A POSSIBLE ROLE OF IRON IN THE RESTLESS LEGS SYNDROME

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Master of Philosophy

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MARCH 1989

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The prevalence of RLS, as assessed by interview, was high in iron deficient (9/36) and rheumatoid subjects (54/151), compared with healthy individuals (10/79). Standard haematological parameters showed that RLS may occur in the absence of iron deficiency There was, however, a significant association between the incidence and frequency of RLS symptoms and iron deficiency in rheumatoid subjects.

Oral ferrous sulphate (200mg three times a day) for 28 days was compared with placebo in a double blind trial involving 12 rheumatoid and two non-rheumatoid subjects with 'classical' RLS occurring at least once a week. No significant change was seen in iron replete non-rheumatoid subjects. In 9/10 rheumatoid subjects, all with concurrent iron deficiency, treatment was associated with significant reduction in frequency of attacks and improvement in subjective patient assessments. Complete remission lasting 1-5 months occurred after only seven days treatment in three individuals, suggesting a non-haematological mechanism.

Findings show iron deficiency is a predisposing factor in development of RLS and that in these subjects oral iron is useful in its treatment. A central aetiology involving dopamine and opiate pathways, where iron is thought to play an integral role, is speculated.

A low-dose iron absorption test was also investigated in which a physiological dose of elemental iron (10mg) was administered to fasted subjects and serum iron measured hourly over four hours. Assessment confirmed the sensitivity of the method in predicting iron status with and without concurrent anaemia. The test was also employed in 25 rheumatoid subjects. Results suggested that in those with absent bone marrow iron stores absorption was increased normally, but that iron absorption was reduced in those with normal iron stores compared with subjects without inflammatory disease. Of more practical relevence, the test differentiated genuine iron deficiency anaemia from the anaemia of chronic disease, thereby providing a potential alternative to bone marrow aspiration.

Key words
Restless legs syndrome
Iron
Rheumatoid arthritis
Iron absorption

ACKNOWLEDGEMENTS

The undertaking of any medical research project involves many disiplines. From the conception of this project many people have offered, not only helpful advice in their own specialist area, but general encouragement and enthusiasm to achieve a successful conclusion. My sincere thanks must go to them all.

I should like to take this opportunity to thank Mr DT Beswick and Dr DA Lewis for all their advice and encouragement throughout this project.

Special thanks go to Professor DR Blake for his guidance and constant support throughout the research and subsequent presentations.

I should like to formally acknowledge the specialist assistance provided by the departments of Haematology and Biochemistry at Selly Oak Hospital in performing all the necessary assay measurements, and Sister V Arthur for performing metrology assessments of rheumatoid subjects.

Guidance from Dr JA Murray, consultant haematologist and Dr AC Williams, consultant neurologist, was greatly appreciated. I should also like to thank Mr A Girling, statistician, for advice relating to statistical analysis of results and to Dr J Donavon and the Computor Advisory Service at Aston University for helping me use the Statistics Package for Social Sciences.

Thanks to Dr P Ireland, general practitioner, for allowing me access to his patients and to junior medical and nursing staff on the rheumatology ward for their helpful co-operation.

I am most grateful for the support and understanding shown by staff within the Pharmacy Department, Selly Oak Hospital.

Finally, my sincere thanks must go to the West Midlands Regional Health Authority who provided the opportunity and sponsorship for this work.

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1.0 INTRODUCTION

> "Wherefore to some, when being a Bed they betake themselves to sleep, presently in the Arms and Leggs, Leapings and Contractions of the Tendons, and so great a Restlessness and Tossings of their Members insue, that the diseased are no more able to sleep than if they were in a Place of the greatest Torture"
>
> Thomas Willis 1685.

Restless legs syndrome (RLS), also known as Ekbom's syndrome is a common, distressing condition, of unknown aetiology. Though described over 300 years ago by Thomas Willis [1], it was first defined in detail by Karl-Axel Ekbom in 1945 [2]. The condition is poorly documented in the literature probably due to reluctance of sufferers to complain of symptoms for fear of appearing ridiculous, and also because it does not affect life span. It has however recently received coverage in the popular press [3,4] and on BBC Radio [5,6].

1.1 CLINICAL FEATURES

The primary manifestation is an ill-defined unpleasant and often distressing lower limb dysaesthesia. The sensation is typically of a creeping or crawling nature, but may be an aching or burning feeling, and occasionally painful. RLS usually occurs bilaterally, deep within the limbs and is most frequently localised between the knee and ankle. It occurs at times of inactivity, characteristically in the evening when sitting down, or on retiring to bed. During the night, RLS is often accompanied by periodic movements, such as myoclonus [7]. The sensation is associated with an irresistable urge to move the legs, which provides almost instantaneous, but temporary, relief. The dysaesthesia may continue for hours, requiring the sufferer to kick or move the legs or to get up and walk around. It is an intermittent condition usually lasting a few weeks or months, but occasionally persisting for years. In its severe form it can lead to significant sleep loss, depression or even suicide [8,9]. It occurs in both

sexes and at any age though more frequently in the middle-aged [9].

1.2 DIAGNOSIS

There are no recognised diagnostic tests [9], or formally accepted diagnostic criteria for RLS. Diagnosis therefore relies heavily on careful history taking [10,11]. It has however been suggested that serum iron and haemoglobin concentrations be measured, and that psychological tests be carried out to exclude a secondary cause [9].

Lack of clear diagnostic criteria has led to frequent misdiagnosis; for example, the characteristic symptoms of "crawling ants" or "an internal itch" described by patients have been mistakenly attributed to parasitophobia [12], neurosis or hysteria [11].

1.3 PREVALENCE

The prevalence of RLS has been assessed in various studies. In general, papers give limited details regarding methods used. In particular the source of subjects, methods of questioning and diagnostic criteria are not fully described.

Prevalence figures reported in normal healthy populations vary between 2-6% [13,14,15,16]. The incidence has also been investigated in many medical conditions. (See Table 1.1). Those associated with an increased incidence of RLS include iron deficiency anaemia (24%) [14], uraemia (15-20%) [15,17], gastric surgery (11%) [15], pregnancy (11-27%) [14,18,19] and rheumatoid arthritis (30%) [16]. These conditions are all characterised by the fact that they may either lead to pure iron deficiency or to redistribution of iron, with increased reticuloendothelial iron stores and the anaemia of chronic disease. This may provide a clue as to the pathogenesis of RLS.

MEDICAL CONDITION	REPORTED INCIDENCE (%)	Ref.
Healthy population	2 - 6%	13,14,15
Iron deficiency anaemia	24%	14
Low serum iron	25%	14
Pregnancy	11 - 27%	14,18,19
Uraemia	15 - 23%	15,17,20
Rheumatoid arthritis	30% (prevalence)	16
Gastric surgery	11 - 12.6%	15,21
Diabetes mellitus	17%	15
Parkinson's disease	7 - 11%	13,14
Psychoneurosis	4%	15
Osteoarthritis	3%	16
Other conditions associated with RLS		
Nocturnal myoclonus		22
Prostatitis		23
Carcinoma		24
Chronic pulmonary disease		8
Primary amyloidosis		25
Telangiectasia		26
Caffeinism		27
Folic acid deficiency		28
Barbiturate withdrawal		29
Phenothiazine administration		14

TABLE 1.1: Association of RLS with various medical conditions

1.4 PATHOGENESIS

1.4.1 Clinical, nerve and muscle examination

In common with most parasomnias [30], the cause of RLS is unknown. Clinical examination [31], nerve conduction studies and electromyography have failed to demonstrate consistent abnormalities [32,33,34].

Callaghan investigated five uraemic patients with RLS and found nerve conduction to be delayed in four, whilst one had overt signs of peripheral neuropathy [20]. Thanh [35] and Frankel [36] also found electromyelogram abnormalities in two patients. In contrast Harriman [34] found no abnormality after in vivo staining of intramuscular terminal endings in ten patients with RLS.

Several cases of RLS have been attributed to peripheral cholesterol crystal microemboli [37] .

1.4.2 Peripheral and central aetiologies

In the absence of any obvious abnormalities both peripheral and central aetiologies have been postulated. Ekbom [14] and Murray [31] thought the most likely cause to be abnormal accumulation of metabolites. This was supported by the association of RLS with anaemia and the beneficial effects of movement, vasodilators and fever. Others have suspected a central origin, perhaps in the spinal cord or basal ganglia.

Central nervous system arousal and subsequent increase in contractility of peripheral striated muscle associated with caffeine intake has been hypothesised as one possible cause of RLS [27].

1.4.3 Association between RLS, akathisia and iron

Recently RLS has been likened to neuroleptic-induced akathisia [38,39], which is characterised by a state of mental and motor restlessness accompanied by an irresistible compulsion to keep moving. This frequently involves leg restlessness similar to that of RLS, but the psychological

component is peculiar to akathisia. The pathogenesis of akathisia has not been established but its association with Parkinson's disease and administration of dopamine antagonists suggest that dopaminergic pathways are involved.

A recent study reported that patients with neuroleptic-induced akathisia have significantly lower serum iron concentration than non-akathisia controls [40]. Iron has also been shown to be an integral part of the dopamine D_2 receptor [41], phenothiazines are known to ligate metal ions [42,43]. These facts have led to the suggestion that akathisia results from post-synaptic dopamine D_2 receptor blockade in the mesocortical dopaminergic pathways, enhanced by a low serum iron concentration [40].

RLS has been associated with iron deficiency and possible involvement of the dopaminergic system is supported by the response of RLS symptoms to levodopa [44,45]. Like akathisia, RLS may also be worsened by dopamine receptor antagonists, such as pimozide [44], promethazine and prochlorperazine [14].

1.4.4 RLS and the endogenous opiate pathway

Another hypothesis which is currently being investigated is based on the effectiveness of opiates in controlling symptoms and worsening of the condition by administration of naloxone [46]. This led to the suggestion that the endogenous opiate system may be involved in the pathogenesis. Radioisotope studies indicate that certain divalent metal ions may form an integral part of opiate receptor sites [47]. Again the high incidence of iron deficiency in subjects with RLS may be relevant.

1.5 TREATMENT

Since the pathogenesis is unclear, treatment of RLS has remained empirical [48]. A large variety of drugs have been used including vasodilators, anxiolytics, anticonvulsants and vitamins. (See Table 1.2).

1.5.1 Treatment of RLS with iron

In the 1950's the high incidence of RLS in pregnancy and iron deficiency prompted the use of iron supplements in the treatment of RLS. Norlander [18] recorded 30 anecdotal cases of RLS with concurrent iron deficiency anaemia, where correction of anaemia improved symptoms. Nineteen patients received 1-3 iron injections and remained symptom free for many months. Several patients had a 1-2 month course of oral iron with equally good effect. Parenteral iron was also found to be effective in cases of RLS without anaemia or low serum iron [18]. These findings were confirmed by Ekbom [14] when he treated iron deficient subjects with parenteral iron. There have since been a number of anecdotal reports relating to iron deficient subjects who obtained relief of RLS following oral iron [58,62]. These findings have not, as far as is known, been substantiated by placebo controlled trials.

Clinical assessment has recently centred around the use of clonazepam, carbamazepine, levodopa and opiates.

1.5.2 Treatment of RLS with anticonvulsants

Clonazepam is well established in the treatment of various myoclonic disorders [63]. The association of nocturnal myoclonus with RLS in some patients prompted its evaluation in RLS. In uncontrolled studies, clonazepam (1-3mg o.n.) was found to reduce or abolish symptoms in 8 patients with idiopathic RLS [53,52], and also in 15 uraemic patients [17]. Reports of two cross-over placebo controlled trials have since been published. In the first involving 6 patients, clonazepam (1mg o.n.) was compared with placebo and a baseline period, each of one week duration [54]. Clonazepam was found to improve quality of sleep and dysaesthesia, though symptoms still remained. The second study, also involving 6 patients did not, however, show clonazepam to be significantly more effective than placebo [55]. In the latter trial the dose of clonazepam was gradually

DRUG	CLINICAL EVALUATION	No. OF PATIENTS	DAILY DOSE	Ref.
Vasodilators				
Carbochol	A			18
Clonidine	A	3	0.1 - 0.3mg on	49
GTN	A		0.5mg s.l.	14,31
m-Inositol hexanicotinate	A			14
Tolazoline	A			18
Beta-blockers				
Propranolol	A		5 - 10mg on	13
Anxiolytics				
Diazepam	A		5 - 10mg on	16
Anticonvulsants				
Carbamazepine	P	6	200 - 600mg	50
	P	174	100 - 300mg	51
Clonazepam	A	3	0.5mgtds & on	52
	A	15	1mg on	17
	O	5	1mg on	53
	P	6	1mg on	54
	P	6	0.5mg qds	55
Levodopa				
As Madopar	A	5	200mg on	44
As Madopar	P	20	50 - 100mg on	45
Opiates				
Codeine	O			46
Methadone	A	3	10mg bd	56
	A	2		57
	O			46
Oxycodone	A		2.5mg hs	56
Propoxyphene	O			46
Vitamins				
Iron	A	10	IV injection	14
	A	19	IV injection	18
	A	2	oral iron	18
	A	3	oral iron	58
Folic acid				28
Ascorbic acid				31
Tocopherol				59
Others				
Orphenadrine	A			60
Oxerutins	P	596	250mg qds	61
Quinine				31

A - anecdotal report
O - open study
P - double-blind placebo controlled trial

TABLE 1.2: Drugs used to treat RLS and extent of clinical evaluation (up to 1986 only)

increased to 0.5mg q.d.s. and each treatment period lasted four weeks. Both trials used a daily point rating scale to monitor intensity of symptoms and quality of sleep.

Carbamazepine has been investigated for RLS following the suggestion that RLS might be due to a lowered excitation threshold at rest. In an initial double blind placebo controlled cross-over trial three out of six patients preferred carbamazepine [50]. Symptoms were reduced in severity and worsened following discontinuation of treatment. More recently 174 patients were recruited to a multi-centre double-blind, between-patient trial lasting five weeks [51]. Carbamazepine and placebo both had a significant effect, though carbamazepine was more effective. In both these studies symptoms were assessed by recording the number of attacks and their severity on a point or visual analogue scale. Factors affecting response were later investigated using linear discriminant analysis [64]. Responders were younger, with a shorter duration of RLS and lower blood pressure.

1.5.3 Treatment of RLS with levodopa

The suggestion that the dopaminergic pathway may be involved in the pathognesis of RLS has led to the investigation of treatment with dopamine agonists. Levodopa and bromocriptine were found to cure symptoms in a small number of patients, though they reappeared on discontinuation of treatment [44]. In a cross-over placebo controlled study involving 20 patients most preferred Madopar (levodopa 100mg, benserazide 25mg o.n.), which completely relieved symptoms [45]. No mention was made to baseline severity of symptoms.

