Reduction of body iron in *HFE*-related haemochromatosis and moderate iron overload (Mi-Iron): a multicentre, participant-blinded, randomised controlled trial

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Summary

Background The iron overload disorder hereditary haemochromatosis is most commonly caused by HFE p.Cys282Tyr homozygosity. In the absence of results from any randomised trials, current evidence is insufficient to determine whether individuals with hereditary haemochromatosis and moderately elevated serum ferritin, should undergo iron reduction treatment. This trial aimed to establish whether serum ferritin normalisation in this population improved symptoms and surrogate biomarkers.

Methods This study was a multicentre, participant-blinded, randomised controlled trial done at three centres in Australia. We enrolled people who were homozygous for HFE p.Cys282Tyr, aged between 18 and 70 years, with moderately elevated serum ferritin, defined as 300–1000 µg/L, and raised transferrin saturation. Participants were randomly assigned, via a computer-generated random number, to undergo either iron reduction by erythrocytapheresis (treatment group) or sham treatment by plasmapheresis (control group). Randomisation was stratified by baseline serum ferritin (<600 µg/L or ≥600 µg/L), sex, and study site. Erythrocytapheresis and plasmapheresis were done every 3 weeks, the number of procedures and volume of red cells or plasma removed determined on the basis of each patient's haemoglobin, haematocrit, and serum ferritin concentration, as well their height and weight. In the erythrocytapheresis group, the target was to reduce serum ferritin to less than 300 µg/L. The number of procedures for the control group was based on the initial serum ferritin and prediction of decrease in serum ferritin of approximately 120 µg/L per treatment. The primary outcome was patient-reported Modified Fatigue Impact Scale (MFIS) score, measured at baseline and before unblinding. Analyses were by intention to treat, including the safety analysis. The trial is registered with ClinicalTrials.gov, number NCT01631708, and has been completed.

Findings Between Aug 15, 2012, and June 9, 2016, 104 participants were randomly assigned to the treatment (n=54) and control (n=50) groups, of whom 94 completed the study (50 in the treatment group and 44 in the control group). Improvement in MFIS score was greater in the treatment group than in the control group (mean difference –6·3, 95% CI –11·1 to –1·4, p=0·013). There was a significant difference in the cognitive subcomponent (–3·6, –5·9 to –1·3, p=0·0030), but not in the physical (–1·90 –4·5 to 0·63, p=0·14) and psychosocial (–0·54, –1·2 to 0·11, p=0·10) subcomponents. No serious adverse events occurred in either group. One participant in the control group had a vasovagal event and 17 participants (14 in the treatment group and three in the control group) had transient symptoms assessed as related to hypovolaemia. Mild citrate reactions were more common in the treatment group (32 events [25%] in 129 procedures) compared with the control group (one event [1%] in 93 procedures).

Interpretation To our knowledge, this study is the first to objectively assess the consequences of iron removal in individuals with hereditary haemochromatosis and moderately elevated serum ferritin. Our results suggest that serum ferritin normalisation by iron depletion could be of benefit for all individuals with hereditary haemochromatosis and elevated serum ferritin levels.

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Introduction

HFE-associated hereditary haemochromatosis is the most common autosomal recessive disease among white European populations, with roughly one in 200 having homozygosity for the p.Cys282Tyr mutation, which places them at increased risk of iron overload.[1] Hereditary haemochromatosis results from elevated dietary iron absorption and subsequent iron deposition in various organs, resulting in potentially severe tissue damage because of the capacity of iron to induce oxidative stress.[2] The most serious, life-threatening clinical manifestations of hereditary haemochromatosis are liver cirrhosis and increased incidence of hepatocellular carcinoma. Iron deposition in the joints, pancreas, pituitary gland, and heart can also lead to arthralgia and arthritis, diabetes mellitus, sexual dysfunction, and cardiac failure.[3] Iron overload has also been reported to cause psychosocial effects, such as fatigue and depression, affecting quality of life.[4,5] Men are particularly at risk of morbidity from hereditary haemochromatosis, with at least 28% of men

Research in context

Evidence before this study

We searched PubMed up to Oct 20, 2017, without language restrictions using the search terms "haemochromatosis", "treatment", "phlebotomy", "venesection", "erythrocytapheresis", and "randomised". Although there are ample data to support the benefit of normalisation of body iron in people with hereditary haemochromatosis due to HFE p.Cys282Tyr homozygosity with serum ferritin of more than 1000 µg/L, few data exist to inform the question of whether treatment is needed for those with serum ferritin above the normal range but less than 1000 µg/L.

