

A randomized trial of iron depletion in patients with nonalcoholic fatty liver disease and hyperferritinemia

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Abstract

AIM: To compare iron depletion to lifestyle changes alone in patients with severe nonalcoholic fatty liver disease (NAFLD) and hyperferritinemia, a frequent feature associated with more severe liver damage, despite at least 6 mo of lifestyle changes.

METHODS: Eligible subjects had to be 18-75 years old who underwent liver biopsy for ultrasonographically detected liver steatosis and hyperferritinemia, ferritin levels ≥ 250 ng/mL, and NAFLD activity score > 1 . Iron depletion had to be achieved by removing 350 cc of blood every 10-15 d according to baseline hemoglobin values and venesection tolerance, until ferritin < 30 ng/mL and/or transferrin saturation (TS) $< 25\%$. Thirty-

eight patients were randomized 1:1 to phlebotomy ($n = 21$) or lifestyle changes alone ($n = 17$). The main outcome of the study was improvement in liver damage according to the NAFLD activity score at 2 years, secondary outcomes were improvements in liver enzymes [alanine aminotransferases (ALT), aspartate aminotransferase (AST), and gamma-glutamyl-transferases (GGT)].

RESULTS: Phlebotomy was associated with normalization of iron parameters without adverse events. In the 21 patients compliant to the study protocol, the rate of histological improvement was higher in iron depleted vs control subjects (8/12, 67% vs 2/9, 22%, $P = 0.039$). There was a better improvement in steatosis grade in iron depleted vs control patients ($P = 0.02$). In patients followed-up at two years ($n = 35$), ALT, AST, and GGT levels were lower in iron-depleted than in control patients ($P < 0.05$). The prevalence of subjects with improvement in histological damage or, in the absence of liver biopsy, ALT decrease $\geq 20\%$ (associated with histological improvement in biopsied patients) was higher in the phlebotomy than in the control arm ($P = 0.022$). The effect of iron depletion on liver damage improvement as assessed by histology or ALT decrease $\geq 20\%$ was independent of baseline AST/ALT ratio and insulin resistance ($P = 0.0001$).

CONCLUSION: Iron depletion by phlebotomy is likely associated with a higher rate of improvement of histological liver damage than lifestyle changes alone in patients with NAFLD and hyperferritinemia, and with amelioration of liver enzymes.

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Key words: Nonalcoholic fatty liver disease; Iron depletion; Randomized controlled trial; Ferritin

Core tip: We compared in a randomized controlled trial iron depletion by phlebotomy ($n = 21$) to standard therapy ($n = 17$) in patients with severe nonalcoholic fatty liver disease and hyperferritinemia despite at least 6 mo of lifestyle changes. Phlebotomy was associated with normalization of iron parameters without adverse events. In the 21 patients compliant to the study protocol, the rate of histological improvement was higher in iron depleted *vs* control subjects ($P = 0.039$). In patients followed-up at two years ($n = 35$), by the end of the study alanine aminotransferases, aspartate aminotransferase, and gamma-glutamyl-transferases levels were lower in iron-depleted than in control patients ($P < 0.05$).

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD), the hepatic expression of the metabolic syndrome^[1], affects 20%-30% of the Western population^[2,3]. When associated with necroinflammation and ballooning, defining nonalcoholic steatohepatitis (NASH), NAFLD may evolve to cirrhosis and hepatocarcinoma^[4]. Although lifestyle modifications improve liver damage^[5], long-term compliance is difficult to achieve^[6], and no pharmacological approach is yet approved for NASH.

Hyperferritinemia with mild hepatic iron accumulation is observed in 20%-30% of patients with NAFLD referred to tertiary centres^[7-10], and is commonly referred as dysmetabolic iron overload syndrome^[11]. Besides directly inducing liver damage^[11], excess iron is involved in the pathogenesis of metabolic syndrome by inducing adipose tissue insulin resistance and modifying the release of adipokines^[11-13]. Furthermore, hyperferritinemia and increased iron stores have been associated with the severity of liver disease and hepatocellular carcinoma in patients with NAFLD^[10,14-17].

Therefore, previous studies have evaluated the therapeutic value of iron depletion in NAFLD. Phlebotomy ameliorated insulin resistance more than lifestyle changes alone in a matched case-control study including 128 patients^[18], whereas in a propensity score adjusted multicenter study with prospective evaluation of 198 patients, it increased the likelihood of alanine aminotransferases (ALT) normalization at the end of follow-up^[19]. Furthermore, in a phase II trial in 31 patients with NAFLD, phlebotomy was associated with and improvement in disease activity^[20].