1.5.4 Treatment of RLS with opiates

Following three anecdotal reports [56] describing relief of RLS with low dose methadone (10mg b.d.) and oxycodone (2.5mg hourly), there have been several open studies with opiates. The first involved only two patients, both of whom had myoclonus combined with RLS, whose symptoms were assessed

on and off methadone or propoxyphene. Patients gained relief from opiates which was reversed by naloxone [57]. The study was later extended to chronic opiate administration in five patients [46]. Here also myoclonus was a dominant feature. Patients reported a marked reduction or complete absence of restlessness and dysaesthesia whilst on methadone, codeine or dextropropoxyphene, without developing addiction or tolerance. The effect was reversed by naloxone. This response to opiates supports the suggestion that the endogenous opiate system might be important in the pathogenesis of RLS.

Most reports in the literature of success in treating RLS are limited to anecdotal reports and uncontrolled studies. Only recently have double blind placebo controlled trials been undertaken, mainly involving small numbers of subjects. Many of the reports make little mention to the severity of symptoms prior to recruitment, or how they are monitored and make little attempt to follow-up patients. All the studies may be criticised for failing to detail diagnostic criteria which, in view of the subjective nature of the condition and lack of objective assessments would appear to be a fundamental omission. The marked placebo effect demonstrated by recent studies and spontaneous remission rate, emphasise the need for placebo controlled trials.

1.6 BACKGROUND TO THE PROJECT

Restless legs syndrome is a common, disturbing and grossly under-diagnosed condition, the pathogenesis and rational treatment of which remain unclear. Prevalence figures suggest that there is a major association between RLS and conditions that lead to pure or functional iron deficiency, as occurs in the anaemia of chronic disease. Current theories in the pathogenesis of RLS favour a central origin either involving the dopaminergic or endogenous opiate pathways. Evidence suggests that iron plays an integral role in

these pathways and subjects developing a related condition, akathisia, have been found to have significantly lower concentrations of iron in their serum. Anecdotal reports suggest that RLS may be treated with iron, even in the presence of a normal haemoglobin. There is therefore evidence implicating iron in the prevalence, pathogenesis and treatment of RLS. The broad aims of this project were to investigate the suggestion that iron deficiency may be the cause or predisposing factor in the development of RLS and that oral iron may be useful in its treatment.

In a recent survey the prevalence of RLS among hospital in-patients with rheumatoid arthritis (RA) was found to be 30% (20/69), compared with 3% (1/30) and 6% (4/70) in patients with osteoarthritis and normal controls respectively [16]. Rheumatoid arthritis is a chronic debilitating disease, and though RLS is not associated with significant morbidity, persistent disturbance at night may significantly affect patients' well-being and ability to cope. Patients with RA characteristically develop the anaemia of chronic disease. A significant proportion [65] (33% [66], 55% [67], 60% [68], 70% [69]) also develop iron deficiency, often related to drug-induced gastrointestinal bleeding.

Due to the high incidence of RLS in RA and the association of the latter with altered iron status, work was undertaken primarily in this group of patients. By concentrating on a patient population in which the incidence of RLS is known to be high it was hoped that mechanistic clues and therapeutic measures be found that would be of value to all sufferers.

It was necessary to give detailed consideration to the role of iron in the body and the development and assessment of iron deficiency. It was also essential to address the diagnostic dilemma posed by iron deficiency and the anaemia of chronic disease in rheumatoid arthritis. These areas are considered in the sub-sections that follow.

1.7 IRON METABOLISM

1.7.1 Body iron requirements

The average healthy adult male contains 4g iron [70]. This may be categorised as functional and storage iron. The relative proportions are shown in Figure 1.1.

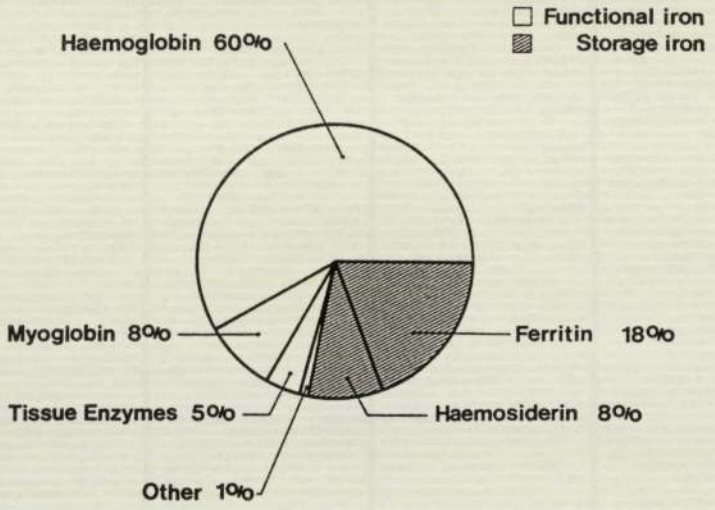


FIGURE 1.1: Body iron distribution

The majority of total body iron is in red blood cells as a constituent of haemoglobin. The quantity of iron in myoglobin and enzyme systems is small but vital for total body function. Most of the remaining body iron is stored as ferritin and haemosiderin in liver parenchyma and in reticuloendothelial cells.

In the normal metabolic state, the majority of total body iron is re-utilised. Daily iron loss in a non-mensturating adult is 0.5 - 1.5mg [71], mainly lost in desquamated epithelium, gut and skin secretions and urine. A mensturating female will lose an additional 0.5 - 2mg per day [72,73]. To remain in iron balance it is essential that sufficient iron is absorbed from the diet to meet these losses.

1.7.2 Iron absorption

Absorption of iron from the gastrointestinal tract involves a complex active transport system. Absorption is dependent on dietary iron content, its availability for absorption and most importantly body iron stores.

The average British diet contains 10 - 20mg iron a day [74,75]. Under normal conditions only 5 - 10% is absorbed [71,74], just sufficient to balance the losses provided they are not excessive. The amount of iron absorbed is increased to 25 - 30% in iron deficiency [71,75].

Dietary iron may be classified a haem iron, found in meat, and non-haem iron as in cereals and vegetables. Most iron in the diet comes from non-haem sources in the form of organic complexes. Absorption of these is poor and affected by inhibitors (eg. tannates, phosphates) and enhancing substances (eg. ascorbic acid, protein sulphydryl groups) [76]. The latter aid conversion to the ferrous form (Fe^{2+}) which is two to three times more readily absorbed than the ferric form (Fe^{3+}) [77,78]. The importance of valency is probably due to the poor solubility of the ferric form once the pH rises above pH 3.

The site of absorption is the upper small intestine, in particular, the duodenum and upper jejunum [79]. Observations in animals suggest that there are specific iron receptors on the brush borders of the gut wall [81]. Receptor number and distribution may therefore affect iron absorption. There are two stages to absorption; 'uptake' into mucosal cells from the gut lumen and 'transfer' from mucosal cells to the systemic circulation. See Figure 1.2.

The precise mechanisms and regulations of these processes are uncertain. Most experimental work in this field has been performed on animals and findings may not apply directly to humans.

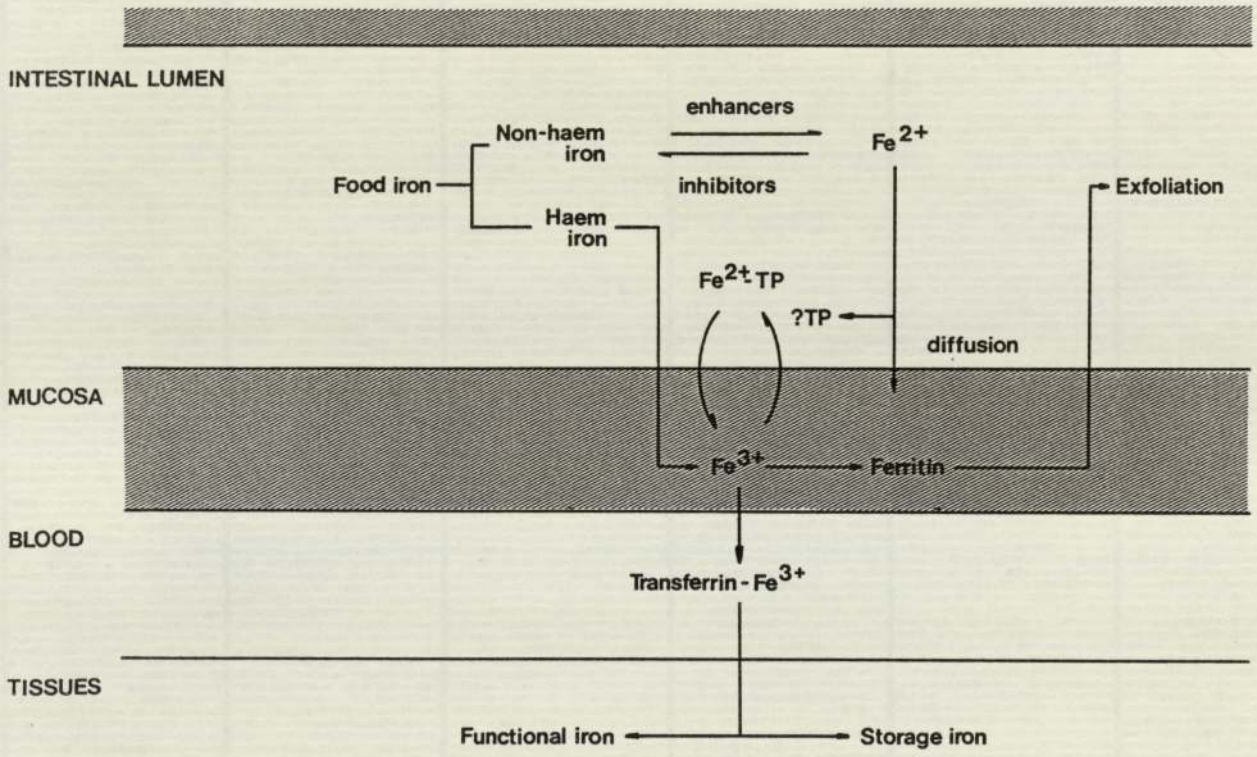


FIGURE 1.2: Scheme of iron transport across the mucosal cells of the duodenum and jejunum

'Uptake' is dependent on the concentration of iron within the gut lumen. While the relationship is linear, the slope varies in accordance with the body's iron requirement [79]. Mechanisms are thought to vary between haem and non-haem iron. The former is absorbed within the porphyrin ring [82], iron being released within the mucosal cell. In contrast, it has been suggested (and rejected [83]) that non-haem iron in the ferrous form (Fe^{2+}) binds to a mucosal protein in the gut lumen [84], which then transports it across the brush borders of the intestinal mucosa. Absorption may therefore be affected by changes in the amount of transport protein present. Once in the mucosal cell, iron is oxidised to the ferric form (Fe^{3+}).

In iron deficiency iron is then rapidly transported into the blood, the rate of transfer being at a maximum during the first 30 minutes [85]. Iron which is not immediately required becomes bound to apoferritin to form ferritin. This iron may then be transferred to the systemic circulation as required, or lost into the gut lumen and excreted.

1.7.3 Iron transport

Iron is transported in the serum bound to iron binding proteins collectively called transferrin. Each transferrin molecule binds two atoms of iron in the ferric form (Fe^{3+}). It has been suggested that the degree of transferrin saturation helps regulate 'transfer' from mucosal cells to the systemic circulation. Transferrin is produced in the liver, synthesis increasing in conditions of hypoxia, for example iron deficiency, and decreasing in iron overload [71].

1.7.4 Iron storage

Excess body iron is stored bound to apoferritin and haemosiderin within the bone marrow, liver, spleen and other body tissues, where it can be mobilised and distributed via serum transferrin if required [79].

Ferritin consists of a spherical apoprotein shell (mol. wt 480,000) and a

core of iron in the form of ferric hydroxyphosphate which may contain up to 4500 atoms of iron [86,87]. The exact structure of ferritin varies depending where it is found. Acidic isoferritins are found in the heart, red blood cells, lymphocytes and monocytes, whilst more basic isoferritns predominate in the liver, spleen and placenta [86]. Synthesis of ferritin is stimulated by iron within body tissues [87].

The other form of storage iron, haemosiderin comprises a group of compounds some of which are derived from ferritin, others from porphyrins, sugars, lipids and proteins [79]. Haemosiderin is insoluble and accumulates in lysosomes [86]. It accounts for about one third of normal iron stores, and more in iron overload.

1.7.5 Role of iron in the body

The major role of iron in the body is as an essential component of haemoglobin, the main function of which is the transport of oxygen from the lungs to the tissues [88]. Iron is similarly bound to the porphyrin complex within myoglobin, which is responsible for oxygen transport and storage in muscle [88]. The other important role of iron is in the functioning of various tissue enzymes, either as an integral part of the enzyme or as a co-factor or activator. The diversity of some of these enzymes and their functions are listed in Table 1.3.

1.8 PURE IRON DEFICIENCY

Iron deficiency is defined as a diminished total body iron content [79]. It is a graded process starting initially with a decrease in iron stores, developing into iron deficient erythropoesis and ultimately into iron deficiency anaemia.

Consideration of the consequences of iron deficiency have traditionally focused on the anaemic stage, with the assumption that symptoms relate primarily to haemoglobin concentration. Symptoms of fatigue, weakness,

ENZYME	FUNCTIONS
HAEM IRON COMPOUNDS	
Mitochondrial cytochromes a,b and c	Electron transfer in oxidative phosphorylation leading to ATP production in mitochondria and cell membranes
Cytochrome P450	Microsomal enzyme involved in hydroxylation and drug metabolism in liver and intestinal mucosa
Catalase	Peroxide production
NON-HAEM COMPOUNDS	
NADH dehydrogenase	
Succinic dehydrogenase	
Xanthine oxidase	
IRON CO-FACTOR	
Tryptophan hydroxylase	Conversion of tryptophan to serotonin
ROLE OF IRON UNCERTAIN	
Ribonucleotide reductase	DNA synthesis
OTHER ENZYMES WITH IRON INVOLVEMENT	
Tyrosine & proline hydroxylase	Amino-acid metabolism Synthesis of dopamine
Monoamine oxidase	Catecholamine metabolism, including dopamine
Glucose-6-phosphate & glucose-6-phospho-gluconate dehydrogenase	Anaerobic metabolism
Metalloflavoproteins	

TABLE 1.3: Iron dependent enzyme [71,89]

swelling of the feet and breathlessness on excertion are often prominent in cases of severe anaemia. Earlier presentation of such symptoms is prevented by adaptive mechanisms, for example greater efficiency in extraction of oxygen from haemoglobin, redistribution of blood flow to essential organs and increased cardiac output. A number of morphological abnormalities of epithelial structures may occur including angular stomatitis, glossitis, oesophageal stricture, changes in gastric mucosa and koilonychia. The exact relationship of some of these to iron deficiency is unclear.