Added value of this study

Our study, to our knowledge, is the first randomised, blinded study of iron depletion therapy by red cell removal compared with sham therapy in people with HFE p.Cys282Tyr homozygosity and moderate iron overload. Our results identified clinical and biochemical benefits of body iron normalisation for these people.

Implications of all the available evidence

This study provides evidence that all individuals with hereditary haemochromatosis who have iron overload, as indicated by serum ferritin above the normal range, could benefit from normalisation of body iron.

homozygous for HFE p.Cys282Tyr in a large, unselected sample of the general population of Australia satisfying criteria for documented iron overload-related disease.[5]

See Online for appendix

Because of the potential complications of hereditary haemochromatosis, all management guidelines recommend removing excess iron from all people with hereditary haemochromatosis who have an elevated serum ferritin concentration, irrespective of whether they are symptomatic or not.[6,7] Treatment is generally by regular venesection, although erythrocytapheresis or iron chelation are occasionally used.[1]

Evidence for the benefit of iron depletion in people with HFE p.Cys282Tyr homozygosity and serum ferritin greater than or equal to 1000 µg/L is strong, as the risk of developing iron overload-related disease, including liver cirrhosis, is high in this group.[5,8] However, the evidence on the need for treatment in those with serum ferritin above the normal range (approximately 300 µg/L) but less than 1000 µg/L (moderate iron overload), the most common group among people with HFE p.Cys282Tyr homozygosity, is less clear. Some experts have suggested that individuals in this category might reasonably be observed rather than treated: the so-called watch and wait approach.[9,10] Although previous cohort studies have not shown evidence of increased risk of morbidity in those with moderate iron overload,[11,12] these studies were not specifically designed to answer the question of whether treatment is clinically beneficial to such individuals. It is unknown whether such individuals benefit from prophylactic treatment. There is, therefore, a need for objective evidence to inform the management of people with hereditary haemochromatosis who have moderate iron overload. We aimed to answer whether reduction of total body iron was beneficial for these individuals, as assessed with patient-reported outcomes, and non-invasive markers of liver fibrosis and oxidative stress.

Methods

Study design and participants

The methods of the Moderately Increased Iron (Mi-Iron) study have been published previously.[13] Mi-Iron was a multicentre, participant-blinded, randomised controlled trial done at three centres in Australia (appendix).

Patient inclusion criteria were HFE p.Cys282Tyr homozygosity, age 18–70 years, serum ferritin between 300 µg/L and 1000 µg/L, and raised transferrin saturation, with no venesection treatment for hereditary haemochromatosis in the 2 years before study entry. Exclusion criteria were other HFE genotypes, pregnancy, or risk factors of concomitant liver disease (hepatitis, alcohol intake ≥40 g per day for women and ≥60 g per day for men, or body-mass index [BMI] >35 kg/m²). Participants were recruited over 4 years through referral from pathology laboratories that did *HFE* testing, the Australian Red Cross Blood Service, medical professionals, and Haemochromatosis Australia, a patient support group. All participants provided written informed consent. The study was approved by the Human Research Ethics Committees of Austin Health, Melbourne, Melbourne Health, and Royal Brisbane and Women's Hospital.

Randomisation and masking

Participants were stratified according to serum ferritin (<600 µg/L or ≥600 µg/L), sex, and study site, and subsequently randomly assigned to either the treatment group to receive erythrocytapheresis (and thus reduction of body iron, while remaining euvolaemic at the end of the procedure) or the control (sham) group to receive plasmapharesis (procedure performed without reduction of body iron). Randomisation was done with a computer-generated random number sequence in permuted blocks of length 6 generated by the main study statistician, LCG. Participants were enrolled by the study coordinators and site investigators who assigned them to trial groups after randomisation. Participants were blinded to the procedure they were undergoing by use of a full-length black curtain to conceal the apheresis machine behind it. Apheresis staff received training to ensure blinding.

Procedures

Erythrocytapheresis and plasmapharesis were done with at least a 3 week interval between each procedure. The

number of treatments and volume of red cells or plasma removed were based on each patient's haemoglobin, haematocrit, and serum ferritin concentrations, as well as their height and weight. Blood sampling was done before each procedure to assess these parameters. In the erythrocytapheresis group, the target serum ferritin concentration was less than 300 µg/L. The number of procedures for the control group was based on the initial serum ferritin and a predicted decrease in serum ferritin of approximately 120 µg/L per treatment, as suggested in a study by Rombout-Sestrienkova and colleagues.[14] All participants were blinded to the results of investigations and blood samples, which were explained as being used for safety testing. A blood test was obtained 1 week before the end of treatment assessment for both groups to ensure that serum ferritin concentrations were less than 300 µg/L for the treatment group.