Aim of this study was to compare the effect of iron depletion by phlebotomy to that of maintenance of lifestyle changes alone (*i.e.*, the standard of care) on histological liver damage evaluated at 24 mo in a randomized controlled phase III trial in subjects with NAFLD and hyperferritinemia (ClinicalTrials.gov Identifier: NCT00658164).

MATERIALS AND METHODS

Eligible subjects had to be 18-75 years old who underwent liver biopsy between January 1st 2007, and December 31st 2010 for ultrasonographically detected liver steatosis and hyperferritinemia despite at least 6 mo of lifestyle modifications, ferritin levels ≥ 250 ng/mL or histological evidence of increased iron stores, and NAFLD activity score (NAS) > 1 (henceforth defined as "severe NAFLD") at a liver biopsy obtained within 6 mo before randomization, as commonly accepted in NAFLD trials^[21]. Subjects had to be willing to maintain diet and exercise during the full course of the study, to give written informed consent, and to comply with all study requirements.

We excluded patients with decompensated liver disease, pregnant or lactating females, type 1 or secondary forms of diabetes, thyroid diseases (by TSH evaluation), alcohol consumption > 20 g/d for females and > 30 g/d for males, BMI ≥ 35 kg/m², other liver diseases, alpha-1-antitrypsin deficiency (alpha-1-antitrypsin levels < 80 mg/dL or PiZ/PiZ or PiZ/PiS genotype), and hereditary hemochromatosis (HFE C282Y/C282Y or C282Y/H63D or hepatic iron index ≥ 1.9), previous or active chronic HCV and HBV hepatitis (HBsAg, ABcAg, and anti-HCV Ab were evaluated in all subjects), congestive heart failure and ischemic heart disease, systolic dysfunction (ejection fraction $\leq 45\%$), ECG abnormalities, malignancy within the last 5 years, serum creatinine levels > 1.5 mg/dL males, > 1.4 mg/dL females, use of drugs known to induce NAFLD, and basal hemoglobin levels < 11 g/dL. Before randomization, all patients underwent electrocardiographic evaluation, and in the presence of hyperglycemia or hypertension also echocardiography. Carriers of *HFE* gene mutation without hemochromatosis were enrolled, as *HFE* mutations are strong risk factors for mildly increased iron stores in NAFLD^[17,22].

Patients were randomized 1:1 by random numbers generation to lifestyle changes alone or lifestyle changes associated with iron depletion by phlebotomy. In the phlebotomy group, iron depletion had to be achieved by removing 350 cc of blood every 10-15 d according to baseline hemoglobin (Hb) values and venesection tolerance, until ferritin < 30 ng/mL and/or transferrin saturation (TS) $< 25\%$. Weekly phlebotomies were allowed for carriers of the C282Y *HFE* mutation, smaller phlebotomies (250 cc) for carriers of beta-thalassaemia trait. Maintenance phlebotomies (as much as required) were then performed to keep iron stores depleted (target: ferritin < 50 ng/mL and $< 25\%$, MCV < 85 fL). Iron removed to

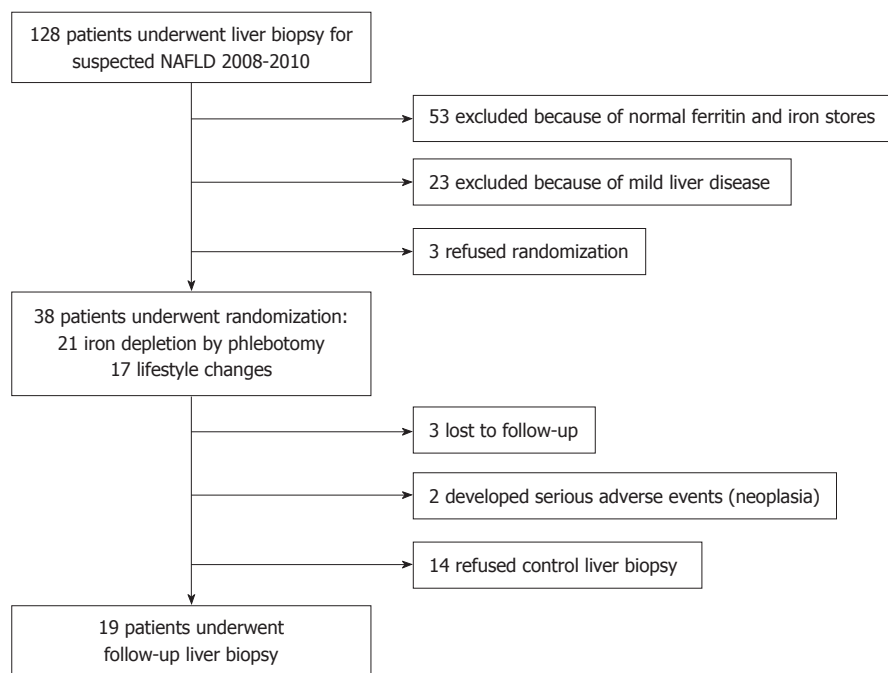


Figure 1 Study flow chart. NAFLD: Nonalcoholic fatty liver disease.

depletion was estimated as previously described^[23].