The clinical effects of iron deficiency on iron-dependent tissue enzymes are poorly defined. A variety of effects have been demonstrated primarily in animals. These include muscle dysfunction [90], growth retardation [91], increased catecholamine levels [92], behavioural abnormalities [93,94], altered resistance to infection [79,80], impairment of dopamine binding to D_2 receptors in the caudate nucleus [95] and of 5HT to brain synaptic vesicles [96]. Depletion of these compounds occurs more readily in some tissues than others [88,97] and depends on turnover. Studies have not yet clearly indicated the degree of deficiency at which such manifestations occur, or the extent of treatment required to reverse them.

1.9 INDICATORS OF IRON STATUS

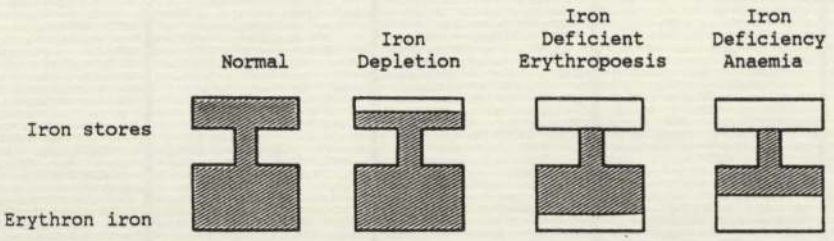
The various stages of iron deficiency are paralleled by changes in different laboratory tests. This sequence of events is shown in Table 1.4.

1.9.1 Reduced iron stores

The initial stage, iron depletion, involves reduction in iron stores. Haemoglobin and red cell indices are not affected at this point. Reduction of body iron stores may be detected by assessment of marrow iron stores, serum ferritin concentration and gastrointestinal iron absorption.

1.9.1.1 Bone marrow assessment of iron stores

Until recently bone marrow aspiration was accepted as the only reliable test for the assessment of iron stores [98]. A sample of haemosiderin from



	Normal	Iron Depletion	Iron Deficient Erythropoesis	Iron Deficiency Anaemia
BM iron stores (grade)	2 - 3+	0 - 1+	0	0
S-ferritin ug/l	> 20	< 20	< 15	< 15
Serum iron umol/l	11 - 30	11 - 20	< 11	< 7
Transferrin saturation %	20 - 50	15 - 20	< 15	< 10
TIBC	Normal	Normal to high	High	High
Iron absorption %	5 - 10	10 - 15	10 - 20	10 - 20
MCV (fl)	80 - 100	80 - 100	< 80	< 70
Erythocytes	Normal	Normal	Normal	Microcytic / Hypochromic

TABLE 1.4: Sequence of events in negative iron balance

within the reticuloendothelial marrow cells is stained using potassium ferrocyanide (also known as Prussian blue or Perl's reaction) [99], then visually assessed and graded as absent, reduced, normal or increased. Subjects with absent or reduced iron stores are assessed as iron deficient.

In common with other histological assessments, the method may give rise to considerable variation in iron staining and interpretation is subjective. Differentiation between reduced and normal iron stores is particularly difficult and sampling technique may result in false negative results. In addition it is an invasive, time consuming and costly procedure, which may be unpleasant for the patient. Nevertheless, bone marrow aspiration remains the reference standard against which other tests are compared.

1.9.1.2 Serum ferritin concentration as a measure of iron stores

The other major storage form of iron is ferritin. Although predominantly intracellular, development of a sensitive immunoradiometric assay has enabled small amounts to be measured in the serum by means of a simple

blood test [104]. Serum ferritin has been found to give an accurate indication of tissue iron stores in normal subjects, and patients with simple iron deficiency or iron overload [101,102,103,104]. In most situations a serum ferritin less than 20ug/l is indicative of iron deficiency [71]. The test has the advantage of producing quantitative and reproducible results and is more sensitive than bone marrow assessment of iron stores [104].

The major limitation of the assay is that levels may be elevated by inflammation and liver disease [105]. Apoprotein, the part of the ferritin molecule which is actually measured by the assay, is an acute phase reactant. Serum ferritin is therefore raised in conditions associated with a high erythrocyte sedimentation rate (ESR), for example malignancy, leukemia and chronic diseases such as rheumatoid arthritis. Some carcinomas and lymphomas secrete cross-reacting ferritins, while acute hepatic necrosis or inflammation result in release of intracellular hepatic ferritin. Levels may therefore appear normal in the presence of iron deficiency. A serum ferritin of less than 15ug/l always means that there is iron deficiency [105,106].

1.9.1.3 Iron absorption as a measure of iron stores

Iron absorption is a function of storage iron [107,108,109]. In general as iron stores become depleted the percentage of gastrointestinal iron absorbed will increase [71,79], even if iron depletion is not severe enough to compromise erythropoesis [79,110]. Absorption may however be impaired with any acute or chronic inflammation [71]. In patients with rheumatoid arthritis there is controversy as to whether absorption reflects iron status. Some authors suggest that iron absorption is defective [111,112,113]. Others have found it to inversely correlate with iron stores [114,115,116], but that patients with concurrent iron deficiency do not increase absorption to the same extent as subjects with simple iron deficiency anaemia [117,118].

Assessment of absorption has been undertaken using a variety of techniques. Methods have mainly been employed to investigate mechanisms of iron uptake and malabsorption syndromes, to compare absorption of therapeutic compounds and to demonstrate interference by food and drugs. Rarely has assessment of iron absorption been advocated in the diagnosis of iron deficiency [119].

Early non-isotopic methods of assessing iron absorption have largely been replaced by those employing radioisotopes. These, however, are time consuming, require specialised counting and scanning equipment and are particularly inappropriate for use in children and women of childbearing age.

By far the simplest method is the iron absorption test in which non-radioactive iron is ingested and serum iron concentration is measured at time intervals over 4 - 8 hours [120]. This method is based on the assumption that the post-dose rise in serum iron is dependent on the total quantity of iron absorbed. Other factors that may affect the iron absorption curve include the rate of absorption from the gastrointestinal tract, the baseline serum iron concentration, the transferrin saturation and rate at which iron is cleared from the plasma. The method has been criticised as lacking sensitivity [79] and showing poor correlation with radioisotope measurements of iron absorption [114]. Previous iron absorption tests utilised a pharmacological dose of iron (50 -250mg), or sometimes 1mg/kg body weight [110]. It has however been suggested that these large doses overwhelm the ability of the intestine to reject available but, unnecessary, iron [110].

Crosby et al showed that performing the test with a physiological dose (5-20mg) provided a sensitive method of differentiating mild iron deficiency from iron repletion in otherwise healthy volunteers [110]. The method has not yet been extended to other medical conditions [121].

1.9.1.4 Serum iron indices

Serum iron concentration (s-iron), total iron binding capacity (TIBC) and % transferrin saturation (% saturation) [% transferrin saturation = (s-iron/TIBC) x 100)] are well established as indices of iron status [105]. They become altered in iron deficiency when iron stores are virtually exhausted [122]; s-iron falling to less than 10umol/l, TIBC being abnormally raised and transferrin saturation falling to less than 16%.

S-iron concentration represents the balance between inflow and outflow of iron in the plasma, in particular it reflects the demands of erythropoesis. It has however been criticised as an indicator of iron status because of its instability [105]. S-iron has been shown to exhibit diurnal variation in healthy subjects. Most individuals experience a morning peak and evening trough [79,123,124], though other workers have shown this to occur at different times of day [125,126]. Biological variation is however thought to diminish in iron deficiency resulting in values that are more consistently low [127,128]. The other limitation of s-iron determination is that a low s-iron may also result from malignancy, inflammation, infection and following myocardial infarction, surgery and trauma [79].

Total iron binding capacity is a measure of the amount of iron transport protein, transferrin, present in plasma. It does not have such a rapid turnover or exhibit diurnal variation and values therfore show less fluctuation. Plasma transferrin concentration is inversely related to the size of body iron stores and hence is raised in iron deficiency. Transferrin levels may also be raised in response to pregnancy and oestrogen therapy [71].

Transferrin saturation (%) tends to be the most useful of the serum iron indices [129]. When the value falls below 16% iron supply becomes inadequate to support basal erythropoesis [130]. Although low levels are

invariably associated with iron deficiency, pregnancy and chronic disease may result in equally low levels [87].

1.9.2 Red blood cells and iron deficiency

The final phase of iron deficiency is the development of anaemia, when supply of iron from recycled haemoglobin and other iron stores is exhausted. Red blood cells become characteristically microcytic and hypochromic, as indicated by low mean cell volume (MCV) and mean cell haemoglobin (MCH).

1.10 ANAEMIA OF CHRONIC DISEASE IN RHEUMATOID ARTHRITIS

Diagnosis of iron deficiency using routine laboratory tests in patients with rheumatoid arthritis is complicated since most also have the anaemia of chronic disease (ACD).

Anaemia of chronic disease is of moderate severity, with haemoglobin rarely below 8g/dl [131]. It is usually normochromic and normocytic, but occasionally hypochromic and microcytic [132]. The anaemia is characterised by decreased serum iron and decreased or normal iron binding capacity in the presence of normal or increased reticuloendothelial iron stores [65]. ACD develops during the first few months of illness [65,78] and thereafter haemoglobin levels tend to vary inversely with erythrocyte sedimentation rate and other markers of disease activity [65,131,132,133].

The pathogenesis of ACD remains unclear despite extensive animal and human studies. Various hypotheses have been investigated including; decreased red cell survival, impaired release of erythropoetin, bone marrow failure, inadequate supply of iron from reticuloenthothelial cells to the bone marrow (termed 'reticuloendothelial iron block'), impairment of iron absorption and humoral or cell mediated suppression of erythroid progenitor cells [131].

In RA iron has been found to be redistributed and sequestered as ferritin

within the cytoplasm and lysosomes of reticuloendothelial cells in the synovial membrane [134]. This may contribute to the functional iron deficiency characteristic of ACD [135]. It has been hypothesised that the accumulation of iron in the joints may promote tissue damage via an oxidative free radical reaction [136].

Currently the only means of treating this type of anaemia is by alleviating the underlying disorder, though this may change in the future with the introduction of a genetically engineered erythopoetin [137].

1.11 DIFFERENTIATION OF THE ANAEMIA OF CHRONIC DISEASE (ACD) AND IRON DEFICIENCY ANAEMIA (IDA).

Differentiation between IDA and ACD in rheumatoid arthritis is difficult by means of routine laboratory tests since many react similarly in both. See Table 1.5.

Parameter	Pure iron deficiency anaemia	Anaemia of chronic disease (ACD)	Iron deficiency with ACD
Hb	Reduced	Reduced (rarely below 8g/dl)	Reduced
MCV MCH MCHC	All reduced in relation to severity of anaemia	Low normal or mild reduction (MCV rarely below 70fl)	Reduced
S-iron	Reduced	Reduced	Reduced
TIBC	Raised	Reduced	High normal
S-ferritin	Reduced	Normal	Reduced or normal
Bone marrow iron stores	Absent	Present	Absent
Response to oral iron	Responds	Refractory	Responds

TABLE 1.5: Diagnosis of iron deficiency and anaemia of chronic disease [131,133]

Clinically the distinction is important, firstly to avoid overlooking a potentially curable source of bleeding and secondly because indiscriminant treatment with iron supplements will not cure ACD and may augment joint inflammation [136].

Attempts have been made to identify a single or combination of laboratory determined variables that will distinguish the two types of anaemia. Correlation of individual red blood cell indices or serum iron indices with marrow iron stores have been found to have low predictive value. It has been suggested that a raised lower normal limit for serum ferritin of 55ug/l [138], 60ug/l [67,139], 100ug/l [68], and <110ug/l [140] is appropriate in rheumatoid arthritis.

In one study where the normal limit was set at 60ug/l, s-ferritin was found to predict iron deficiency accurately in 83% of anaemic patients [139]. Simultaneous presence of low s-ferritin and high TIBC was found to increase the predictive value (91%), but with loss of specificity. In another study a good response to oral iron, and hence indication of iron deficiency was associated with s-ferritin less than 60ug/l and TIBC greater than 55ug/l [69].

Beck et al constructed a multivariant algorithm to aid diagnosis of iron deficiency [141]. They combined the correlation of laboratory variables (s-ferritin MCV, s-iron and TIBC) with bone marrow iron stores. Later ESR was incorprated to extend the usefulness of the programme to include patients with ACD. They reported accurate prediction of iron status in 96% (71/74) of cases, though details of concurrent medical condition were not given. The approach has not been specifically adapted for a rheumatoid population.

Despite attempts to solve the diagnostic dilemma posed by IDA and ACD in rheumatoid arthritis bone marrow assessment of iron stores remains the only

reliable way to accurately determine iron status in RA [142].

1.12 TREATMENT OF IRON DEFICIENCY

In a clinical situation it is essential that the cause of iron deficiency is identified and treated to prevent recurrence. Common causes include gastrointestinal blood loss, menorrhagia and malabsorption. Dietary deficiency is rarely implicated in Western countries. The aim of treatment then is to correct any anaemia and replenish iron stores.

There are few indications for administration of iron via the parenteral route and discussion will therefore concentrate on oral therapy. Ferrous iron is used to correct iron deficiency since it is readily absorbed [78]. There is no significant difference in absorption of the various ferrous salts; sulphate, lactate, fumarate and gluconate having been shown to be absorbed to approximately the same extent [143]. Nevertheless, ferrous sulphate is generally regarded as the treatment of choice [73,144], being comparatively well absorbed, well tolerated, effective and cheap.

The optimum dose for therapy is not well defined [145]. The amount of iron absorbed continues to increase as the dose administered increases, though the percentage absorbed decreases [73,146]. Some authors advocate administration of large daily doses of iron (250 - 400mg elemental iron) to fuel the increased rate of erythropoesis [71,79], while others in the absence of life threatening deficiency favour smaller doses (50 - 100mg elemental iron) [147,148]. In practice the dose used is a compromise between the therapeutic effect required and development of adverse effects. The standard recommended dose of ferrous sulphate is 200mg t.d.s. [144]. Claims that slow release perparations are equally effective and better tolerated than conventional tablets have not been substantiated by controlled studies [79,149] and do not justify the extra cost [150,151]. Similarly, use of compound preparations containing absorption enhancing agents (eg. ascorbic acid, succinic acid) is not recommended [78].

Duration of treatment depends on the severity of anaemia and tissue depletion. Using the standard dose of ferrous sulphate it is predicted that haemoglobin should rise by 0.1-0.2g/dl/day [144]. It has been suggested that iron therapy be continued for 8 - 10 weeks to correct anaemia [71] and then for a further eight weeks to replenish iron stores [152]. Haematological parameters return to normal in the reverse order to development of iron deficiency; haemaglobin and red cell indices first, s-ferritin and bone marrow iron stores last.