Participants completed questionnaires at baseline and the end of treatment (prior to unblinding), including the Modified Fatigue Impact Scale (MFIS),[15] Medical Outcomes Study Health Survey Version 2 (SF36v2),[16] Hospital Anxiety and Depression Scale (HADS),[17] and Arthritis Impact Measurement 2 Short-form Scale (AIMS2-SF).[18] At the end of treatment, before unblinding, participants were asked whether they thought they were in the treatment or control group.

We used surrogate biomarkers to assess liver fibrosis, including transient elastography with Fibroscan (Echosens, Paris, France),[19] Hepascore,[20] and Fibrometer 2G, 3G, and VCTE (Echosens).[21,22] We assessed oxidative stress by measuring urinary and plasma F_2-isoprostane concentrations with mass spectrometry as previously described.[2,23–27] Surrogate biomarkers were measured at baseline and at the end of treatment, before unblinding.

Outcomes

The primary outcome was change in MFIS score between baseline and the end of treatment. Secondary outcomes were patient-reported outcomes, as measured by SF36v2, HADS, and AIMS2-SF; assessment of liver fibrosis according to transient elastography, Hepascore, Fibrometer, and oxidative stress; and the fidelity of blinding. Safety and adverse events were recorded during procedures by the apheresis nursing staff who administered the erythrocytapheresis and plasmapheresis procedures. Other adverse events were recorded by the research team members at study visits.

Statistical analysis

The sample size calculation has been described in detail previously.[13] Briefly, using an SD of 18, a sample size of 50 in each treatment group would deliver a statistical power of 80% to detect a treatment effect of a mean difference of 10 MFIS units with an α of 0·05. With an SD of 0·20, a sample size of 50 will ensure statistical power of 85% to detect a treatment effect of 0·12 on the Hepascore scale with an α of 0·05.

We compared differences in scores at baseline and the end of treatment between the control and treatment groups. The analyses were based on the intention-to-treat principle in participants who had completed the study, with estimates of the treatment effect generated by fitting a linear regression model to data from all participants adjusting for the stratification factors, serum ferritin concentration (300–599 µg/L and 600–999 µg/L), sex, and study site. Fibrometer 2G, 3G, and VCTE results were log-transformed for analysis. Unless otherwise stated, within-group summary statistics are presented as the sample mean and SE. We did the statistical analyses with Stata software (version 13.1). This study is registered with ClinicalTrials.gov, number NCT01631708.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Aug 15, 2012, and Jun 9, 2016, 128 individuals were screened and 104 participants were randomised (figure). Of the 24 individuals screened but not included in randomisation, 22 were ineligible for the study and two were eligible but withdrew before randomisation. Ten participants withdrew after randomisation, of whom two in the treatment group and five in the control group withdrew for personal reasons (eg, not having time to be in the trial) without starting treatment, and two in the treatment group and one in the control group withdrew after starting the study but without completing the end-of-trial assessment.

Of the 94 participants who completed the study, 50 were randomly assigned to the treatment group and 44 to the control group. 93 of the 94 participants were newly diagnosed with hereditary haemochromatosis and thus had not had treatment for the condition before the study. One participant in the control group had undergone venesection treatment more than 2 years before study entry. 75 participants were diagnosed through routine blood tests or family history (38 in the treatment group and 37 in the control group) and 18 participants presented with symptoms including lethargy, fatigue, or generally feeling unwell (11 in the treatment group and seven in the control group). Data on mode of diagnosis were missing from one individual in the treatment group (appendix).

Table 1 shows the baseline demographics of all participants. The mean number of treatments for participants was similar between the two groups (2·6 [SD 1·9], 95% CI 2·1 to 3·1 in the treatment group *vs* 2·1 [1·1], 1·8 to 2·5 in the control group, p=0·18). The difference in mean serum ferritin between baseline and the end of

