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Title: Pilot study: randomized, placebo-controlled comparator trial of IV vs oral iron treatment of RLS with Iron deficiency anemia. (IVOR-IDA)

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INTRODUCTION

Summary: The restless legs syndrome (RLS), also known as Willis-Ekbom Disease, occurs in 40-50% of patients with iron deficiency (ID) or its end stage, iron deficiency anemia (IDA) (Achebe, AJH, 2023). Treatment correcting the ID is expected to also be effective for reducing or eliminating the RLS with IDA. Two accepted treatments for IDA, oral ferrous sulfate and ferumoxytol were compared for efficacy and speed of response for treatment of RLS occurring with IDA (RLS-IDA). The original plan for this study was the accrual of 70 RLS-IDA patients to be randomly assigned 35 each to oral or IV iron using double-blind, double-dummy procedures. The primary outcome was determined at 6 weeks of treatment. Following initial evaluation, due to complications of performing a clinical trial during the pandemic and the death of the principal investigator, it was determined that the patient and clinical global impression scores, primary outcomes for the trial, were missing in 30 patients. As a result, in order to maintain the prespecified statistical analysis, an additional 30 patients were accrued.

Background: Low iron status is associated with RLS. RLS has been documented to have significant deceased iron concentrations in the substantia nigra (Allen, Neurology, 2001) and other areas (Rizzo, Movement Disorders, 2013). These associations have been documented into studies using both magenetic resonance imaging (Allen, Rizzo, Earley Sleep Med 2006) and ultrasound (Schmidauer, Ann Neuro 2005). Peripheral iron status also relates to RLS severity (Sun, Sleep 1998) and both oral and IV iron treatments significantly reduce RLS symptoms compared with placebo (Allen, Sleep Med 2011 and Wang, Sleep Med 2009).

RLS occurs commonly with IDA. In the single institution in which the study was performed the prevalence of clinically significant RLS was approximately 45% (Achebe, AJH). This is approximately seventeen times the prevalence of any RLS in the general population. Standard treatments for IDA also treat RLS without IDA. Both oral and IV iron are considered standard treatments for IDA (Schrier, UTD 2013). These same treatments also reduce RLS symptoms. Oral iron as ferrous sulfate containing 60 mg of elemental iron per table, taken twice daily produced significant reduction of RLS symptoms in a blinded placebo-controlled study (Wang, Sleep Med). IV iron administered as ferric carboxymaltose in two doses of 750 mg spaced five days apart, also reduced RLS symptoms significantly more than placebo (Allen, Sleep Med).

Rationale for study: It is assumed the treatments which reverse IDA will also reduce the RLS symptoms occurring with IDA. This is supported by the benefit observed in RLS patients without IDA. However, this benefit has never been demonstrated in a prospective controlled trial, especially in a blinded study. The iron stores, however, are less in those with IDA than in the general population and

therefore IDA-RLS may require more aggressive treatment than is used for RLS without IDA and possibly even more aggressive therapy than is usually administered for IDA. In particular, despite the recovery of anemia after six weeks of oral iron therapy, the actual iron stores remain low. In contrast the IV iron treatment with 1000 mg of IV iron corrects the anemia and provides a better increase in iron parameters over a 6-week period (Schrier UTD, 2013). Accordingly, if the IDA-RLS results more from the low iron than the anemia, it may benefit significantly more from IV than oral iron [BUT IT DIDN'T—DAMNIT!].

Our major problem in testing the treatment benefits for IDA-RLS for either oral or IV iron was not knowing the actual effect size of either treatment. We had as an initial estimates the effect sizes from clinical experience with oral and IV iron therapy for IDA-RLS. Thus, we set the sample size to give adequate power to differentiate IV from oral iron assuming these estimates, without knowing if the estimates were accurate. The decision not to do a placebo control is based on the assumption that the treatment effect size can be adequately determined without a placebo. The decision to use a blinded comparator trial was based on the assumption that we would see differences between the treatments.