Side effects are largely confined to the gastrointestinal tract and include constipation, diarrhoea, heartburn, nausea, epigastric discomfort and distension. In a double blind controlled study comparing ferrous sulphate, gluconate and fumarate (222mg elemental iron daily) with placebo, the incidence of side effects with each salt was 25% compared with 13% for placebo [153]. In another study involving a similar dose of iron, 15% reported gastrointestinal intolerance [78]. The incidence of constipation appears to be unrelated to dose, whereas nausea and epigastric pain occur more frequently as the quantity of soluble iron in the gut increases [78,144].

There are conflicting views as to when iron supplements should be taken in relation to food. The bioavailability is reduced by 40-75% when taken with food [73,78]. Food, however, improves the gastrointestinal tolerance [71].

1.13 AIMS AND OBJECTIVES

The main aim of the research was to investigate the suggestion that iron deficiency may be the cause or predisposing factor in the development of Restless Legs Syndrome and, that oral iron may be useful in its treatment. The diagnostic dilemma posed by iron deficiency and the anaemia of chronic disease in rheumatoid arthritis was also addressed by an investigation of a low dose iron absorption test.

The objectives are represented schematically in Figure 1.3 and were as follows:

1. To investigate the prevalence of RLS in three populations; iron deficient, rheumatoid and healthy.

2. To investigate the iron status of subjects with RLS using standard haematological methods.

3. To evaluate the efficacy of oral iron in the treatment of RLS by means of a double blind placebo controlled trial.

4. To assess a low dose iron absorption test, described by Crosby et al. [110], as a method of differentiating iron deficiency and anaemia of chronic disease in patients with rheumatoid arthritis.

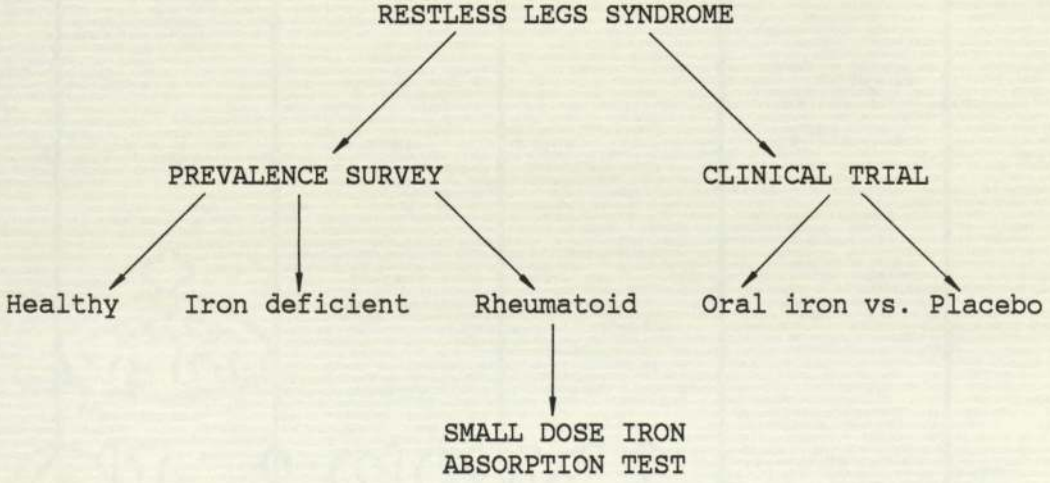


FIGURE 1.3: Schematic representation of research

2.0 MATERIALS AND METHODS

This chapter has been arranged into three main sections, thereby conforming with the schematic research plan included at the end of the previous chapter. Section 2.1 gives details of the survey which investigated the prevalence of restless legs syndrome. Section 2.2 deals with the clinical trial evaluating oral ferrous sulphate in the treatment of restless legs syndrome. Finally, section 2.3 explains the method of the low-dose iron absorption test used firstly, to assess iron absorption in rheumatoid disease and secondly, as a potential method of differentiating iron deficiency anaemia from the anaemia of chronic disease.

2.1 PREVALENCE SURVEY

The survey was designed to assess the prevalence of RLS, (defined as the number of subjects experiencing symptoms during the previous 12 months), in selected populations and to compare iron status between subjects with and without RLS. In view of the poor understanding of the condition additional information was also collected permitting classification of the type, frequency and duration of sypmtoms; evaluation of the influence of hereditory factors, age and sex; correlation with concurrent disease and medication; and assessment of the level of inconvenience experienced and medical advice sought and received.

2.1.1 Ethical approval

The study protocol was submitted to and approved by the Ethics Committee, South Birmingham Health Authority.

2.1.2 Diagnosis of RLS

Diagnostic criteria were defined based on the detailed description of RLS by Ekbom [1]. Criteria used were briefly as follows:

1. Discomfort in the legs.
2. Symptoms occurring at rest.
3. Association with an irresistable need to move the legs, which provides some relief.

Differentiation was made between 'classical' and other forms of symptoms. 'Classical' symptoms were defined as those where the discomfort involves a crawling sensation, localised between the knee and ankle and where symptoms occur bilaterally.

Individuals with known neurological disorders, Parkinson's disease, varicose veins, leg ulcers or on neuroleptic medication were excluded from the study. Full criteria are detailed in Appendix A1.

Diagnostic criteria were approved by a consultant neurologist (AW) and consultant rheumatologist (DRB), and are largely in agreement with those that have since appeared in the literature [154]

2.1.3 Selection of subjects

The survey was undertaken in three populations:

i) consecutive in-patients and out-patients with classical or definite RA (in accordance with American Rheumatism Association criteria shown in Appendix A2), under the care of the Rheumatology Department, Selly Oak Hospital, Birmingham.

ii) consecutive out-patients with iron deficiency or iron deficiency anaemia (as defined in Appendix A3) attending iron deficiency or haematology clinics at Selly Oak Hospital.

iii) Healthy individuals comprising; hospital staff, relatives of rheumatology in-patients, patients and relatives attending an early evening general practioner (GP) surgery, and GP referrals attending the Haematology Department at Selly Oak Hospital for a blood test. This population was defined as having no known underlying disease pathology, excluding common ailments, and receiving no regular drug therapy.

2.1.4. Subject interviews

The primary method of data collection was by patient interview. Formal data collection was preceded by a short period of unstructured interviewing in a rheumatology clinic where approximately thirty patients were seen. The main aim of this was to improve understanding of RLS and of rheumatoid arthritis and practice interview technique whilst under close medical supervision.

During the main phase of the survey, subjects were interviewed by the investigator in a structured manner, using a specially designed questionnaire (see Appendix A4). The questionnaire content was based on literature review and on information gathered from preliminary unstructured interviewing. This was approved by rheumatology and neurology consultants. Interviews lasted 3 - 15 minutes. For dialogue from a typical unstructured interview, see Appendix A4.

2.1.5 Assessment of iron status

At the time of interview a blood sample (5ml fresh, 10ml clotted) was taken from subjects for assessment of iron status by means of standard haematological indices. In the case of hospital patients this was part of routine investigation.

Haemoglobin (Hb, g/dl), red blood count (RBC, $x10^{-9}/l$), mean cell volume (MCV, fl), mean cell haemoglobin (MCH, pg) and packed cell volume (PCV, l/l) were measured on a Coulter counter, Model S880, Coulter Electronics Ltd. Red blood count and mean cell volume are determined based on the Coulter method [155]. As each red blood cell passes through an aperture it changes the resistance producing a voltage, the magnitude of which is proportional to the mean cell volume. The analyser also determines haemoglobin concentration. This involves a reaction with potassium cyanide and ferrocyanide to form cyanomethaemoglobin, the absorbance measured using a photoelectric colorimeter [156]. This then allows automatic calculation of packed cell volume (RBC x MCV / 1000) and mean cell haemoglobin (Hb x 10

/RBC).

Iron indices, serum iron concentration (s-iron) and total iron binding capacity (TIBC) were determined by an automated deproteinisation-ferrozine colorimetric assay [157]. Transferrin saturation (%) is calculated automatically (s-iron x 100/ TIBC). Serum ferritin concentration (s-ferritin) was determined by single-incubation two-site immunoradiometric assay [158] (Quantimune Ferritin IRMA, Biorad assay). The method uses ^{125}I-labelled antibody to ferritin as the tracer and ferritin antibodies immobilised on polyacrylamide beads as the solid phase.

All measurements were performed by the Haematology Department, Selly Oak Hospital.

2.1.6 Assessment of rheumatoid disease activity

Disease activity of rheumatoid subjects was assessed by clinical and biochemical measurements.

Both subjective and objective clinical assessments were used. These were: duration of morning stiffness (mins); Ritchie articular index, which is a numerical measurement of joint tenderness [159]; grip strength (mmHg), measured using an adapted sphygnomanometer; and pain score assessed using a 0 - 10 visual analogue scale (0=no pain, 10=worst pain). These assessments were performed by a metrology nurse (VA).

Biochemical parameters measured included erythrocyte sedimentation rate (ESR, mm/1st hour) by Westergren method [160], in which blood is mixed with trisodium citrate and left to settle for an hour in a standard column (140mm x 2mm), the height of clear plasma being measured after one hour. C-reactive protein (CRP, mg/l) was determined by single radial immunodiffusion [161], and rheumatoid factors (RF) by the Waaler-Rose assay [162, 163] and latex slide test [164]. Biochemical assays were performed by the Division of Rheumatology Biochemistry, Selly Oak Hospital.

Normal limits for haematological and biochemical parameters are listed in Appendix A5.

2.1.7 Data Analysis

In view of the large volume of data produced by the survey, questionnaire results were coded, then transferred to magnetic tape and verified by trained operators (Datapron, Erdington, Birmingham) before being entered onto the Vax Cluster computer system at Aston University. Data was subsequently analysed utilising SPSS* (Statistical Package for the Social Sciences), version 3.1. This is a computer program which has been designed especially for handling large amounts of data produced by questionnaires. It enables manipulation and analysis of data using standard statistical methods.

2.2 CLINICAL TRIAL OF ORAL IRON IN RLS

2.2.1 Aims of the study

The primary aim of the trial was to assess the efficacy of oral iron in the treatment of RLS. The trial also aimed to establish the duration of treatment required to obtain relief, to determine the length of time patients remained symptom free and to investigate the effect of changing iron status on RLS symptoms.

The trial was based at a rheumatology out-patient clinic, at Selly Oak Hospital, under the medical supervision of a consultant rheumatologist (DRB). The trial protocol (see Appendix A6) was submitted to, and approved by the Ethics Committee, South Birmingham Health Authority.

2.2.2 Selection of subjects

Prospective trial patients were mainly identified during the prevalence survey of rheumatoid and healthy individuals. Specific referrals from doctors who were aware of the study were also considered. However, in view of the relatively high incidence of RLS previously observed amongst rheumatoid patients [16], it was decided to direct recruitment to this group.

Entry criteria stipulated that subjects should have experienced **classical symptoms** of RLS (see Appendix A1) **at least once a week** during the month prior to recruitment. Exclusion criteria for the trial are detailed in the trial protocol. They included subjects already on iron supplements, those symptomatic of iron deficiency and therefore requiring active treatment, subjects with recent gastrointestinal disease and patients with thalassaemia or sideroblastic anaemia at risk of iron overload.

2.2.3 Trial design

The choice of trial design was governed by a number of factors:

1) For the study to advance on previous clinical observations in the

literature, a controlled design, rather than an open design, was essential.

2) Due to the subjective nature of RLS a randomized double-blind design was necessary to avoid patient and investigator bias.

3) Lack of an existing recognized treatment for RLS required that oral iron be compared with placebo. As a significant placebo response had been noted by previous trials, it was important that this be fully evaluated.

It was therefore decided to undertake a double-blind, randomised, between patient, placebo controlled trial comparing ferrous sulphate tablets (200mg three times a day) with placebo (one tablet three times a day). The trial design is represented schematically in Figure 2.1

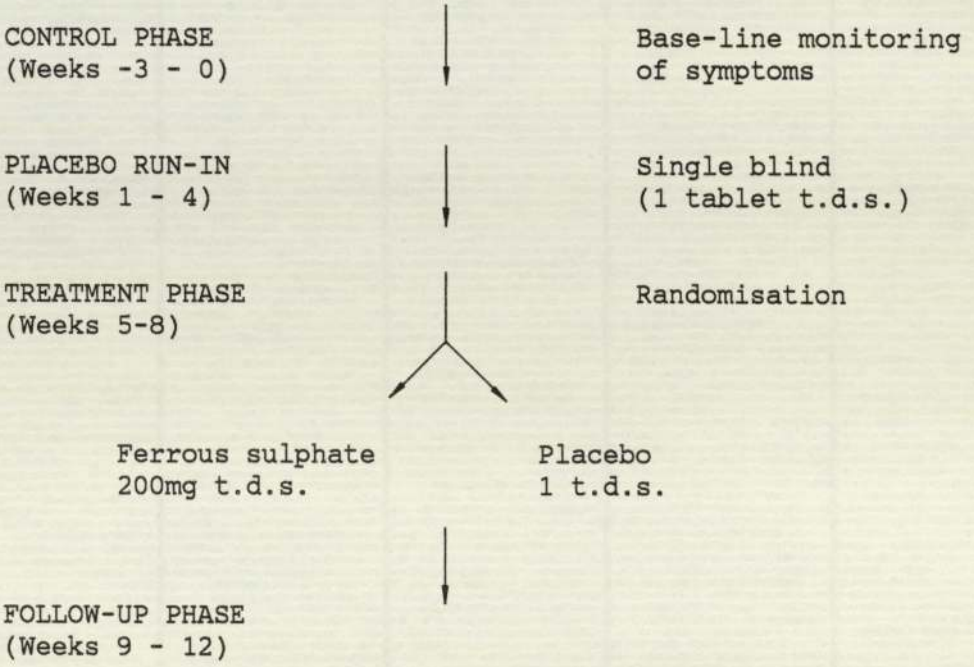


FIGURE 2.1: Initial trial design (used for the first 6 months of trial)

The treatment phase was preceded by two run-in periods; a control phase to monitor baseline symptoms, confirming eligibility to the trial and a single blind placebo run-in period for assessment of placebo response.

Randomisation to iron or placebo used a method of random permuted blocks to help ensure equal numbers on each treatment. Copies of randomisation codes were kept in Pharmacy and Rheumatology departments for use in emergency. Each phase of the trial was of four weeks duration. Having completed the treatment phase, subjects were followed-up for a further four weeks, giving a total trial duration of 4 months.

Six month interim review of recruitment and analysis of results prompted an alteration in the trial, to a within-patient two period crossover design. There were two main reasons for this change. Firstly, the rapid improvement in RLS syptoms seen in two patients whilst on iron suggested that the mode of action was not related to correction of haematological iron deficiency. Secondly, during this period, only six patients were recruited. The altered design allowed within patient comparison, so reducing the number of patient variables and numbers of subjects required to assess the difference between treatments. Patients who were subsequently recruited to the trial received both iron and placebo medication. Pre-treatment run-in periods remained unchanged. The modified trial design is shown in Figure 2.2.