Visits were scheduled at week: -2, baseline (day 1), and at months 6, 12, 18, 24, for physical examination, evaluation of vital signs and anthropometric measures (weight, abdominal circumference), laboratory evaluation, including complete blood count, liver enzymes, iron parameters, C reactive protein, glucose, insulin, serum lipids. Hepatic ultrasonography was performed at baseline, year 1, year 2.

Adverse events were reported at each visit, and when notified by the patients. Changes in diabetes medication dosage or start of new therapy (metformin) have been allowed for HbA1C values < 6% or ≥ 7%.

The primary outcome of the study was improvement of histological damage related to NAFLD, as defined by any improvement of NAS (based on the severity of steatosis graded 0-3, lobular necroinflammation graded 0-3, and hepatocellular ballooning graded 0-2) without worsening of liver fibrosis, or an improvement of liver fibrosis without worsening of NAS^[21]. Liver biopsies have been reviewed in a blinded fashion by an expert pathologist (Maggioni M). Hepatic iron concentration was quantified by atomic absorption spectrometry^[24], and scored according to Deugnier *et al.*^[25]. Secondary outcomes included the evaluation of the effect of phlebotomy on liver enzymes [ALT, aspartate aminotransferases (AST), and gamma-glutamyl-transferases (GGT) levels].

The protocol was approved by the Ethical Committee of the Fondazione IRCCS Ca' Granda Ospedale Policlinico Milano, and conforms to the principles of the Declaration of Helsinki. Informed written consent has been recorded for each subject participating in the study. The study flow chart is presented in Figure 1. The clinical features of enrolled patients are shown in Table 1.

Statistical analysis

The sample size was based on the estimate that 20% of the patients would spontaneously improve liver histology in the lifestyle changes group. To detect a 45% response rate among the treatment patients, using an alpha level of 0.05 with 80% power, we estimated we needed to include 62 patients per group. However, the study was closed before the completion of enrolment because of the difficulties in accepting 24 mo control liver biopsies. Therefore, we report the results obtained in patients enrolled in the proposing center (Fondazione IRCCS Ca' Granda Milano) during the predefined time period (2007-2010).

Data are expressed as mean ± SD, median (interquartile range), and frequency (%) according to distribution. Study outcomes were compared between the two treatment arms by χ^2 test, Student *t* test or Wilcoxon, as required according to data distribution. Improvement in histological indices of liver damage activity were compared by Wilcoxon signed-rank test. Logistic regression analysis considering the treatment arms and major clinical confounders was conducted to adjust the effect of treatment on histological improvement and ALT normalization for clinical factors (included variables are reported in the results section). *P* values were considered significant when ≤ 0.05 (two-tailed).

RESULTS

Study cohort

We enrolled 38 subjects: 21 were randomized to phlebotomy and 17 to the lifestyle changes arm (Figure 1). Patients were mostly middle-aged overweight men with central distribution of adiposity, moderate to severe ste-

Table 1 Baseline features of 38 randomized subjects with nonalcoholic fatty liver disease and hyperferritinemia/ increased iron stores according to treatment allocation

Baseline features	Lifestyle changes alone (n = 17)	Phlebotomy (n = 21)	P value
Age (yr)	53 ± 11	55 ± 8	0.45
Gender (female)	3 (18)	1 (5)	0.31
Waist circumference, cm	103 ± 11	102 ± 10	0.70
BMI (kg/m ²)	28.2 ± 4.7	27.7 ± 3.5	0.71
HOMA-IR	4.1 ± 2.5	4.4 ± 2.3	0.67
HDL chol (mg/dL)	47 ± 18	48 ± 8	0.88
Triglycerides (mg/dL)	141 ± 87	129 ± 53	0.61
Type 2 diabetes	1 (6)	4 (19)	0.23
Ferritin (ng/mL)	642 (505-907)	710 (548-997)	0.40
TS (%)	40 ± 14	42 ± 13	0.67
HIC (μg/100 mg dry tissue)	234 (98-358)	303 (238-570)	0.23
TIS	6 (4-12)	9 (6-11)	0.19
ALT (UI/mL)	39 ± 29	43 ± 25	0.61
AST (UI/mL)	30 ± 14	31 ± 14	0.70
GGT (UI/mL)	40 (24-56)	28 (21-41)	0.36
US Steatosis grade > 1	17 (100)	17 (81)	0.11
NAS ≥ 3	11 (65)	13 (63)	1.00
Fibrosis stage 2-4	3 (18)	7 (33)	0.46
HFE genotype			
C282Y+ or H63D +/+	2 (11)	2 (10)	0.82
Beta-thalassemia trait	4 (23)	4 (19)	1.00