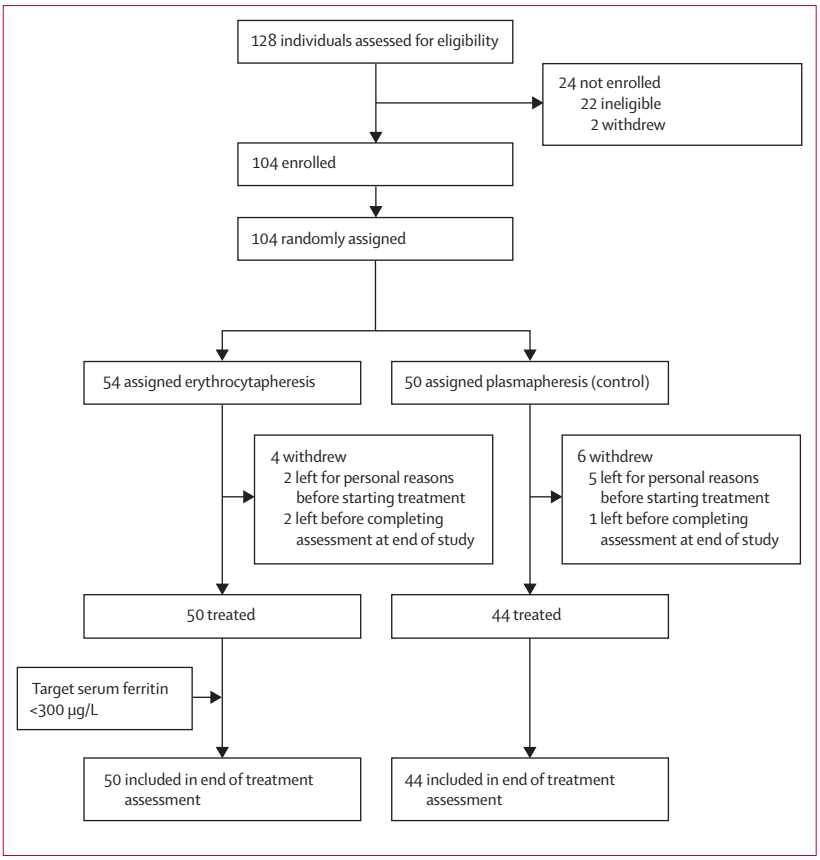

Figure: **Trial profile**

treatment was significant for the treatment group (decrease of 314·9 µg/L [185·5], –367·7 to –262·2, p<0·0001) but not for the control group (decrease of 30·8 µg/L [138·6], –73·0 to 11·3, p=0·15). Similarly, there was a significant reduction in mean transferrin saturation in the treatment group (baseline transferrin saturation 63·5% [17·0], 58·8 to 68·1 *vs* end of treatment transferrin saturation 45·4% [15·9], 40·9 to 50·0, p<0·0001) but not for the control group (baseline transferrin saturation 64·2% [18·6], 59·0 to 69·5 *vs* end of treatment transferrin saturation 61·7% [18·1], 95% CI 56·2 to 67·2, p=0·64). The mean baseline alanine aminotransferase (ALT) and aspartate aminotransferase (AST) concentrations were in the normal range and similar in both groups (table 1). ALT and AST concentrations remained in the normal range for both groups at the end of treatment (treatment group ALT 22·6 U/L [8·9], 20·1 to 25·1 *vs* control group ALT 28·4 U/L [15·7], 23·7 to 33·2; treatment group AST 20·9 U/L [6·5], 19·1 to 22·8 *vs* control group AST 23·3 U/L [8·1], 20·8 to 25·7). The mean weight of erythrocytes removed per treatment in the treatment group (mean 436·5 g [112·3], 404·6 to 468·5) was similar to the weight of plasma removed per procedure in the control group (434·7 g [121·6], 397·7 to 471·7; p=0·77). The mean total weight of iron removed was about 1·2 g with erythrocytapheresis, whereas the control group had close to no iron removed (mean total 0·0008 g).

The mean decrease in MFIS score, the primary outcome, was greater in the treatment group (–6·8 [SE 1·6], 95% CI –10·0 to –3·6) than for the control group (–1·4 [1·7], –4·8 to 2·1, p=0·013; table 2). Of the MFIS subscales, the only significant difference between the groups was in the cognitive component (treatment –3·9 [0·78], –5·5 to –2·4 *vs* control –0·80 [0·83], –2·5 to 0·86, p=0·0030; table 2).

There was no significant difference between change in scores for the mental (p=0·44) and physical (p=0·31) components of SF36v2 between the groups (table 2). Similarly, there were no significant difference in change in score between the groups for the total HADS score, which was generally less than 10 at baseline (p=0·26; table 2). Among the five components of the AIMS2-SF, the mean affect component was the only one that was significantly different between the treatment group and the control group. The AIMS2-SF affect component improved by about half a unit on a baseline mean of about two units in the treatment group, whereas there was no change in the mean component for controls (p=0·034; table 2).