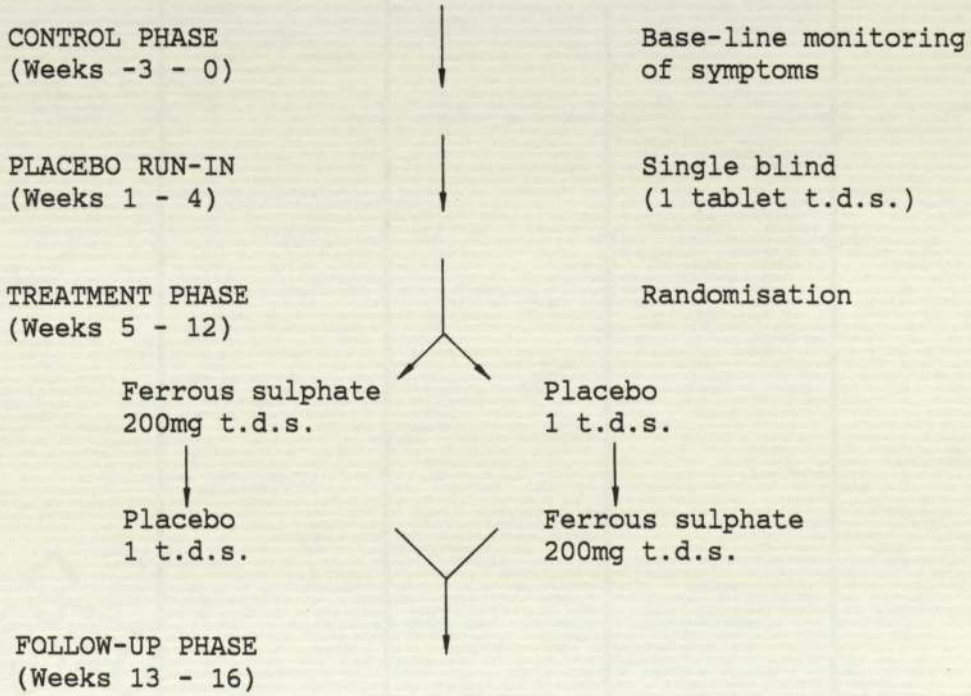


FIGURE 2.2: Modified trial design (used for the latter part of the trial)

2.2.4 Recruitment and clinic visits

Subjects were formally recruited to the trial after the control phase. They were given both verbal and written explanation of the trial (see Appendix A6) informing them that there would be two types of tablet, only one of which was expected to give relief. In accordance with the Declaration of Helsinki [165] subjects were also informed that they were free to withdraw from the trial at any time and without explanation. Signed consent was obtained from all participants. At the time of recruitment subjects' legs were examined (DRB) for pulses, perfusion, power, reflexes and sensation, to exclude any obvious neurological abnormality.

Subjects were asked to attend the clinic every four weeks, at the beginning of each trial phase and at the end of the four week follow-up phase. At each visit they were seen by the investigator and supervising consultant.

2.2.5 Trial medication

Ferrous sulphate tablets (200mg) and matching placebo tablets were kindly supplied by Thomas Kerfoot & Co.

Medication was dispensed for rheumatoid subjects in wide-mouthed plastic containers with broad screw-top closures to aid opening [166,167]. Each bottle contained 100 tablets, sufficient for four weeks treatment plus 16 extra to assess compliance.

In order to minimize the side-effects from ferrous sulphate subjects were advised to take tablets with or after food and with a drink of water. Special instructions were also given to those taking penicillamine [168,169] to avoid interference with absorption. Subjects were otherwise instructed to continue with all other medication as before.

2.2.6 Evaluation of patient response

In view of the subjective nature of RLS, strictly objective measurements of

symptom severity and duration were not possible. Instead the **number of attacks** of RLS and their **duration** (hours) were recorded every day by each subject on a diary card (see Appendix A6). This was presented at the monthly clinic visits. At each visit subjects were also asked to classify their symptoms during the previous month as **'worse'**, **'unchanged'**, **'slightly improved'**, **'much improved'** or **'cured'**. Use of these two approaches allowed both quantitative and qualitative data collection.

2.2.7 Assessment of the effect of iron status and rheumatoid disease activity (where applicable) on RLS symptoms

At each clinic visit subjects had a blood test for assessment of iron status by standard haematological indices as detailed in section 2.1.5. Rheumatoid disease activity was assessed by measurement of ESR and both subjective and objective clinical assessments performed by a metrology nurse (see section 2.1.6).

2.2.8 Evaluation of patient compliance with treatment and side-effects

Patient compliance was assessed by tablet count on returned medication bottles.

The incidence of side-effects to trial medication was assessed by recording voluntary patient information and that elicited by direct questioning; 'Have the tablets disagreed with you in any way ?'

2.2.9 Statistical analysis

Response to oral iron and placebo tablets was compared statistically by paired Student's t test and Wilcoxon's rank sums test.

2.3 A LOW-DOSE IRON ABSORPTION TEST

The main aim of this area of research was to evaluate the small-dose iron absorption test described by Crosby et al [110], as a non-invasive alternative to bone marrow aspiration in the differentiation of iron deficiency anaemia and the anaemia of chronic disease in rheumatoid arthritis. The test was also employed to investigate the effect of rheumatoid arthritis on iron absorption. In his original study, Crosby compared test doses of 5, 10 and 20mg elemental iron, but found that 10mg provided the greatest difference between normal iron repletion and iron deficiency [110]. A 10mg test dose was therefore employed in the current work.

The work took place primarily on the rheumatology ward at the base hospital under close medical supervision and with co-operation from junior medical and nursing staff. Ethical approval for the work was also obtained.

2.3.1 Selection of subjects

Five subject groups were investigated:

i) Normal healthy volunteers, recruited from hospital and technical staff associated with the Rheumatology department. These subjects were neither iron deficient or anaemic as shown by s-ferritin >15ug/ and Hb >11.2g/dl.

ii) Non-rheumatoid subjects with pure iron deficiency indicated by s-ferritin < 20ug/l, with or without anaemia. Those with iron deficiency anaemia were under investigation at an iron deficiency clinic to establish a cause for their anaemia. Subjects with concurrent chronic inflammatory disorders were excluded.

iii-v) Subjects with clinically active rheumatoid arthritis and shown by recent sternal bone marrow aspiration to have (iii) absent, (iv) reduced or (v) normal or increased iron stores. Subjects had bone marrow aspiration performed as part of routine investigation

following the finding that one or more haematological parameters were suggestive of iron deficiency.

2.3.2 Bone marrow aspiration

Assessment of bone marrow iron stores was by sternal aspiration [170]. This procedure takes approximately 30 minutes and is performed on the ward or in an out-patient clinic. Marrow is withdrawn from the sternum, and a thin film of marrow placed on a glass slide for examination under a microscope. Slides are inspected for marrow cellularity and to assess the relative proportions of the four cell lines; erythroid, granulocytes, lymphocytes and eosinophils. Abnormalities in these may suggest an alternative cause for anaemia other than iron deficiency. Iron stores within reticuloendothelial marrow cells and developing erythroblasts were visualised by staining with Prussian blue (also known as Perl's reagent) [171]. The amount of iron was graded subjectively by comparison with a normal control slide. Iron content was classified as 0 (absent), 1 (reduced), 2 (normal) or 3 (increased) [172].

Aspiration, staining and microscopic examination were all performed by a consultant haematologist (JAM), thus minimising variation in technique and assessment.

2.3.3 Test procedure

The test procedure was explained fully to all participants. Subjects were starved from midnight the night before the test. Where possible medication was withheld for the duration of the test, especially drugs thought to interfere with iron absorption such as tetracyclines [173], antacids [174], ascorbic acid [76], penicillamine [168,169] and sulphasalazine [175]. Oral prednisolone was allowed in cases of steroid dependancy and paracetamol was given for joint pain.

At time 0 a blood sample (5ml fresh, 10ml clotted blood) was taken (by the investigator or junior medical staff) for measurment of baseline red blood

cell indices (haemoglobin, red blood count, mean cell volume, mean cell haemoglobin, packed cell volume) and iron indices (s-iron concentration, transferrin saturation, total iron binding capacity, s-ferritin concentration). Erythrocyte sedimentation rate was also measured in rheumatoid subjects, as an indicator of inflammatory activity. Methods used were as outlined earlier in sections 2.1.5 and 2.1.6. Immediately after this, subjects were administered 10mg elemental iron, in the form of a freshly prepared solution of ferrous sulphate B.P. in distilled water.

Further blood samples (5ml clotted blood) were taken for assay of s-iron at 0.5, 1, 1.5, 2, 3, and 4 hours post dose. Initially a sample was also taken at six hours after iron administration, but this was not found to give any additional information and was therefore discontinued. In order to reduce the number of venepunctures patients had a 24 gauge canulla or butterfly needle inserted. This was kept patent by flushing through with 2ml heparin sodium (10 units in 1ml) after each blood sample. The first 2ml of each sample was then discarded. The test was commenced at approximately 8.30am to reduce patient variation due to possible diurnal variation [79,123,124, 125,126]. For full test procedure see protocol in Appendix A7.

2.3.4 Calibration and inter-run variation of s-iron assay

Serum iron measurements were performed as detailed in section 2.1.5. The autoanalyser was calibrated each day using a calibration solution, Autoset H. The between-run analytical coefficients of variation were calculated from daily s-iron determinations on pooled serum at three levels of iron status. Results are shown in Table 2.1

Approx s-iron of pooled serum	No. of samples	Mean s-iron umol/l	SD	Coefficient of variation
5 umol/l	20	5.7	0.400	7.0%
15 umol/l	20	15.2	0.680	4.5%
30 umol/l	20	29.2	0.619	2.1%

TABLE 2.1: Variation of between-run serum iron determination

2.3.5 Data analysis

Change and percentage change in s-iron were plotted against time for each subject, to obtain iron absorption test curves. Statistical analysis of results was by non-parametric un-paired Wilcoxon's rank sums test.

3.1 PREVALENCE SURVEY RESULTS

3.1.1 Data analysis

Interview responses and laboratory data collected from the prevalence survey were coded and entered into a data file on the Vax Cluster computer, and analysed using SPSS* on an interactive basis. A print out of the SPSS* data list created to define survey data, together with a listing of the data file are shown in Appendix A8.

For reasons that will be identified, a complete data set was not collected for every subject. SPSS* allows identification of missing data and options for handling such cases when undertaking statistical procedures. The two tests of statistical significance used during the analysis were Chi-square test for comparison of frequency data and Mann Whitney U test, a non-parametric test for score data. By default SPSSx deletes cases with missing values on a table-by table basis for Chi-square and for the latter on a test-by-test basis. All figures and tables throughout this Section state the number of subjects from which the information was derived.

3.1.2 Survey population

Interviewing for the prevalence survey was undertaken during the period Dec 1985 - May 1987. The study population comprised three sub-groups; 151 rheumatoid patients, 36 subjects with iron deficiency and 79 healthy individuals. Details of age and sex distribution are summarised in Table 3.1.

Subgroup	n	Sex ratio Female	Male	Mean age (yrs)	Age range
Rheumatoid	151	109 (72.2%)	42 (27.8%)	57.1	22 - 82
Iron deficient	36	24 (66.7%)	12 (33.3%)	62.0	38 - 83
Healthy	79	48 (60.8%)	31 (39.2%)	37.7	18 - 75

Table 3.1: Summary of age and sex distribution of survey sub-groups

3.1.2.1 Age - sex matching of subgroups

In order that comparisons between RLS prevalence figures and other prognostic factors could be made, sub-groups were age and sex matched where possible. Since rheumatoid and iron deficient subjects were selected on a consecutive basis, matching was primarily directed at selection of the healthy sub-group. This was not entirely acheived since rigid matching would have further restricted subject numbers.

Age distributions for the three groups are depicted by bar chart in Figure 1.3. Age ranges of the three groups were similar, however the mean (and median) age of the healthy group was significantly lower than that of the rheumatoid or iron deficient groups ($p<0.0001$, 2-tailed Mann-Whitney U test, on both counts). The main factors contributing to this difference were the large number of hospital staff interviewed and recruitment from an early evening GP surgery.

The female : male ratios of the three sub-groups were as follows; rheumatoid 2.6 : 1, iron deficient 2 : 1 and healthy 1.6 : 1. The ratio of the healthy sub-group was not significantly different to that in the rheumatoid ($p >0.1$, Chi-square test with Yate's correction) or iron deficient sub-groups ($p >0.9$). The higher proportion of female subjects in the rheumatoid and iron deficient groups reflects the documented sex distribution in these disease states, and the fact that the survey selected consecutive patients in these groups. There was a relatively higher proportion of men in the healthy sub-group. This may partly be explained by timing of the GP surgery, which tended to attract working men, and partly that since rheumatoid arthritis affects a disproportionate number of women, visitors recruited from the hospital rheumatology ward were therefore often men.

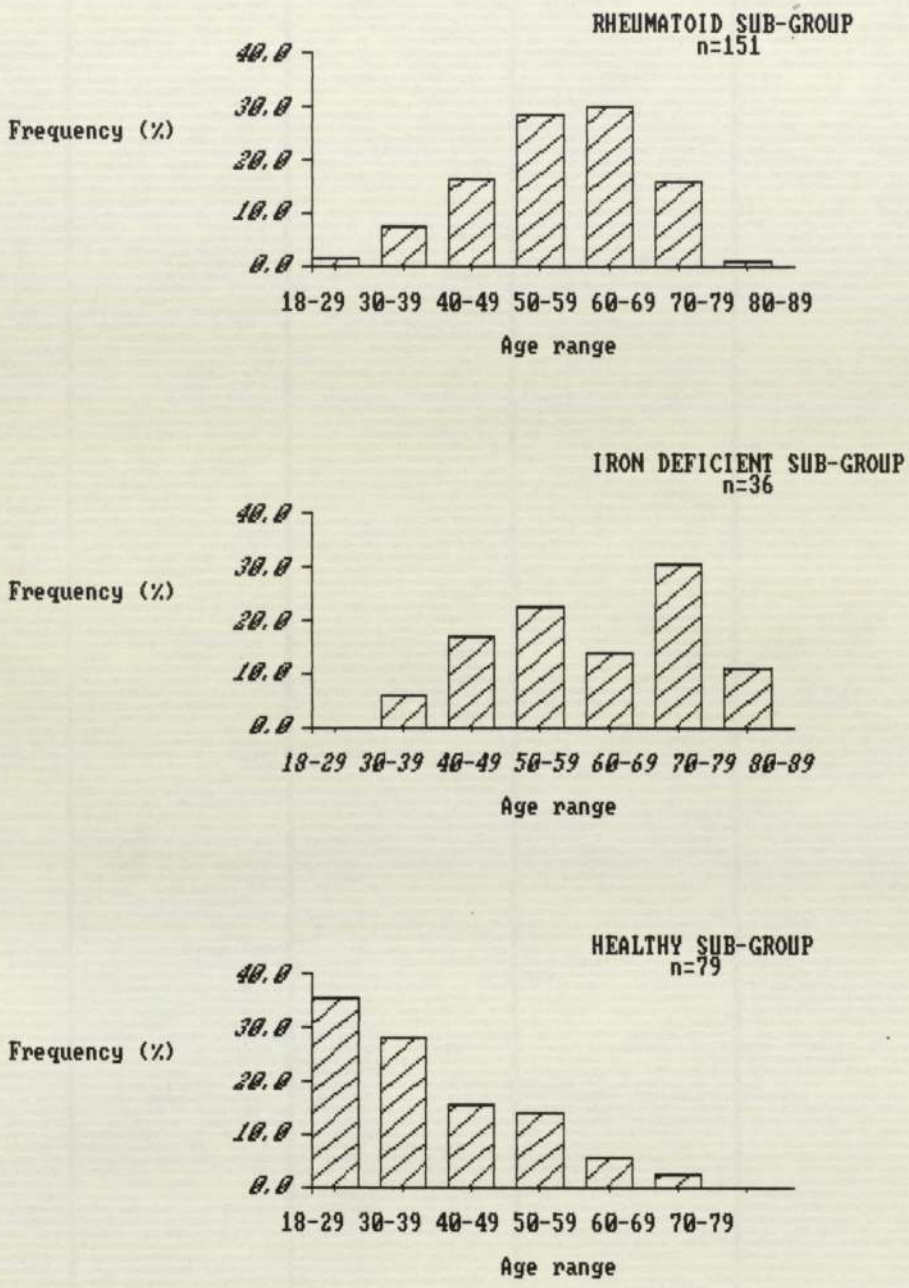


FIGURE 3.1: Bar charts showing age distribution of survey sub-groups.