METHODS

The study was a randomized, comparative, double-blind study to evaluate effect size and time course of treatment response for RLS-IAD over six weeks. Two medications and two placebos were used with equal random assignment to both groups:

1. *FCM group*: Oral vitamin C, 500 mg tablets taken twice a day (placebo pill) and Ferumoxytol intravenous (IV) 1020 mg in two divided doses over 15 minutes 2-7 days apart
2. *FeSO4 group*: Ferrous sulfate 325 mg (oral) tabs morning and evening and IV saline given over 15 minutes 2-7 days apart (placebo infusion)

IV ferumoxytol, 1020 mg was be offered to those not significantly responding (CGI-I scores less than much or very much improved) at 6 weeks after oral iron and still meeting all the requirements for entry to study.

All patients who presented to a single outpatient Hematology clinic for treatment for their iron deficiency anemia (IDA), completed the Cambridge-Hopkins diagnostic questionnaire (CDQ) as part of their routine clinical evaluation, which was not limited to its use in this study. Those identified by the questionnaire as having RLS were checked for meeting the other inclusion criteria. The research coordinator reviewed the responses to the questionnaire, their clinical history and recent blood values to ensure inclusion criteria were met. If all criteria were met, the research coordinator consented the

patient for enrollment in the study. After consenting, a protocol number was assigned and appropriate blood samples obtained. Diagnosis of RLS was then confirmed by Hopkins-Hening Telephone Diagnostic Interview (HTDI) conducted by Dr. Allen or Dr. Earley.

A xcel-generated randomization table was used to randomly assign subjects to one of two treatment conditions. Blinding was done by a separate and independent "treatment nurse" who used the randomize table, made the assignments on the day of treat and then completed the appropriate treatment. The randomization was in 7 blocks of 10 with equal treatments in each block. The research coordinator did not know the treatment administered and was not present during treatment and did not have access to files indicating, which treatment was administered. During IV infusions, the patients wore sleep masks that fully covered their eyes to ensure they did not notice any color differences between the iron and placebo administration. All investigators and the Analysts were blind to the assignment until completion of the analysis.

Two IV treatments spaced 2-7 days apart were administered. These were either ferumoxytol or placebo. At the time of the first infusion, an opaque bottle containing either vitamin C or ferrous sulfate was given to the subject. The patient was instructed to take each of the pills twice a day for the next six weeks. Enough pills to last 7 seven weeks were given to allow for ongoing treatment for the follow-up appointment at six weeks after the first IV infusion.

For IV iron treatment, the commercially available ferumoxytol was used. Ferumoxytol is marketed as an iron replacement formulation indicated for the treatment of iron deficiency anemia and is FDA approved for this indication. Ferumoxytol (30 mg/mL) is available for IV injection in single use vials containing 510 mg of elemental iron in 17 mL. Ferumoxytol is stored at controlled room temperature (20-25^{o} C). Pre-treatment medications were not administered. 510 mg of ferumoxytol was diluted in 100 ml normal saline and administered with an infusion pump over a 15-minute period. Vital signs were measured and recorded immediately prior to drug administration and were observed for 60 minutes following completion of the infusion with observation for signs or symptoms of adverse events. For the placebo (normal saline) administration was exactly the same procedure as the IV iron formulation.

For oral iron, ferrous sulfate 325 mg tablets containing 65 mg of elemental iron were used. The pills were taken twice daily on an empty stomach. If significant gastrointestinal perturbation occurred the medication could be taken with food. The oral placebo was standard vitamin C 250 mg tablets given in the same bottle as oral iron.