As could be expected in an unselected group of people with HFE p.Cys282Tyr homozygosity and serum ferritin less than 1000 µg/L, there was little evidence of liver fibrosis as measured by transient elastography in either group at baseline (table 1). The mean change in transient elastography scores between treatment and control groups was also similar between groups (table 2). There was no evidence of liver fibrosis as measured by Hepascore in either group at baseline (table 1). However, after treatment, Hepascore decreased in the treatment group and increased in the control group (p=0·049; table 2). There was little evidence of advanced liver fibrosis as measured by Fibrometer 2G, 3G, or VCTE in either group at baseline (table 1) and little evidence of change in either group after treatment, so there was no discernible difference in change between groups (table 2). The mean change in plasma F_2-isoprostanes was larger for the treatment group than the control group (p=0·038), although this effect was not seen for urinary F_2-isoprostanes (table 2).

There were no differences between groups when participants were asked about which group of the study they believed they had been assigned to, indicating successful blinding of participants to the randomised treatment allocation (appendix).

Apheresis treatments were well tolerated, with no serious adverse events reported in either group. One participant in the control group had a vasovagal event and 17 participants (14 in the treatment group and three in the control group) had transient symptoms assessed as related to hypovolaemia. Mild citrate reactions were more common in the treatment group (32 events [25%] in 129 procedures)

	Erythrocytapheresis (n=54)	Plasmapheresis (control; n=50)
Sex		
Male	34 (63%)	32 (64%)
Female	20 (37%)	18 (36%)
Mean age (years)		
Male	37·3 (14·2; 32·3–42·3)	43·5 (13·1; 38·8–48·2)
Female	49·1 (13·3; 42·8–55·3)	45·3 (15·3; 37·7–52·9)
Mean body-mass index (kg/m²)		
Male	25·4 (3·8; 24·1–26·8)	26·8 (3·8; 25·4–28·2)
Female	26·3 (4·7; 24·2–28·5)	26·6 (3·6; 24·8–28·4)
Serum ferritin 600–1000 µg/L (n=30)		
Male	14 (26%)	13 (26%)
Serum ferritin (µg/L)	723·5 (93·3; 669·6–777·4)	705·5 (69·2; 663·7–747·3)
Female	4 (7%)	1 (2%)
Serum ferritin (µg/L)	715·5 (173·6; 439·2–991·8)	861
Serum ferritin 300–599 µg/L (n=64)		
Male	20 (37%)	19 (38%)
Serum ferritin (µg/L)	403·5 (77·7; 367·1–439·0)	436·2 (87·0; 394·5–478·4)
Female	16 (30%)	17 (34%)
Serum ferritin (µg/L)	414·3 (72·5; 375·6–452·9)	402·3 (82·6; 359·8–444·8)
Serum markers		
Transferrin saturation (%)	63·5 (17·0; 58·8–68·1)	64·2 (18·6; 59·0–69·5)
Alanine aminotransferase (U/L)	29·3 (13·4; 25·4–33·1)	32·6 (23·9; 25·4–39·9)
Aspartate aminotransferase (U/L)	23·9 (9·0; 21·4–26·5)	26·0 (13·2; 22·0–30·0)
Patient-reported outcome measures		
MFIS		
Total	26·5 (17·1; 21·8–31·2)	24·9 (17·1; 20·1–29·8)
Cognitive	13·2 (8·7; 10·8–15·6)	11·7 (7·3; 9·7–13·8)
Physical	11·3 (8·2; 9·1–13·6)	10·9 (8·9; 8·4–13·5)
Psychosocial	2·0 (1·9; 1·5–2·5)	2·2 (2·2; 1·6–2·9)
SF36v2		
Mental component summary	48·3 (12·3; 44·9–51·7)	47·4 (14·6; 43·2–51·6)
Physical component summary	49·6 (7·4; 47·6–51·7)	51·0 (7·7; 48·8–53·2)
(Table 1 continues in next column)		