3.1.2.2 Disease characteristics of the rheumatoid sub-group

Rheumatoid subjects included in the prevalence survey had definite or classic disease as judged by a clinician, and all were under hospital care. Characteristics of disease duration, activity, serology and treatment at time of interview are summarised in Table 3.2.

Metrology assessments were not done on all subjects. This was not always practical within the confines of a busy out-patient clinic. In addition, for conformity, it was necessary to use the same metrologist and therefore assessments were not carried out when she was away.

Characteristic	n	Mean value	Range
Disease duration	146	14.6 yrs	Less than 1yr - 40yrs
Serology	137	Latex negative 39 Latex positive 98 Rose Waaler titre >1/64 48	
Clinical assessments of disease activity			
Duration of early morning stiffness	58	117.8 mins	0 - all day
Ritchie articular index (max of 84)	55	14.9	0 - 79
Grip strength(mmHg) (Normal adult >300)	53	99.5	40 - 245
Pain score	54	2.5	0 - 9
Laboratory assessments of disease activity			
Erythrocyte sedimentation rate (mm/1st hour)	145	42.1	1 - 146
C-reactive protein (ug/l)	59	31.1	2 - 129
Medication			
Analgesics	97	79 (81%)	
NSAIDS	144	112 (78%)	
Second-line agents	144	82 (59%)	
Steroids	144	38 (26%)	

TABLE 3.2: Characteristics of rheumatoid subjects included in survey

3.1.2.3 Disease characteristics of the iron deficient sub-group

The iron deficient sub-group comprised 33 subjects with iron deficiency anaemia (as defined in Appendix A3) and three who were not anaemic but who had serum ferritin less than 20ug/l, indicative of iron deficiency. All subjects were attending a hospital iron deficiency clinic and were interviewed prior to initiation of any iron supplements.

On investigation causes of iron deficiency were found to include: oesophagitis (2), angiodysplasia (1), duodenal ulcer (1), colonic polyps (1), rectal bleeding (1), gastrointestinal carcinoma (3), adult coeliac

disease (2), vaginal blood loss (3), aspirin of NSAID-induced erosions (3) and dietary deficiency (1). No cause was found in 13 patients despite thorough investigation, and three refused investigation. Haematological parameters for this sub-group are summarised in Table 3.3, and individual details may be found in Appendix A8.

Parameter	n	Mean value	Range
Hb	36	8.9	2.9 - 12.5
MCV	36	65.94	49.7 - 84.2
MCH	36	21.21	16.0 - 27.9
s-iron	29	8.9	1.0 - 42.2
TIBC	29	82.0	52.7 - 98.9
saturation %	29	11.2	1.2 - 56.6
s-ferritin	29	10.9	1.0 - 70.8

TABLE 3.3: Haematological parameters of the iron deficient sub-group

3.1.2.4 Characteristics of the healthy sub-group

This control group comprised 28 hospital staff, 10 relatives of rheumatology in-patients, and 41 individuals attending an early evening G.P. surgery or hospital haematology department either because of minor ailments or accompanying a relative. Subjects had no known disease pathology and were on no regular medication.

3.1.3 Prevalence of RLS

The prevalence, defined as the number of people who had experienced RLS during the previous 12 months, was assessed in each sub-group. The incidence, or number of subjects who had had RLS during the previous month was also determined. Results are shown in Table 3.4.

Sub-group	n	PREVALENCE* of RLS (%)	95% limits	INCIDENCE** of RLS (%)	95% limits
Rheumatoid	151	54 (35.8%)	± 7.6%	41 (27.2%)	± 7.1%
Iron deficient	36	9 (25.0%)	± 14.2%	7 (19.4%)	± 12.9%
Healthy	79	10 (12.7%)	± 7.3%	7 (8.9%)	± 6.3%

* based on no. of subjects with symptoms during the previous year
** based on no. of subjects with symptoms during the previous month.

TABLE 3.4: Prevalence and incidence of RLS in the three survey sub-groups

The prevalence of RLS was significantly higher in rheumatoid sub-group compared with the healthy control group (p 0.00006, Chi-square with Yate's correction). While the figure for the iron deficient sub-group was much higher than the control, the difference was not statistically significant due to small subject numbers (p 0.072). Incidence figures were similarly significantly different in the rheumatoid sub-group (p 0.0006) and higher but not significantly so in iron deficiency (p 0.098).

Small numbers of subjects with RLS from the iron deficient and healthy sub-groups limited interpretation of detailed assessment of symptoms and other features in these groups. This was however possible with rheumatoid RLS sufferers.

3.1.4. Classification of RLS symptoms

One of the aims of the survey was to collect data regarding the type, frequency and duration of symptoms in order to permit a greater understanding of the condition.

3.1.4.1 Sensation

All subjects were able to dissociate RLS symptoms from any other lower leg problems, including joint involvement in those with rheumatoid arthritis. Subjects were asked to describe dysaesthesic symptoms. Between a third and a half of subjects in each group included 'crawling' as a description of

discomfort felt. This was considered to be a feature of the classical presentation. Others descriptions included sensations of itching, burning, or aching. Only four individuals described symptoms as painful. A small proportion of subjects merely experienced an irresistable need to move the legs. The incidence of each of these categories is shown in Figure 3.2.

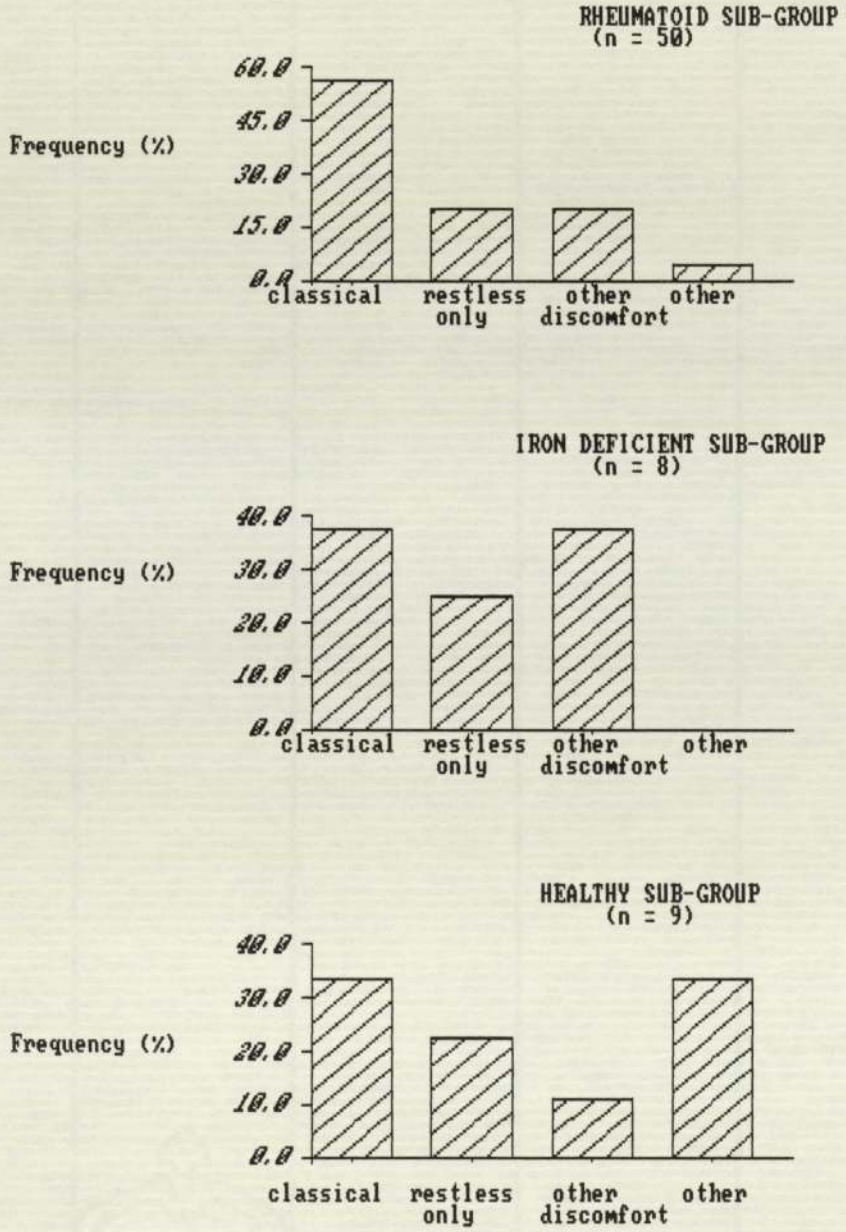


FIGURE 3.2: Classification of dysaesthesic symptoms associated with RLS

3.1.4.2 Site

Three questions were asked to identify the area of the legs affected, the depth of the sensation and whether it occurred bilaterally. Results are shown in Tables 3.5 - 3.7

Area affected	No. of subjects affected (valid %) Rheumatoid n=50	Iron deficient n=9	Healthy n=8
Between knee and ankle	21 (42%)	2 (22.2%)	3 (37.5%)
Between knee and ankle, and other areas of leg	21 (42%)	5 (55.6%)	3 (37.5%)
Ankles, knees or thighs only	3 (6%)	1 (11.1%)	2 (25%)
Feet only	2 (4%)	0	0
Arms and legs	3 (6%)	1 (11.1%)	0

TABLE 3.5: Area affected by dysaesthesic symptoms

Depth of sensation	No. of subjects affected (valid %) Rheumatoid n=46	Iron deficient n=7	Healthy n=5
Deep inside	32 (69.6%)	6 (85.7%)	5 (100%)
On skin surface	9 (19.6%)	1 (14.3%)	0
Don't know	5 (10.9%)	0	0

TABLE 3.6: Subjective assessment of depth of dysaesthesia

Symmetry of RLS	No. of subjects affected (valid %) Rheumatoid n=51	Iron deficient n=9	Healthy n=8
Bilateral	44 (86.3%)	6 (66.6%)	5 (62.5%)
Unilateral	7 (13.7%)	3 (33.3%)	3 (37.5)

TABLE 3.7: Symmetry of RLS

Discomfort was similar in all three sub-groups. In at least 75% of cases from each sub-group the site affected included the area between the knee and ankle. Very rarely did discomfort originate in the arms as well as the legs. Most subjects described the sensation as being deep inside the limb, rather than superficial, and symptoms were predominantly bilateral.

In his original monograph [2] Ekbom described features of classical RLS as: dysaesthesia of a crawling nature, felt within the limb, affecting the area between the knee and ankle bilaterally, occuring at rest and relieved by movement. Such a presentation was described by 17/54(31.5%)rheumatoid, 2/9 (22%) iron deficient and 1/10 (10%) healthy subjects. The numbers meeting all these requirements were therefore relatively small.

3.1.4.3 Time of day, duration and frequency of RLS symptoms

Subjects were asked when attacks most commonly occurred, how long they lasted and regarding the frequency of attacks. Results are summarised in Table 3.8 and Figures 3.3 and 3.4.

No. of subjects affected (valid %) Time of day	Rheumatoid n=50	Iron deficient n=9	Healthy n=8
Sitting in the evening	8 (16%)	1 (11.1%)	0
On retiring to bed	9 (18%)	2 (22.2%)	2 (25%)
At night	2 (4%)	2 (22.2%)	0
During the evening and at night	25 (50%)	2 (22.2%)	3 (37.5%)
During the day-time and evening	6 (12%)	2 (22.2%)	3 (37.5%)

TABLE 3.8: Time of day at which attacks of RLS occurred.

No subject had RLS at the time of interview, which were mainly carried out in the morning, afternoon or early evening. RLS occurred only in the

evening or after retiring to bed in 63 - 88% of subjects. The average duration of attack varied from a few minutes to several hours. Duration appeared worst in iron deficient subjects, being over one hour in 75% of this group compared with 24 % and 25% of rheumatoid and healthy subjects, respectively. Frequency of attack was also greatest for iron deficient subjects; 78% experiencing at least one attack each week; while 67% of rheumatoid and only 37.5% of healthy subjects had at least one attack per week. Several rheumatoid patients reported that RLS symptoms only occurred when sitting for a prolonged period in a confined space, for example on a coach journey, at the theatre or in church. These were included in an 'other' frequency category.