Data are expressed as absolute numbers (percentage), mean ± SD or median (interquartile range). ALT: Alanine aminotransferases; AST: Aspartate aminotransferases; BMI: Body mass index; Chol: Cholesterol; GGT: Gamma-glutamyl-transferases; HDL: High density lipoprotein; HFE: Hemochromatosis gene; HIC: Hepatic iron concentration; HOMA-IR: Homeostasis model assessment insulin resistance index; NAS: Nonalcoholic fatty liver disease activity score; TIS: Tissue iron score; TS: Transferrin saturation; US: Ultrasonographic.

atosis and insulin resistance (Table 1). At baseline, about two thirds of patients had NASH, and roughly a quarter moderate to severe fibrosis (stage 2-4; Table 1). There were no significant differences in basal demographic, clinical, histological and genetic features between the two treatment arms (Table 1). The majority of patients (22/38, 58%) had already lost > 5% of body weight after clinical presentation, while the other were not able to modify body weight despite at least 6 mo of lifestyle counseling. All patients had hyperferritinemia at baseline. Four patients (10%) had hepatic iron concentration within the upper reference range (150 μg-100 mg), but all were positive for stainable iron at liver biopsy.

Effect of treatment on iron metabolism and body mass

In patients randomized to iron depletion, phlebotomy achieved a reduction in biochemical iron parameters (Figure 2A, B, $P < 0.0001$). Ferritin levels dropped progressively towards the treatment target, whereas TS was already reduced to low to normal levels at 6 mo and remained stable throughout the study period. In phlebotomized patients, iron depletion was reached after a median of 16 phlebotomies, interquartile range (IQR) 12-18, corresponding to a median of 4.3 g of iron, IQR 2.4-5.6. In patients with follow-up liver biopsies, tissue iron score

was significantly decreased in phlebotomized patients (median -9, IQR -9/-1, $P = 0.018$), but not in controls (median -2, IQR -5/0, $P > 0.05$).

No patient reported adverse events nor developed anemia (Hb levels during the study are shown in Figure 2C), or alterations in renal function tests (not shown).

No significant loss in body weight was achieved and maintained during the study in either arms ($P > 0.7$), and there was no significant difference in body mass between treatment groups at the end of the study (27.2 ± 3.9 kg/m² in the phlebotomy arm *vs* 27.6 ± 5.2 kg/m² in the control arm, $P = 0.8$).

Effect of treatment on histological damage

Follow-up liver biopsy was performed in 19/38 patients (50%). Two patients developed severe adverse events (neoplastic diseases, all in the control group), precluding follow-up liver biopsy. Therefore, 21 patients were evaluated in a per protocol analysis. Of the remaining patients, three were lost to follow-up, and 14 refused control liver biopsy (Figure 1). Reasons most commonly reported for retiring consent from control liver biopsy were complete normalization of iron indices and liver function tests in the phlebotomy group, and the possibility to undergo phlebotomy for persistently abnormal iron indices in the control group.

Histological improvement of liver damage in an intention to treat analysis considering all randomized patients was non-significantly higher in iron depleted *vs* control patients (Figure 3A, $P = 0.067$), but the difference was statistically significant in the per protocol analysis (Figure 3B, $P = 0.039$).

Variations in histological indices of liver damage activity in individual patients subdivided according to treatment arm are presented in Figure 4. Patients randomized to iron depletion had a significantly better improvement in steatosis grade to that observed in the control group ($P = 0.02$), whereas differences in the improvement of necroinflammation and ballooning were not statistically significant.

Effect of treatment on liver enzymes

The time course of liver enzymes during the study period is shown in Figure 5. At later time points, ALT, AST, and GGT levels were significantly lower in phlebotomized patients than in controls ($P < 0.05$).

At receiver-operating characteristic curve analysis conducted in the 19 patients who underwent both baseline and follow-up liver biopsy (not shown), a decrease in ALT levels $\geq 20\%$ from baseline to the end of the study represented the best cut-off among the available biomarkers for predicting improvement of histological damage (sensitivity = 100%, specificity = 78%, positive predictive value = 83%, negative predictive value = 100%, positive likelihood ratio = 4.54, negative likelihood ratio = 0.00).