Following the first IV infusion, telephone evaluations were performed at 7, 14 and 28 days. At four weeks after the first of the pair of IV treatment were completed, blood tests were performed to include hemoglobin concentration and iron parameters (serum iron, total iron binding capacity, serum ferritin, percent transferrin saturation). A six weeks after the first of the pair of IV treatment were completed, another clinic visit was scheduled so a CGI form could be completed by involved medical personnel. At 6 weeks, non-responders were given the option of receiving ferumoxytol as an open-label infusion. At the 6-week clinic visit, repeat blood tests (see above), adverse event reporting and clinical evaluation of the RLS was performed.

Inclusion criteria were as follows:

1. Aged ≥ 18 years
2. Diagnosis of RLS based on CHDQ and confirmed by HTDI
3. International RLS Study Group Severity Scale (IRLS) score > 15.
4. Iron deficiency anemia defined as ID either ferritin < 20 mcg/l, TSAT <19%, anemia Hb <13 g/dL for both males and females.
5. Willingness to use contraceptive to avoid pregnancy: Women have to be surgically sterile, post menopausal or use one of the following contraceptives during the whole study period and after the study has ended for at least 5 times plasma biological half-life of the investigational medicinal product: intrauterine devices or hormonal contraceptives (contraceptive pills, implants, transdermal patches, hormonal vaginal devices, or injections with prolonged release).
6. Willingness to participate and signing the informed consent form

Exclusion criteria were as follows:

1. Iron overload or disturbances in utilization of iron (e.g. haemochromatosis and hemosiderosis)
2. Decompensated liver cirrhosis or active hepatitis (ALAT > 3 times upper limit of normal)
3. Serum ferritin > 500 ng/mL or transferrin saturation >40 %
4. Active acute or chronic infections (assessed by clinical judgment that may be indicated by white blood cells (WBC) and C-reactive protein (CRP) when these are available)
5. Rheumatoid arthritis with symptoms or signs of active inflammation
6. Pregnant and nursing women
7. History of multiple allergies
8. Known hypersensitivity to parenteral or oral iron or any excipients in the drug products
9. Previous IV iron treatment for RLS
10. Other iron treatment or blood transfusion within 4 weeks prior to the screening or treatment visit
11. Planned elective surgery during the study
12. Current (past 4 weeks) use of drugs that treat RLS, e.g. opioids, alpha-2-delta anti-depressants, dopaminergic agents (dopamine promoters, dopamine antagonists/blockers)

Any other medical condition that, in the opinion of Investigator, may cause the subject to be unsuitable for the completion of the study or place the subject at potential risk from being in the study, e.g. a malignancy, uncontrolled hypertension, unstable ischemic heart disease, or uncontrolled diabetes mellitus.

The following endpoint were used for the study:

1. At evaluation in the clinic at about 6 weeks after the first IV iron treatment.

2. Termination of participation because of significant adverse effect from the IV infusion in the hour observed after the infusion.

3. Significant adverse event after starting the iron treatment and before the 6 week follow-up evaluation that was judged to require ending the study.

4. Subject decision not to continue in the study after the treatment and before the final evaluation.

5. Physician's decision that it would be harmful to the patient to continue in the study.

6. Patient is non-compliant with study requirements and was judged the non-compliance was enough to significantly alter evaluation of the treatment. (Note that stopping the oral medication because of intolerance to the treatment is not considered non-compliance, but rather an outcome for the study). Patients discontinuing oral medication for adverse effects may qualify for repeat IV treatment.

Subjects could terminate the study at any time for could be terminated from the study for non-compliance or if continuation in the study was judged by the PI (Dr. Auerbach) to be harmful. Reasons for termination were recorded. 21 patients were terminated or lost to follow-up. Some of these terminations may have been due to hiatuses in the study caused by the untimely death of the principal investigator (Dr. Allen) and the untimely occurrence of the COVID pandemic, interfering with patient visits. A list of those terminated or lost to follow-up are reported in Table ___. As a result, an additional 30 patients were accrued above the planned prespecified number, to achieve appropriate statistical power.