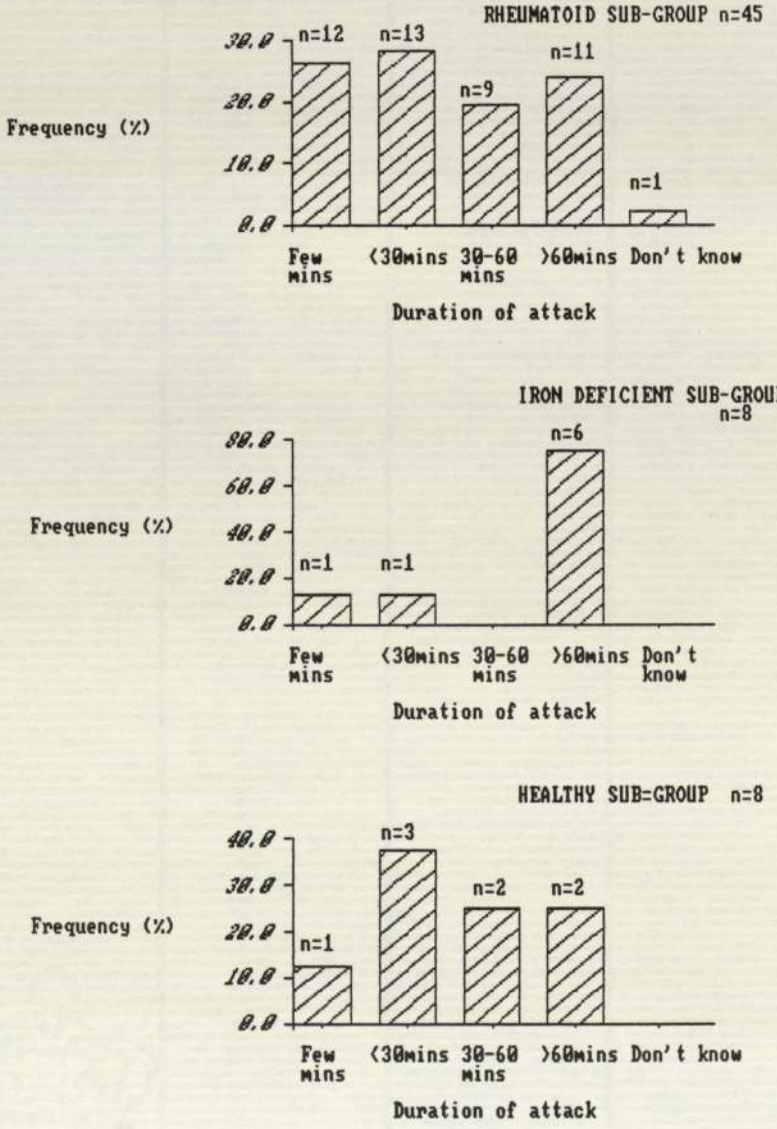


FIGURE 3.3: Average duration of attacks for the three survey sub-groups

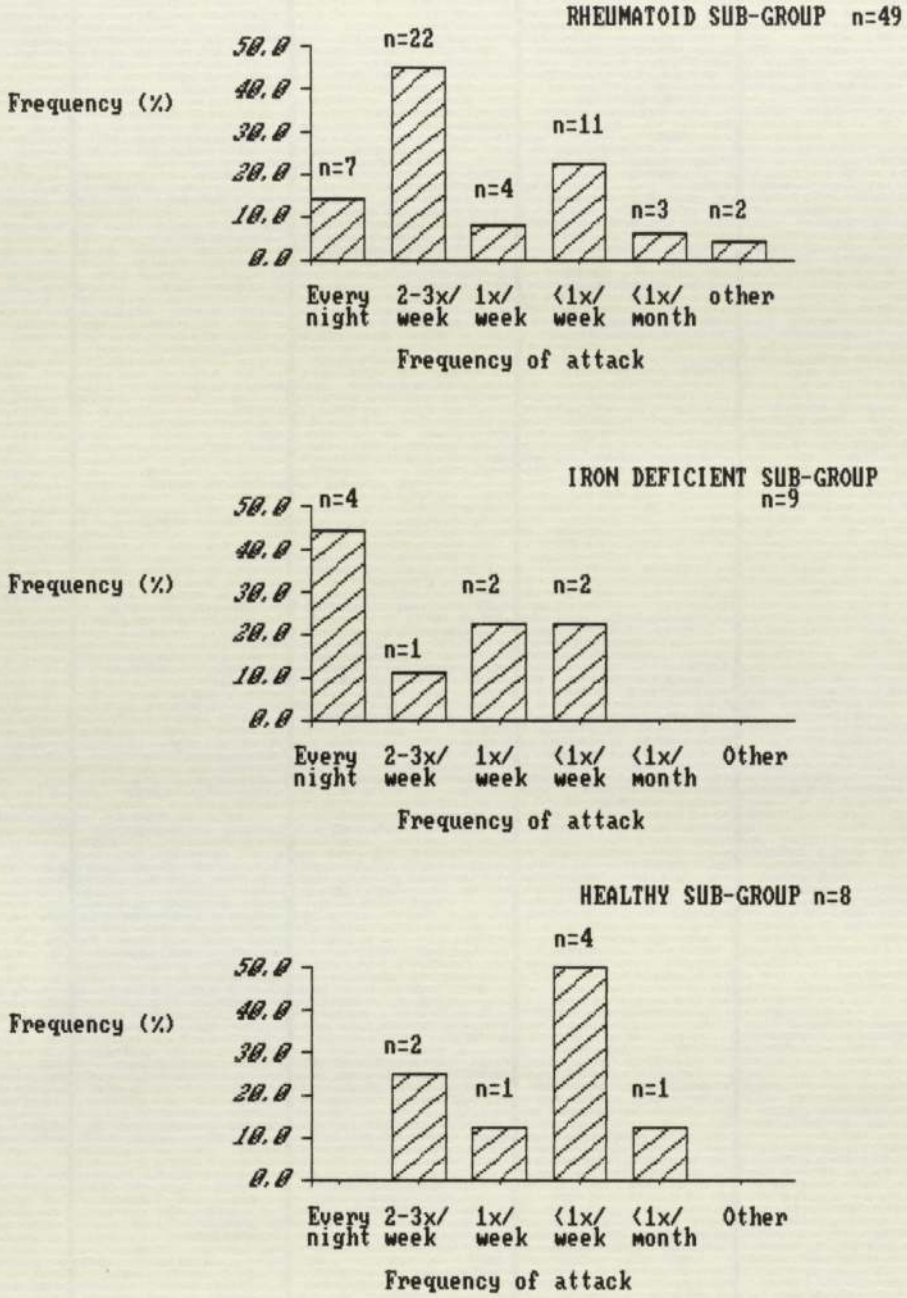


FIGURE 3.4: Frequency of RLS attacks in the three survey sub-groups

3.1.4.4 Exacerbating and relieving factors

Subjects were asked to identify conditions, actions or medicines which aggravated or conversely improved RLS symptoms. Rheumatoid subjects were also asked whether there was any correlation with rheumatoid disease activity.

Factor	No of subjects Rheumatoid n=42	Iron deficient n=6	Healthy n=8
Aggravating factors			
Tiring day	18	0	3
Exercise	0	1	0
Heat	0	1	0
Damp	1	0	0
Nothing specific	23	3	4
Other	0	1	1
Relieving factors			
Moving legs	16	1	2
Walking	13	3	2
Walking and moving	17	3	3
Heat	2	0	0
Cold	0	0	1
Rubbing legs	8	1	1
Hanging legs out of bed	3	1	0
Hot drink	3	0	0

TABLE 3.9: Factors found to aggravate and relieve RLS symptoms

A variety of factors including a tiring day, exercise, heat and damp were reported to exacerbate RLS. Approximately 50% of subjects in each sub-group did not associate RLS with any particular factor.

Moving the legs or walking provided some relief in all subjects, though often only temporarily. Other relieving factors included heat, cold, rubbing the legs, hanging them out of bed and a hot drink.

Correlation with rheumatoid disease activity

According to rheumatoid subjects joint disease did not correlate well with frequency or severity of RLS; only 21% suggested that there was a positive relationship. See Table 3.10.

Correlation with RA disease activity	No. of subjects (Valid %) n = 33
Positive correlation	7 (21.2%)
No correlation	22 (66.7%)
Don't know	4 (12.1%)

TABLE 3.10: Subjective impression of RA and RLS disease correlation

3.1.4.5 Level of inconvenience and interference with sleep,

Subjects were asked several questions to try to obtain a measure of the inconvenience and suffering caused by the condition. Results are summarised below in Tables 3.11 and 3.12.

Level of inconvenience	No of subjects (valid %) Rheumatoid n=45	Iron deficient n=7	Healthy n=7
Extremely irritating	21 (46.7%)	3 (42.9%)	4 (57.1%)
Mildly irritating	20 (44.4%)	3 (42.9%)	2 (28.6%)
Accepted as normal	4 (8.9%)	1 (14.3%)	1 (14.3%)

TABLE 3.11: General level of inconvenience caused by RLS symptoms

Interference with sleep	No of subjects (valid %) Rheumatoid n=45	Iron deficient n=6	Healthy n=8
Frequently	9 (20%)	1 (16.7%)	1 (12.5%)
Occasionally	16 (35.6%)	3 (50%)	4 (50%)
Never	19 (42.2%)	2 (33.3%)	3 (37.5%)

TABLE 3.12: Interference with sleep from RLS

RLS symptoms were judged as being mildly or extremely irritating in 86 - 91% of cases in all sub-groups. At least half of these subjects expressed extreme irritation. Interference with sleep occurred in 56% of rheumatoid subjects, 67% of iron deficient and 63% of normal individuals. Higher figures were not to be expected since symptoms did not always occur whilst in bed.

3.1.4.6 Referral/response to and by the medical profession

In view of the lack of recognition of RLS amongst the medical profession, subjects were asked if they had specifically mentioned RLS symptoms to their general practitioner or hospital doctor, and if so what the response had been.

Despite the apparently high level of suffering attributed to RLS, only six (19.4%) rheumatoid, two (28.6%) iron deficient, and one (14.3%) healthy individuals had mentioned RLS symptoms to a doctor. When asked why they had not told a doctor about their RLS symptoms, many were afraid of the response that the doctor might have to an apparently trivial sounding problem. Rheumatoid subjects did not think of mentioning these symptoms at the rheumatology clinic since it was not a joint problem. Of the nine subjects who had asked their doctor for help, six were given neither explanation or treatment. Three rheumatoid subjects had been prescribed oral iron therapy.

3.1.4.7 Other lower limb problems

In order to help ensure that subjects were not confusing RLS with other common leg problems a specific question was included in the questionnaire concerning those leg symptoms experienced regularly. Results are summarised in Table 3.13.

Leg symptom	No. of subjects affected (valid %) Rheumatoid n=116	Iron deficient n=32	Healthy n=72
Cramp	20 (17.2%)	11 (34.3%)	11 (15.2%)
Spontaneous jerking	12 (10.3%)	3 (9.3%)	6 (8.3%)
Pins and needles	3 (2.6%)	1 (3.1%)	3 (4.2%)
Other leg symptoms	7 (6.0%)	3 (9.3%)	0
No leg symptoms (except those with RLS)	74 (63.8%)	14 (43.7%)	52 (72.2%)

TABLE 3.13: Incidence of other leg problems in the three sub-groups

Other leg symptoms, excluding arthritis, were present in 54% iron deficient subjects, 36% rheumatoid and 27% healthy individuals. There was no significant association between these problems and RLS ($p > 0.5$ in each sub-group, Chi-square test with Yate's correction).

3.1.5 Influence of various factors on the occurrence of RLS

The influence of demographic characteristics, hereditary factors, iron status and, in the case of rheumatoid subjects, disease activity and drug therapy was assessed.

3.1.5.1 Relationship between age and occurrence of RLS

The age of subjects in each sub-group with and without RLS was compared, and statistical difference assessed by 2-tailed Mann-Whitney U test as shown in Table 3.14.

Sub-group	n	Mean age (yrs)	Range	Mann-Whitney U test 2-tailed probability	Significance
Rheumatoid					
RLS +ve	54	55.9	32 - 82	0.236	NS
RLS -ve	97	57.9	22 - 79		
Iron deficient					
RLS +ve	9	57.2	39 - 72	0.159	NS
RLS -ve	27	63.6	38 - 83		
Healthy					
RLS +ve	8	39.0	24 - 55	0.795	NS
RLS -ve	71	37.8	18 - 75		

NS - Null hypothesis is accepted, there is no statistically significant difference

TABLE 3.14: Influence of age on occurrence of RLS

Statistical analysis showed that within each sub-group there was no statistically significant difference in age between those with RLS and those without RLS. This is demonstrated further in Figure 3.5

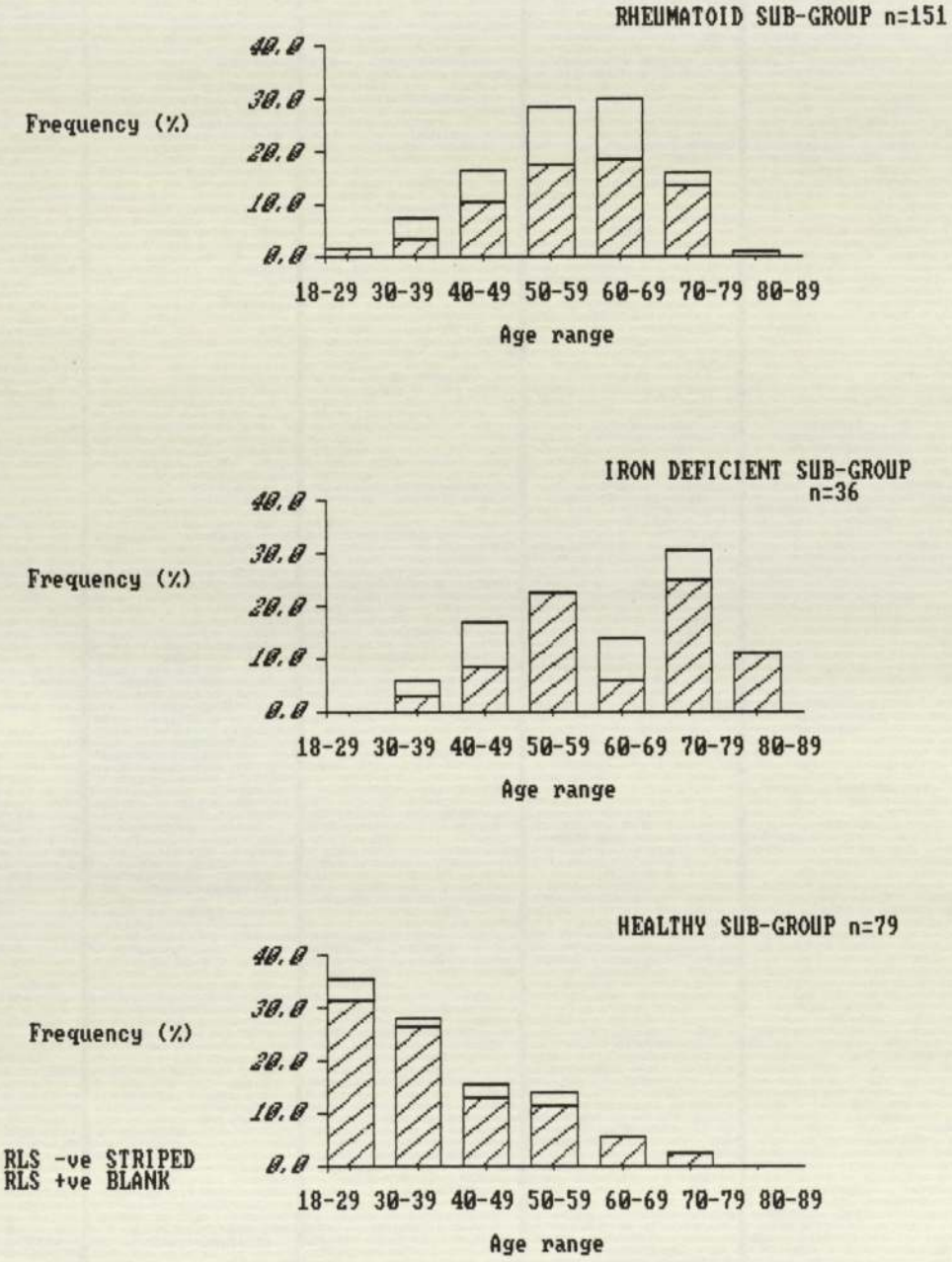


FIGURE 3.5: Comparison of RLS +ve and RLS -ve age distribution

3.1.5.2 Relationship between sex difference and prevalence of RLS

The sex distribution of those with and without RLS was compared for each sub-group, by four-fold Chi-square test with Yate's correction* for rheumatoid and healthy sub-groups. Due to small subjects numbers, a 2-tailed Fisher's exact test (* see table) was used when assessing the

difference in the iron deficient sub-group. Results are shown in Table 3.15.

