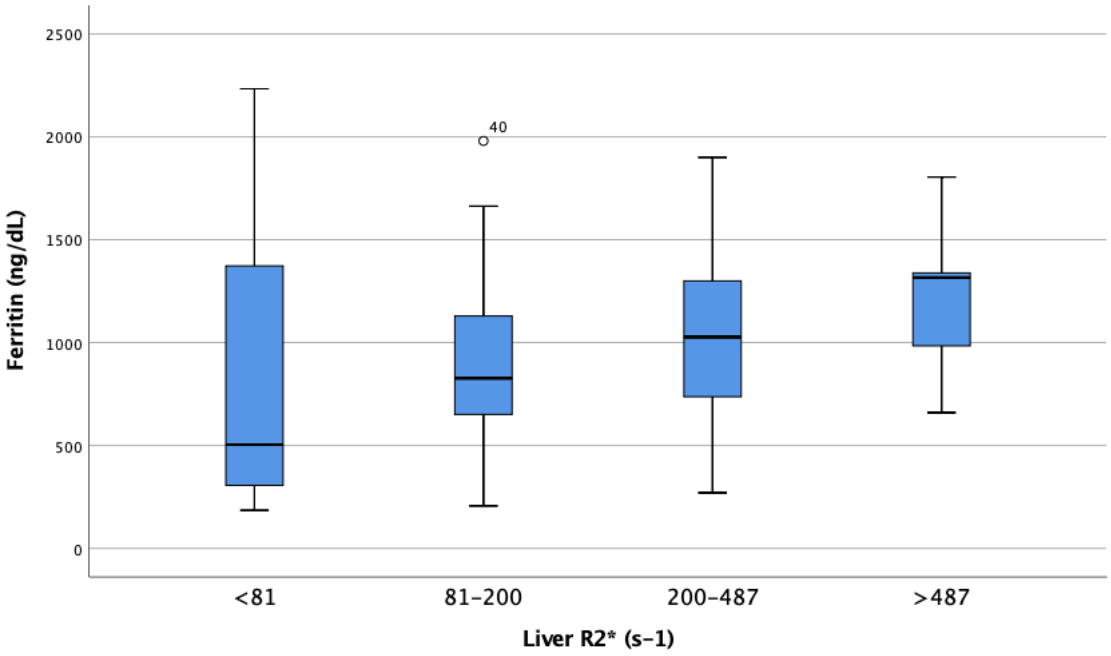

Figure 2 - Boxplot with ferritin distribution in different liver R2 groups showing the trend towards higher values of ferritin in the groups with higher liver R2*.*

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