The prevalence of subjects with improvement in histological damage or, in the absence of liver biopsy, ALT decrease $\geq 20\%$ (overall liver damage assessment) was

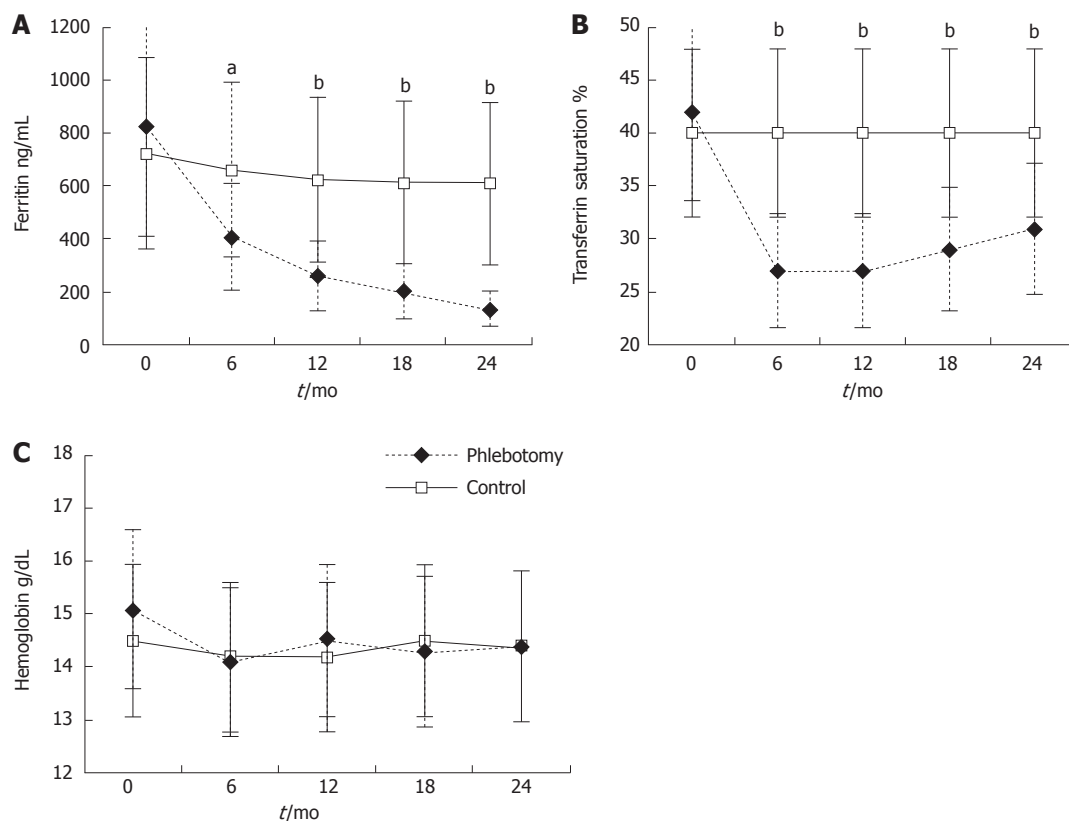


Figure 2 Effect of treatment on iron status and erythropoiesis. Effect of treatment on the levels of ferritin (A), transferrin saturation (B), and hemoglobin (C). Phlebotomy: dashed lines and black squares; Lifestyle changes alone: continuous lines and empty squares. ^a $P < 0.05$, ^b $P < 0.01$ between phlebotomy and lifestyle changes alone treatment arms.