There were two co-primary outcome measures: Clinician Global Inventory-Improvement (CGI-I) assessment at week 6 and the change-in-baseline at 6 weeks in International RLS Study Group Severity Scale (IRLS) score. The Patient Global Inventory-Severity (PGI-S) was a secondary outcome measure (PGI-S). The IRLS and PGI-S were obtained at the clinic visits and at each telephone evaluation, while the CGI-i was only obtained at the Week-6 study endpoint.

Statistical analysis

Baseline and follow up laboratory values were described overall and within each study group. Means and standard deviations and medians and ranges were produced for continuous variables, and frequency counts and percentages were generated for describing variables that were dichotomous or

polytomous in nature. Changes in laboratory values from baseline to follow up were calculated and the mean change in values was compared using t-tests.

Results

Baseline lab values of all subjects and by Group are found in Table 1. There was a significant difference between baseline ferritin and TSAT values. Group A had a higher baseline ferritin and TSAT values than Group B.

Change in hemoglobin and iron parameters for all subjects and by group are shown in Table 3. The mean change in hemoglobin from baseline to follow up was 1.2 (SD 1.3) for Group A and 6.0 (17.2) for Group B. The mean change in Ferritin from baseline to follow up was 200.3 and 23.3 for Group A and Group B, respectively. The mean change in TSAT from baseline to follow up was 16.5 and 12.2 for Group A and Group B, respectively. The mean change in MCV from baseline to follow up was 24.2 and -17.6 for Group A and Group B, respectively. There was a significant difference between follow up ferritin and TSAT values. Group A had a higher follow up ferritin values (p=0. <0.0001) and higher TSAT (p=0.007) values compared to Group B.

Table 4 shows the changes in patient severity scores from baseline to follow up, and the follow up patient and clinical global impression scores for all subjects and by group. No significant differences between the 2 groups in terms of change in (1) patient severity score from baseline to follow up, (2) follow up patient global impression score, or (3) clinical global impression score were observed.

Table 5 shows the change in FSS from baseline to follow up. There were no significant differences between the 2 groups in terms of change in FSS.

Table 6 shows the change in IRLS score from baseline to follow up for all subjects and by group. Group B had a greater reduction in IRLS score from baseline to follow up compared to Group A

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Commented [VS1]: These are results from my original analysis. I still need to double check the results. However, before doing so, I want to confirm that there are no new data to analyze.

Commented [VS2]: Will define "Group A" and "Group B" once I know which group is which

TABLES

Table 1. Baseline hemoglobin and iron parameters for all subjects and by group.

	Total	Group A	Group B	P-value[1]
Hemoglobin	N=93	N=47	N=46	0.14
Mean (SD)	11.1 (1.5)	11.3 (1.3)	10.8 (1.6)	
Median (IQR)	11.6 (10.2, 12.2)	11.7 (10.8, 12.3)	11.6 (9.7, 12.1)	
Min, Max	7.0, 13.1	7.2, 13.1	7.0, 13.0	
Ferritin	N=91	N=45	N=46	0.017
Mean (SD)	17.9 (16.7)	22.6 (19.6)	13.5 (12.1)	
Median (IQR)	11.0 (7, 24)	19 (7, 29)	10.0 (7-15)	
Min-Max	3, 102	4, 102	3.0, 59.0	
TSAT	N=92	N=46	N=46	0.025
Mean (SD)	10.4 (6.2)	11.4 (5.9)	9.5 (6.5)	
Median (IQR)	9.0 (6, 13)	10.0 (8, 15)	8.5 (6.0-11.0)	
Min-Max	2, 36	2, 36	2, 32	
MCV	N=93	N=47	N=46	0.233
Mean (SD)	88.7 (77.3)	82.2 (7.8)	95.4 (110.4)	
Median (IQR)	83 (75.5, 87.0)	83.9 (77.9, 86.3)	81.5 (71.6-87.0)	
Min-Max	56.5, 825.0	58.7, 100.0	56.5, 825.0	

[1] P value comparing Group A vs. Group B using Kruskal-Wallis Test.