	Erythrocytapheresis (n=54)	Plasmapheresis (control; n=50)
(Continued from previous column)		
HADS		
Total	9·0 (6·8; 7·1–10·9)	9·2 (7·6; 7·0–11·4)
Anxiety	5·7 (3·9; 4·6–6·8)	5·5 (4·3; 4·3–6·8)
Depression	3·3 (3·3; 2·4–4·2)	3·7 (4·1; 2·5–4·9)
AIMS2		
Physical	0·59 (0·65; 0·41–0·77)	0·66 (0·75; 0·45–0·88)
Affect	2·0 (1·9; 1·5–2·5)	2·1 (2·2; 1·4–2·7)
Symptom	0·85 (1·8; 0·37–1·3)	0·90 (1·5; 0·47–1·3)
Social	4·9 (1·5; 4·5–5·3)	4·8 (2·2; 4·2–5·4)
Work	1·1 (2·0; 0·47–1·7)	1·9 (3·2; 0·93–2·9)
Hepatic fibrosis markers		
Transient elastography score (kPa)	4·9 (1·6; 4·5–5·4)	4·9 (1·3; 4·5–5·2)
Hepascore*	0·21 (0·12; 0·18–0·25)	0·20 (0·12; 0·16–0·24)
Fibrometer2G (log)*	0·16 (0·091; 0·13–0·19)	0·18 (0·13; 0·14–0·22)
Fibrometer3G (log)*	0·18 (0·11; 0·15–0·22)	0·21 (0·14; 0·16–0·25)
Fibrometer VCTE (log)*	0·12 (0·067; 0·10–0·14)	0·14 (0·14; 0·10–0·19)
Oxidative stress markers		
F_2-isoprostanes plasma (pmol/L)*	929·2 (201·1; 871·5–987·0)	884·4 (255·8; 804·7–964·1)
F_2-isoprostanes urine (pmol/L)*	396·5 (163·5; 350·1–443·0)	382·9 (219·8; 315·2–450·5)

Data are mean (SD; 95% CI) or n (%). MFIS=Modified Fatigue Impact Scale. SF36v2=Medical Outcomes Study 36-item short form version 2. HADS=Hospital Anxiety and Depression Scale. AIMS2-SF=Arthritis Impact Measurement Scales 2 short form. *Data available for participants who completed the study.

Table 1: **Baseline demographics and clinical characteristics**

compared with the control group (one event [1%] in 93 procedures).

In a post-hoc analysis, we also compared participants in the treatment group who were diagnosed as having symptoms at baseline with those who were asymptomatic. The change in MFIS in response to ferritin normalisation was not significantly different (–2·7 [SE 3·7], 95% CI –10·1 to 4·6, p=0·46) between treatment group participants with symptoms (–8·8 [3·2], –15·3 to –2·3) and those who were asymptomatic and were diagnosed because of family history or by screening blood tests (–6·1 [1·7], –9·6 to –2·6).

Discussion

Our results showed that iron depletion in individuals with hereditary haemochromatosis with moderately elevated serum ferritin improved mental wellbeing. Iron depletion was also associated with improvement in Hepascore and plasma F_2-isoprostanes.

Hereditary haemochromatosis is common in white European populations, but who should be treated with iron depletion therapy and how aggressively they should