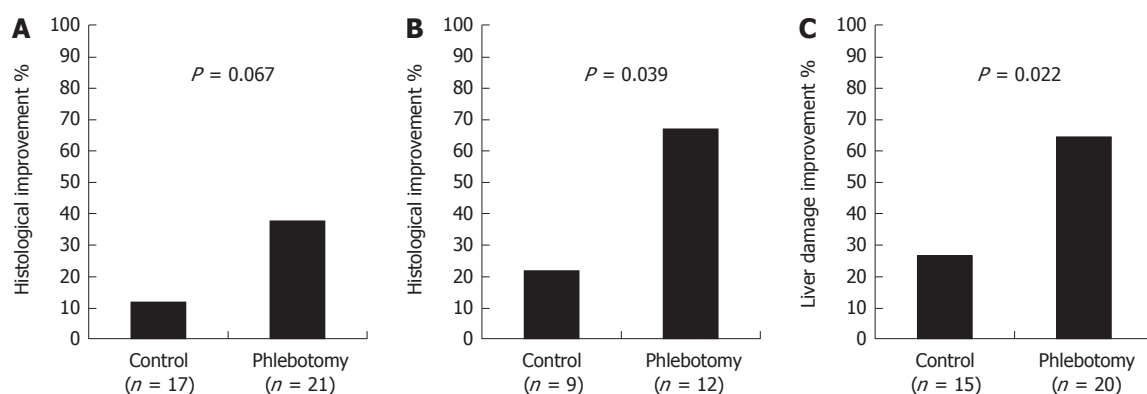


Figure 3 Effect of treatment on liver damage. Prevalence of demonstrated histological improvement of liver damage at 2 years (any improvement in NAS score without worsening of fibrosis stage; main outcome of the study) in all patients who underwent randomization (panel A; $n = 38$; intention to treat analysis), and in patients compliant to the study protocol [panel B; $n = 21$ (19 biopsied and 2 failures due to serious adverse events), per protocol analysis]. The effect of treatment on overall liver damage assessment [improvement in liver histology or alanine aminotransferases (ALT) decrease $\geq 20\%$ at the end of the study ($n = 35$, three patients could not be assessed because lost to final follow-up evaluation)]; direct and indirect analysis of liver damage evolution in all patients that completed follow-up] is shown in panel C.

higher in the phlebotomy than in the control arm ($P = 0.022$; Figure 3C).

Independent predictors of liver damage progression

Independent predictors of liver damage improvement (overall assessment) by multivariate logistic regression analysis considering variables most strongly associated at univariate analysis ($P < 0.01$) are shown in Table 2. Iron

depletion was strongly associated with improvement in liver damage independently of confounders. In addition, higher baseline AST/ALT ratio (a validated non-invasive index of liver fibrosis) and insulin resistance (homeostatic metabolic assessment insulin resistance: HOMA-IR index) were negative predictors of a favourable outcome. There was no significant influence of age, gender, baseline iron parameters and hepatic iron stores, and

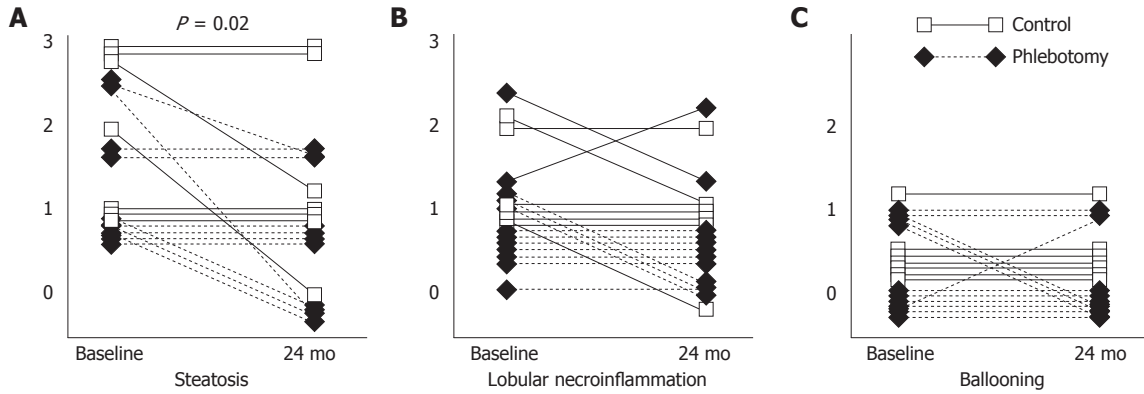


Figure 4 Variations in histological indices of liver damage activity in individual patients subdivided according to treatment arm. A: Steatosis grade ($P = 0.02$ at Wilcoxon signed-rank test); B: Necroinflammation ($P > 0.05$); C: Ballooning ($P > 0.05$).