Table 2. Follow-up hemoglobin and iron parameters for all subjects and by group.

	Total	Group A	Group B	P-value[1]
Hemoglobin	n=70	N=36	N=33	.833
Mean (SD)	14.4 (11.8)	12.5 (1.0)	16.6 (17.0)	
Median (IQR)	12.4 (11.7, 13.3)	12.6 (11.9, 13.2)	12.3 (11.7, 13.4)	
Min-Max	9.7, 105.0	10.4, 14.3	9.7, 105.0	
Ferritin	n=70	N=36	N=33	<0.0001
Mean (SD)	132.8 (136.7)	223.4 (136.6)	37.1 (29.5)	
Median (IQR)	66.5 (27.0, 214.0)	209.5 (127.5, 308.5)	33.0 (21.0-40.0)	
Min-Max	7.0, 620.0	11.0, 620.0	7.0, 159.0	
TSAT	n=70	N=36	N=33	0.026
Mean (SD)	24.4 (13.3)	27.7 (10.0)	21.4 (15.5)	
Median (IQR)	24.0 (14.0, 31.0)	28.0 (20.5, 32.0)	16.0 (12.0-28.0)	
Min-Max	7.0, 89.0	13.0, 59.0	7.0, 89.0	
MCV	n=70	N=36	N=33	0.21
Mean (SD)	95.1 (93.7)	106.4 (130.2)	83.3 (10.6)	
Median (IQR)	86.0 (82.0-88.6)	86.8 (82.7, 88.8)	85.1 (80.0-88.1)	
Min-Max	36.8, 864.0	36.8, 864.0	38.7, 101.0	

[1] P value comparing Group A vs. Group B using Kruskal-Wallis Test.

Table 3. Change in hemoglobin and iron parameters from baseline to follow up for all subjects and by group.

	Total	Group A	Group B	P-value[1]
Hemoglobin	N=70	N=36	N=33	0.17
Mean (SD)	3.5 (12.0)	1.2 (1.3)	6.0 (17.2)	
Median (IQR)	1.4 (0.2-2.3)	1.4 (0.2, 1.8)	1.7 (0.3, 3.3)	
Min-Max	-0.9, 95.6	-0.9, 5.0	-0.9, 95.6	
Ferritin	N=67	N=34	N=33	<0.0001
Mean (SD)	113.1 (130.4)	200.3 (133.0)	23.3 (21.2)	
Median (IQR)	35.0 (18, 579)	190.0 (103.0, 290.0)	20.0 (9.0, 27.0)	
Min-Max	-7, 579	-1.0, 579.0	-7.0, 102.0	
TSAT	N=68	N=35	N=33	0.007
Mean (SD)	14.4 (12.6)	16.5 (7.5)	12.2 (16.3)	
Median (IQR)	13.5 (6.5, 19.4)	16.0 (11.0, 20.0)	7.0 (3.0, 18.0)	
Min-Max	-17.0, 82.0	-5.0, 37.7	-17.0, 82.0	
MCV	N=70	N=36	N=33	0.49
Mean (SD)	4.2 (129.9)	24.2 (130.9)	-17.6 (129.5)	
Median (IQR)	3.2 (-0.2, 8.2)	3.3 (-0.4, 7.6)	3.1 (1.2, 9.5)	
Min-Max	-736.9, 784.4	-58.4, 784.4	-736.9, 21.1	

[1] P value comparing Group A vs. Group B using Kruskal-Wallis Test.

Table 4. Change in patient severity scores from baseline to follow up, and follow up patient and clinical global impression scores for all subjects and by group.