	Patients assessed	Change in erythrocytapheresis group	Change in plasmapheresis (control) group	Adjusted mean difference	p value
Patient reported outcome measures					
MFIS					
Total	93	−6·8 (1·6; −10·0 to −3·6)	−1·4 (1·7; −4·8 to 2·1)	−6·3 (2·5 ; −11·1 to −1·4)	0·013
Cognitive	94	−3·9 (0·78; −5·5 to −2·4)	−0·80 (0·83; −2·5 to 0·86)	−3·6 (1·2 ; −5·9 to −1·3)	0·0030
Physical	93	−2·3 (0·83; −4·0 to −0·70)	−0·60 (0·89; −2·4 to 1·2)	−1·9 (1·3; −4·5 to 0·63)	0·14
Psychosocial	94	−0·58 (0·22; −1·0 to −0·15)	−0·068 (0·23; −0·52 to 0·39)	−0·54 (0·33; −1·2 to 0·11)	0·10
SF36v2					
Mental component summary	88	2·1 (1·3; −0·41 to 4·6)	1·2 (1·4; −1·5 to 3·9)	1·5 (1·9; −2·3 to 5·4)	0·44
Physical component summary	88	1·4 (0·87; −0·29 to 3·2)	0·30 (0·94; −1·6 to 2·2)	1·4 (1·4; −1·3 to 4·1)	0·31
HADS					
Total	91	−2·0 (0·64; −3·3 to −0·72)	−1·1 (0·69; −2·5 to 0·28)	−1·1 (0·95; −3·0 to 0·80)	0·26
Anxiety	92	−1·5 (0·42; −2·3 to −0·64)	−0·49 (0·45; −1·4 to 0·40)	−0·98 (0·61; −2·2 to 0·24)	0·12
Depression	93	−0·62 (0·30; −1·2 to 0·022)	−0·51 (0·33; −1·2 to 0·13)	−0·28 (0·46; −1·2 to 0·63)	0·54
AIMS2-SF					
Physical	93	−0·071 (0·11; −0·28 to 0·14)	0·044 (0·11; −0·18 to 0·27)	−0·13 (0·16; −0·46 to 0·19)	0·42
Affect	89	−0·48 (0·15; −0·78 to −0·17)	0·00 (0·17; −0·33 to 0·33)	−0·51 (0·24; −0·98 to −0·038)	0·034
Symptom	94	0·00 (0·21; −0·41 to 0·41)	0·11 (0·22; −0·32 to 0·55)	−0·19 (0·31; −0·81 to 0·43)	0·54
Social	93	−0·13 (0·20; −0·51 to 0·26)	0·00 (0·21; −0·42 to 0·42)	−0·21 (0·30; −0·80 to 0·40)	0·48
Work*	67	0·48 (0·53; −0·58 to 1·5)	−0·68 (0·54; −1·8 to 0·39)	1·2 (0·82; −0·47 to 2·8)	0·16
Hepatic fibrosis markers					
Transient elastography score (kPa)	94	0·052 (0·21; −0·37 to 0·48)	−0·12 (0·23; −0·57 to 0·34)	0·16 (0·33; −0·49 to 0·82)	0·63
Hepascore	94	−0·012 (0·017; −0·091 to 0·0073)	0·030 (0·018; −0·0062 to 0·065)	−0·051 (0·026; −0·10 to −0·00018)	0·049
Fibrometer 2G (log)	88	0·075 (0·050; −0·026 to 0·17)	0·034 (0·057; −0·078 to 0·15)	0·068 (0·077; −0·086 to 0·22)	0·38
Fibrometer 3G (log)	88	0·055 (0·050; −0·045 to 0·15)	−0·026 (0·060; −0·14 to 0·085)	0·11 (0·075; −0·034 to 0·26)	0·13
Fibrometer VCTE (log)	89	0·15 (0·062; 0·023 to 0·27)	−0·050 (0·069; −0·19 to 0·088)	0·19 (0·097; −0·0015 to 0·39)	0·052
Oxidative stress markers					
F_2-isoprostanes plasma (pmol/L)	91	−62·9 (34·7; −131·8 to 6·1)	37·6 (37·5; −36·9 to 112·1)	−113·7 (53·9; −220·9 to −6·5)	0·038
F_2-isoprostanes urine (pmol/L)	92	−1·4 (30·8; −62·5 to 59·8)	4·5 (33·6; −62·2 to 71·3)	−26·7 (46·7; −119·6 to 66·1)	0·57

Data are mean (SE; 95% CI). Changes in the erythrocytapheresis and plasmapheresis control groups were calculated as the mean difference in scores between baseline and follow-up. Mean difference was the comparison of the change between control and treatment groups, adjusted for serum ferritin groups, sex, and site. MFIS=Modified Fatigue Impact Scale. SF36v2=Medical Outcomes Study 36-item short form version 2. HADS=Hospital Anxiety and Depression Scale. AIMS2-SF=Arthritis Impact Measurement Scales 2 short form. *The work component of AIMS2-SF was only assessed in the subset of participants who were still in paid employment (not retired, or doing unpaid, volunteer , or carer work).

Table 2: **Outcome measures**

be treated remain important clinical questions. There are surprisingly few objective data on this topic and no evidence from randomised controlled trials. Our study, to our knowledge, the first randomised, blinded study of iron depletion therapy by red cell removal compared with sham therapy, provides good evidence that normalising body iron stores has a clinical benefit for people with HFE p.Cys282Tyr homozygosity and moderate iron overload.

Change in total MFIS was greater in the treatment group than in the control group, which was mainly due to improvement in the cognitive component. It is unknown what change in MFIS score is clinically meaningful. Although the baseline mean MFIS score in this study was lower than the minimum MFIS score for a diagnosis of fatigue (38),[28] this does not necessarily mean that treated individuals did not obtain benefit from the treatment. A difference of six units in the MFIS score between the treatment and control groups could represent psychosocial benefit in the treated cohort. It is possible that the higher baseline MFIS in the treatment group than in the control group, despite not being a significant difference, contributed to the greater change detected in the treatment group. However our finding of an improvement in the affect component of AIMS2-SF in the treatment group supports the notion that normalisation of iron can positively affect mental wellbeing. These patient-reported findings were accompanied by improvement in the non-invasive hepatic fibrosis marker Hepascore and plasma F_2-isoprostanes, a marker of oxidative stress, in the treatment group.