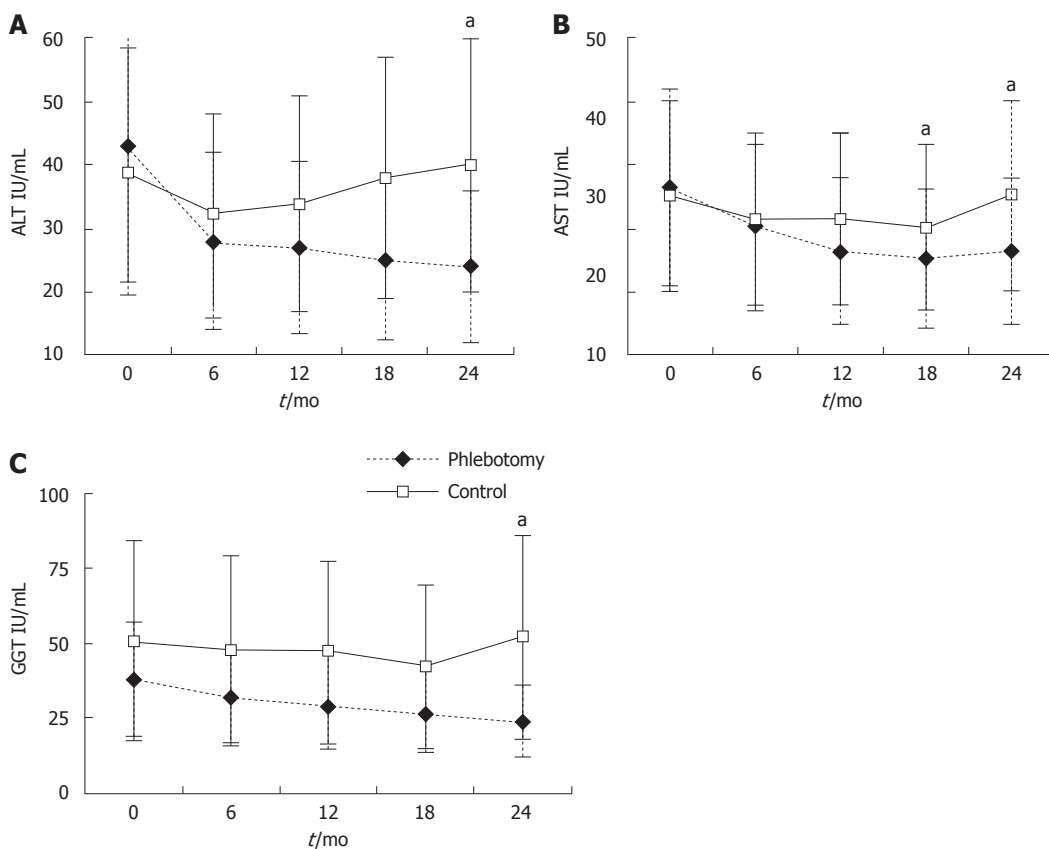


Figure 5 Effect of treatment on liver enzymes. Time course of liver enzymes: alanine aminotransferases (ALT, A), aspartate aminotransferases (AST, B), and gamma-glutamyl-transferases (GGT, C) during the study. Phlebotomy: Dashed lines and black squares; Lifestyle changes alone: Continuous lines and empty squares. $^aP < 0.05$ between phlebotomy and lifestyle changes alone treatment arms.

the genetic background on liver damage improvement (not shown). The outcome was worse in patients with higher baseline abdominal circumference, but the effect was not independent of insulin resistance (not shown). There was no significant interaction between treatment arm and either AST/ALT ratio or HOMA-IR on the improvement of liver damage.

DISCUSSION

In this paper, we present the results of a phase III ran-

domized controlled trial of iron depletion by phlebotomy on histological liver damage in patients with severe NAFLD and hyperferritinemia, a commonly observed clinical syndrome associated with a substantial risk of progressive liver disease^[10,11,14-17].

Difficulties in recruiting patients due to the requirement of follow-up liver biopsy, and to the low rate of compliance to this procedure, reduced the statistical power of the study. Notwithstanding, results, that for the first time are derived from a randomized trial with a histological outcome, are consistent with improvement

Table 2 Independent predictors of improvement of liver damage evaluated by multivariate logistic regression analysis at the end of the trial in 35 patients with nonalcoholic fatty liver disease and hyperferritinemia/increased iron stores ($P < 0.0001$ for model)

Independent predictors ¹	OR	95%CI	P value
Iron depletion by phlebotomy	265	8.9-1 × 10 ⁶	0.0001
AST/ALT ratio	0.86	0.72-0.94	< 0.0001
HOMA-IR index	0.28	0.05-0.65	0.0003

¹Liver damage included direct or indirect evaluation of liver damage in all patients that completed follow-up. AST/ALT: Alanine aminotransferases/aspartate aminotransferases; HOMA-IR: Homeostasis model assessment insulin resistance index.

of histological damage in patients who underwent iron depletion, also in line with previous results based on the assessment of non-invasive markers of liver damage and on the results of uncontrolled studies^[18-20].

Indeed, despite iron depletion was not associated with a significant improvement of liver damage at the intention to treat analysis, there was a higher rate of histological improvement of NAFLD activity score in patients randomized to iron depletion who attained to the clinical protocol. The difference in the improvement rate (roughly 65% *vs* 25% in the control group) was maintained when liver damage improvement was estimated by a non-invasive predictor of liver damage evolution, *i.e.*, serum ALT decrease $\geq 20\%$ compared to baseline, which showed a high accuracy in the prediction of histological improvement in patients with follow-up liver biopsy. The limited rate of improvement in the control group is consistent with the selection of patients that were already resistant to lifestyle changes at baseline^[6], and by the persistence of altered iron metabolism during the study.