	Total	Group A	Group B	P-value[1]
Change in patient severity score[2]	N=57	N=30	N=27	0.24
Mean (SD)	-1.9 (1.8)	-1.7 (1.8)	-2.1 (1.9)	
Median (IQR)	-2.0 (-3.0, -1.0)	-2.0 (-3.0, -1.0)	-3.0 (-4.0, -1.0)	
Min-Max	-5.0, 2.0	-5.0, 2.0	-5.0, 2.0	
Follow up patient global impression score[3]	N=77	N=40	N=37	.72
Mean (SD)	2.1 (1.2)	2.1 (1.1)	2.1 (1.2)	
Median (IQR)	2.0 (1, 3)	2.0 (1, 3)	2 (1, 3)	
Min-Max	1-6	1-5	1-6	
Follow up clinical global impression score[3]	N=72	N=38	N=34	.80
Mean (SD)	2.1 (1.3)	2.1 (1.2)	2.1 (1.4)	
Median (IQR)	2.0 (1, 3)	2 (1, 3)	1.5 (1, 3)	
Min-Max	1-6	1-5	1-6	

[1] P value comparing Group A vs. Group B using Kruskal-Wallis Test.
[2] Assessed at baseline and 6 weeks post-baseline.
[3] Assessed at 1-6 weeks post-baseline.

NOTE: If missing 6 wk fup use most recent data point. Do not report change in impression patient and clinical scores - report 6 wk fup score

Table 5. Change in FSS.

	Total	Group A	Group B	P-value[1]
Change in FSS	N=61	N=32	N=29	0.11
Mean (SD)	-10.9 (14.6)	-7.8 (14.2)	-14.3 (14.5)	
Median (IQR)	-10 (-17, -2)	-6 (-17, 3)	-12 (-17, -6)	
Min-Max	-50, 17	-34, 17	-50, 9	

[1] P value comparing Group A vs. Group B using Kruskal-Wallis Test.
[2] Assessed at baseline and 6 weeks.

Table 6. Change in IRLS score from baseline to follow up for all subjects and by group.

	Total	Group A	Group B	P-value[1]
Change in IRLS score[2]	N=74	N=37	N=36	0.047
Mean (SD)	-10.1 (8.8)	-8 (7.7)	-12.2 (9.5)	
Median (IQR)	-11 (-15, -3)	-10 (-13, -3)	-12 (-18, -5)	
Min-Max	-34-10	-24-10	-34-7	

[1] P value comparing Group A vs. Group B using Kruskal-Wallis Test.
[2] Follow up periods: 1 week n=2 ; 2 weeks n=4 ; 4 weeks n=5 ; 6 weeks n=61 .

Adverse Events

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Baseline

Group A	6 subjects	• Constipation • Diarrhea • Mouth burns • Chest pain • Flushing • Sneezing • Scratchy throat
Group B	7 subjects	• Fatigue • Constipation • stomach issues, constipation • unable to tolerate pills, stomach cramps, nausea • constipated, stopped pills • nausea, vomiting

Day 7

Group B	3	• stomach, headache • stomach, diarrhea, nausea • stomach, constipation

Day 28

Group A	1	• stomach issues, constipation
Group B	1	• stomach issues and dyspnea

Week 6

Group A	6	• mild stomach issues • Stomach upset, stomach pain, constipation, diarrhea, nausea, vomiting, dizziness, hyptotension, headache, chest pain, back pain, cough, prexia, rash, Back pain, muscle spasms, Stomach upset, stomach pain, constipation, diarrhea, nausea, vomiting, dizziness, hypotension, peripheral edema, edema, headache, chest pain, back pain, cough, pruritus, Prexia, muscle spasms, Dyspnea, Rash, Diarrhea, Peripheral Edema, Back Pain, Cough, Muscle Spasms
Group B	10	• Mild edema • Constipation • Mild constipation • Constipation • Diarrhea, Nausea, Dizziness, Headache, Chest Pain, Back Pain, Cough, Muscle Spasms • Constipation, Diarrhea

		• Stomach, Constipation • Headache, hives, stuffy noses • Diarrhea, Nausea, Muscle Spasms • Stomach issues

Month 3

Group B	1	• Constipation

Month 12

Group A	1	• Constipation