There was no change in any outcome measure that was significantly greater in the control group than in the treatment group. This finding suggests that the normalisation of body iron in people with HFE p.Cys282Tyr homozygosity is unlikely to be harmful. Indeed, we have shown in this study that, in many instances, treatment is beneficial for those with moderate iron overload. Overall, we considered 21 comparisons between treatment and control interventions in eight domains (four each that were patient-reported and surrogate markers). Of these comparisons, six of eight domains and 16 of 21 comparisons favoured the treatment over control including 13 of 14 patient-reported outcomes, although not all differences were statistically significant. Notwithstanding the positive correlation between comparisons in some domains, such summary statistics are unlikely if the treatment offered no benefit in any domain.

The mechanism by which venesection results in reduced fatigue is uncertain. Reduction in body iron leading to reduced oxidative stress is thought to be the most likely explanation and is supported by our observation of a reduction in plasma F_2-isoprostanes in the treatment group. Also supporting this mechanism is the finding of increased F_2-isoprostanes in individuals with chronic fatigue compared with controls.[29] It is also possible that the mechanism is unrelated to iron levels and could be a non-specific effect of red blood cell removal.

Apheresis staff and other study staff were trained to use neutral language to ensure that unblinding did not occur through accidental reference to whether a participant was undergoing erythrocytapheresis or plasmapheresis. The effectiveness of the blinding of participants to treatment was supported by similar proportions of participants in each group who believed they were treated, were in the sham group, or were unsure to which group they had been assigned (appendix). Double blinding was not possible because apheresis staff needed to implement different procedures for the treatment and control groups.

About 70% of the participants in the study were asymptomatic and diagnosed through family history or routine iron studies as part of a health check. It is therefore unlikely that our results are due to offering treatment only to individuals with the most severe and advanced forms of clinical disease. Moreover, in an exploratory analysis, we did not find a difference in the primary outcome, the MFIS, in response to normalisation of serum ferritin in participants who were diagnosed as a result of having symptoms compared with those who were asymptomatic. This finding suggests that serum ferritin normalisation should be recommended for all individuals with hereditary haemochromatosis and raised serum ferritin, irrespective of how the diagnosis is made. This recommendation is supported by results from a cohort study that showed decreased cardiovascular and extrahepatic cancer-related mortality in individuals with HFE-related hereditary haemochromatosis with moderately elevated serum ferritin that was normalised by venesection therapy.[30]

There is uncertainty as to the appropriate target serum ferritin concentration after iron depletion therapy for hereditary haemochromatosis. Some experts recommend a lower limit of 50 µg/L[9] whereas others recommend 100 µg/L.[31] We chose an endpoint of serum ferritin less than 300 µg/L for this study on the assumption that if a clinical benefit of iron normalisation exists, then it should be seen by reducing serum ferritin into the normal range and should not require reduction to the lower serum ferritin levels recommended in various guidelines. We were also concerned that aiming for a serum ferritin endpoint of 50–100 µg/L could result in iron deficiency anaemia and fatigue that might have confounded the outcome of the study. Our study was not designed to answer the question of how low the concentration of serum ferritin should be to signal the end of treatment for iron overload due to hereditary haemochromatosis. It would be interesting to do a similar study with sufficient power to allow for some treated individuals to be assigned to have their serum ferritin lowered to just below 300 µg/L and others to around 50 µg/L to assess any differences in outcomes between these groups.

Debate continues as to whether screening for hereditary haemochromatosis should be instituted.[32,33] There are roughly 1 million people each in the USA and Europe, and almost 100 000 in Australia who have or will get moderately elevated serum ferritin due to *HFE* mutations.[5] Our data suggest that people with HFE p.Cys282Tyr homozygosity with moderate iron overload, who are apparently asymptomatic, can benefit from normalisation of body iron. These findings add weight to the case for introducing screening for hereditary haemochromatosis in the community.

The limitations of this study include the fact that the trial was, unavoidably, single blinded rather than double blinded. It is also possible that a larger sample size would have allowed clearer differences between the groups to be identified. Additionally, reduction of final serum ferritin to 50–100 µg/L in the treatment group might also have resulted in clearer differences between the groups.

In conclusion, our results show both patient-reported and surrogate marker evidence of benefit from normalisation of iron levels in people with HFE p.Cys282Tyr homozygosity with moderate iron overload and support recommendations for the treatment of all individuals in this category, irrespective of the means of diagnosis.

Contributors

LCG, AJN, EMW, GJA, GAR, KJA, JKO, DC, PG, LWP, and MBD conceived and designed the study. SYO and LCG analysed the data. SYO, LD, JD, MW, LER, SD, LWP, and MBD acquired the data. All authors interpreted the data and contributed to the manuscript.

Declaration of interests

We declare no competing interests.

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