Strengths of the study include the high comparability of clinical features in the two treatment arms at baseline, and the fact that all the secondary outcomes related to improvement of liver enzymes (which could be evaluated in the majority on randomized patients) were met. Finally, at multivariate logistic regression analysis the favourable effect of iron depletion on liver damage improvement was independent of the other identified predictors, including the AST/ALT ratio, reflecting baseline severity of liver damage, and of insulin resistance.

Notably, phlebotomy normalized iron parameters and hepatic iron stores without adverse effects, whereas two severe adverse events were observed in the control group, consisting in diagnosis of neoplastic diseases after 1 year of follow-up. The difference is likely due to chance, even if increased iron stores have been linked with cancer risk^[26], and iron depletion may reduce carcinogenesis^[27].

Limitations related to the limited power of the present study must be weighted against the lack of available pharmacological treatments for progressive liver disease in NAFLD and NASH, which is at least partly related to the difficulty of conducting trials requiring short-term follow-up liver biopsies^[28]. The difficulties in maintaining proto-

col compliance were amplified in the treatment arm by the positive effect of phlebotomy on biochemical indices, and in the controls by the possibility to switch to active treatment (which can be prescribed even in the absence of evidence of efficacy on liver damage progression) without the need of control biopsy. We believe that these difficulties are inherent to the lack of possibility to perform a phlebotomy trial in blinded fashion, and to the availability of active treatment outside the research setting.

Finally, we think that the choice of considering any improvement of NAFLD activity score without worsening fibrosis as primary outcome (instead of higher cutoffs), and liver enzymes as secondary outcomes was appropriate for this study. Indeed, the pathophysiology of liver damage progression is different in patients with increased hepatic iron stores than in those with classic NAFLD, in that in the presence of hepatocellular iron liver damage is associated with lesser severity of steatosis and is not invariably dependent on apoptosis^[11-13,29,30], but also on other forms of cellular damage including ferroptosis^[31]. Given the evidence that, when associated with histopathological signs of liver damage, even steatosis without a diagnosis of definite NASH frequently progress to fibrosis^[32], we also believe that the inclusion in the trial of subjects without definite NASH, but with iron overload coupled with severe steatosis or necroinflammation/hepatocellular damage, was appropriate.

In conclusion, iron depletion by phlebotomy is probably associated with a higher rate of improvement of liver damage improvement than the maintenance of lifestyle changes alone in patients with severe NAFLD and hyperferritinemia, who did not normalize liver enzymes after at least 6 mo of lifestyle counseling. In addition, iron depletion ameliorates liver enzymes, with a possible impact on the natural history of the disease. The present and previous results^[18-20] suggest that, in the absence of pharmacological treatments, larger trials evaluating phlebotomy treatment may be conducted in patients with severe NAFLD and hyperferritinemia resistant to lifestyle changes.

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COMMENTS

Background

Hyperferritinemia associated with mildly increased hepatic iron stores is frequently observed in nonalcoholic fatty liver disease (NAFLD), the hepatic manifestation of the metabolic syndrome and now a leading cause of liver disease, and has been associated with more advanced liver damage. Pathophysiological studies suggested that iron stores may be involved in the pathogenesis of insulin resistance and liver disease progression, and controlled studies indicated that iron depletion by phlebotomy may decrease insulin resistance and liver enzymes in patients with NAFLD.

Research frontiers

However, the effect of iron depletion on the progression of liver damage as evaluated by histological examination, the gold standard for assessment, has

not been previously tested in a randomized controlled trial in patients with NAFLD.

Innovations and breakthroughs

In a selected population of Italian patients with NAFLD and hyperferritinemia which persisted despite at least 6 mo of lifestyle counseling, who were found to have mild hepatic iron overload, iron depletion by phlebotomy was well tolerated and induced a more marked amelioration of liver enzymes, non-invasive markers of liver damage, than lifestyle counseling alone. In addition, in a subgroup of patients who underwent follow-up liver biopsy at 2 years, iron depletion was associated with a higher probability of improvement of the histological activity of NAFLD.

Applications

These findings suggest that iron depletion by phlebotomy should be further studied, as it represents as an attractive therapeutic approach for patients with NAFLD with persistent hyperferritinemia despite a trial of lifestyle change/additional therapies or with increased iron stores.

Peer review

Authors present their findings on a randomized controlled Phase III trial investigating the therapeutic efficacy of phlebotomy to treat fatty liver disease associated with high ferritin. The concept is intriguing. The article is well written and clearly presented. The low power does not allow us to draw any definitive conclusions, though the results support a large Phase III trial. To their credit, the authors fully acknowledge the limitations.

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