Sub-group	n	No of subjects Male	Female	Probability*	Significance
Rheumatoid					
RLS +ve	54	4	50	0.00007	S
RLS -ve	97	38	59		
Iron deficient					
RLS +ve	9	2	7	0.690	NS
RLS -ve	27	9	18		
Healthy					
RLS +ve	8	1	7	0.211	NS
RLS -ve	71	30	41		

S - Null hypothesis disproved, there being a statistically significant difference.
NS - No statistically significant difference

TABLE 3.15: Assessment of the influence of gender on occurrence of RLS

Gender was found to have no appreciable influence on the occurrence of RLS amongst iron deficient and healthy sub-groups. It is not possible to know whether this result is a true reflection of these populations since the size of the iron deficient sub-group was small and the prevalence of RLS in healthy individuals was low.

In contrast, in the rheumatoid sub-group the female:male ratio for those with RLS was 12.5:1, a difference which was found to be highly statistically significant.

3.1.5.3 Effect of hereditary tendency on the prevalence of RLS

Evidence of a possible genetic predisposition was assessed by asking subjects whether any blood relatives had suffered with RLS. Data for this question is incomplete due to this line of questioning being introduced after interviewing began.

Sub-group		n	No. of relatives with RLS
Rheumatoid	RLS +ve	12	3
	RLS -ve	31	3
Iron deficient	RLS +ve	6	3
	RLS -ve	24	0
Healthy	RLS +ve	4	2
	RLS -ve	61	9

TABLE 3.16: Relationship of RLS in relatives and prevalence in survey group

On comparing the numbers of subjects in the survey population as a whole, with and without RLS who had close relatives also with RLS, it was found that this was significantly higher in those survey subjects with RLS ($p < 0.005$, Chi-square test with Yate's correction).

3.1.5.4 Relationship between iron status and incidence of RLS

Iron status was assessed by determination of Hb, MCV, MCH, s-iron, transferrin saturation (%), TIBC, and s-ferritin. Blood tests were performed on 145/151 rheumatoid subjects, 52/79 healthy individuals, and all iron deficient subjects. Tests were not performed on the 27 subjects interviewed at a G.P. surgery (though individuals were by definition well apart from minor ailments, and gave no history of iron deficiency anaemia).

For the purpose of analysis, only blood test results of those who had experienced RLS during the previous month were compared with those subjects with no RLS. This therefore comprised 132 rheumatoid subjects (38 RLS +ve, 94 RLS -ve), 34 iron deficient individuals (7 RLS +ve, 27 RLS -ve) and 52 healthy individuals (7 RLS +ve, 45 RLS -ve). Statistical analysis was by Mann-Whitney U test and Chi-square test. Larger subject numbers in the

rheumatoid sub-group permitted more extensive analysis to be performed; inparticular, investigation of the affects of the anaemia of chronic disease as well as iron deficiency. Correlation of haematological indices with frequency and duration of RLS attack, and occurrence of classical symptoms was also assessed.

First, values of haematological variables were compared between RLS +ve and RLS -ve subjects in each sub-group.

1.3.5.5 Iron status in healthy individuals with and without RLS

Haematological values for the healthy sub-group are summarised in Table 3.17.

Variable		n	Mean value	Range	2-tailed Mann-Whitney U test probability	Significance
Hb	**RLS +ve**	**7**	**13.1**	**12.0 - 14.2**	**0.015**	**S**
	RLS -ve	43	14.0	9.5 - 16.2		
MCV	**RLS +ve**	**7**	**88.6**	**81.5 - 93.6**	**0.493**	**NS**
	RLS -ve	43	90.0	70.9 - 102.5		
MCH	**RLS +ve**	**7**	**29.7**	**27.2 - 32.7**	**0.742**	**NS**
	RLS -ve	43	29.9	22.8 - 34.5		
s-iron	**RLS +ve**	**7**	**16.6**	**7.7 - 23.6**	**0.379**	**NS**
	RLS -ve	43	19.7	5.2 - 41.8		
satn (%)	**RLS +ve**	**7**	**23.5**	**11.8 - 35.6**	**0.528**	**NS**
	RLS -ve	43	26.9	5.6 - 59.6		
TIBC	**RLS +ve**	**7**	**70.6**	**60.0 - 90.3**	**0.335**	**NS**
	RLS -ve	43	73.9	45.8 - 92.3		

TABLE 3.17: Summary of haematological indices in the healthy sub-group

Overall mean values for haemaglobin, MCV, MCH, s-iron, and transferrin saturation (%) were lower in those healthy subjects with RLS, but only the difference for haemoglobin reached statistical significance.

Inspite of these apparent differences, none of the subjects in this sub-group were anaemic, with the exception of one who had been a blood donor and who did not have RLS. Serum iron and transferrin saturation were below normal limits in 2 subjects with RLS and 5 without.

3.1.5.6 Iron status of iron deficient subjects with and without RLS

Table 3.18 summarises haematological indices for the iron deficient sub-group.

Variable		n	Mean value	Range	2-tailed Mann-Whitney U test probability	Significance
Hb	**RLS +ve**	**7**	**8.6**	**6.2 - 11.2**	**0.403**	**NS**
	RLS -ve	27	9.1	2.9 - 12.5		
MCV	**RLS +ve**	**7**	**64.7**	**50.0 - 74.8**	**0.708**	**NS**
	RLS -ve	27	66.6	49.7 - 84.2		
MCH	**RLS +ve**	**7**	**20.3**	**16.4 - 24.1**	**0.403**	**NS**
	RLS -ve	27	21.5	16.0 - 27.9		
s-iron	**RLS +ve**	**5**	**7.4**	**3.6 - 13.3**	**0.650**	**NS**
	RLS -ve	22	9.6	1.0 - 43.2		
satn (%)	**RLS +ve**	**5**	**9.3**	**4.8 - 14.8**	**0.565**	**NS**
	RLS -ve	22	12.1	1.2 - 56.6		
TIBC	**RLS +ve**	**5**	**80.4**	**64.8 - 90.1**	**0.976**	**NS**
	RLS -ve	22	81.3	52.7 - 98.9		
Ferritin	**RLS +ve**	**5**	**19.8**	**5.0 - 70.8**	**0.344**	**NS**
	RLS -ve	22	9.4	1.0 - 38.5		

TABLE 3.18: Summary of haematological indices in the iron deficient sub-group

Mean values of Hb, MCV, MCH, s-iron, and transferrin saturation were lower for those with RLS, but not significantly so, and small patient numbers may have hindered discovery of any real differences.

3.1.5.7 Iron status in the rheumatoid subjects with and without RLS

Haematological results are shown in Table 3.19.

Haemoglobin, MCH, s-iron, transferrin saturation and s-ferritin were significantly lower in the rheumatoid sub-set with RLS. In addition the MCV was lower and TIBC higher in this sub-set, although not significantly so.

Variable		n	Mean value	Range	2-tailed Mann-Whitney U test probability	Significance
Hb	**RLS +ve**	**38**	**11.5**	**7.0 - 15.3**	**0.021**	**S**
	RLS -ve	94	12.5	5.6 - 17.1		
MCV	**RLS +ve**	**36**	**80.1**	**55.9 - 96.3**	**0.065**	**NS**
	RLS -ve	94	83.1	60.8 - 95.1		
MCH	**RLS +ve**	**37**	**26.1**	**16.8 - 33.4**	**0.032**	**S**
	RLS -ve	94	27.6	19.6 - 33.4		
s-iron	**RLS +ve**	**34**	**8.75**	**2.9 - 21.6**	**0.044**	**S**
	RLS -ve	75	11.1	3.8 - 25.1		
satn (%)	**RLS +ve**	**34**	**13.4**	**4.6 - 29.7**	**0.010**	**S**
	RLS -ve	75	17.5	5.3 - 34.7		
TIBC	**RLS +ve**	**34**	**65.5**	**41.2 - 86.9**	**0.168**	**NS**
	RLS -ve	75	62.4	35.5 - 94.0		
Ferritin	**RLS +ve**	**34**	**50.3**	**1.0 - 191**	**0.015**	**S**
	RLS -ve	84	78.7	3.9 - 370		

TABLE 3.19: Summary of haematological indices in the rheumatoid sub-group

As detailed earlier (see section 1.10), interpretation of haematological parameters in this population with respect to iron status is complicated by the presence of the anaemia of chronic disease in most patients with active disease. Further analysis was therefore undertaken to try to differentiate ACD from iron deficiency, and thereby assess whether either

of these haematolgical disorders were likely to be contributary factors in the occurrence of RLS.

Relationship between anaemia and RLS

The correlation between anaemia, of what ever cause, and the incidence of RLS was assessed. Anaemia was defined as Hb < 11.4g/dl in women and <13.4g/dl in men, in accordance with the normal ranges used at the study hospital. Of the 38 rheumatoid subjects with RLS who had had blood tests, 19 (50%) were anaemic, while 43/94 (46%) of those without RLS were classified as being anaemic. This difference was not statistically significant (p 0.657, 2-tailed Chi-square test).

Influence of anaemias due to iron deficiency and chronic disease

As determined above, s-iron and transferrin saturation values were significantly lower in rheumatoid subjects with RLS. Whilst this could be a reflection of iron deficiency, it could equally be attributed to the anaemia of chronic disease. Clarification of this was attempted by referral to s-ferritin values and where available, recent bone marrow results. A s-ferritin value of less than 20 ug/l is considered a good indicator of iron deficiency, whether or not an inflammatory process is present. It has also been suggested that in the presence of inflammation a s-ferritin value of less than 55 ug/l is an appropriate lower limit. The presence of definite (s-ferritin <20ug/l) or probable (s-ferritin < 55ug/l) iron deficiency, with or without anaemia was compared in rheumatoid subjects with and without RLS. This is shown schematically in Figures 3.6 and 3.7, and summarised in Table 3.20. There was disparity between bone marrow iron stores and s-ferritin results in 7/17 RLS+ve subjects and 4/23 RLS-ve subjects. This only affected differentiation between probable iron deficiency and anaemia of chronic disease.

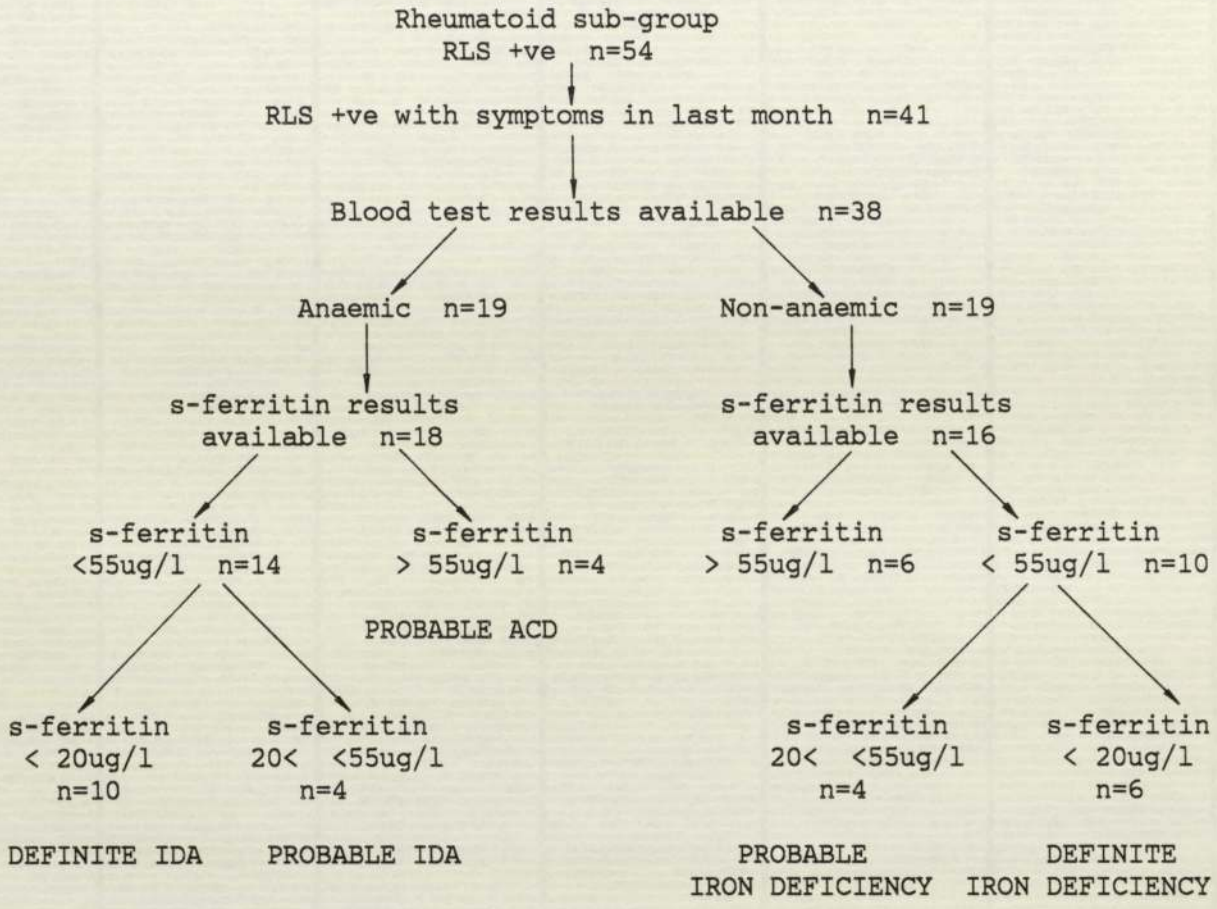


FIGURE 3.6: Classification of iron status in rheumatoid subjects with RLS based on s-ferritin values alone

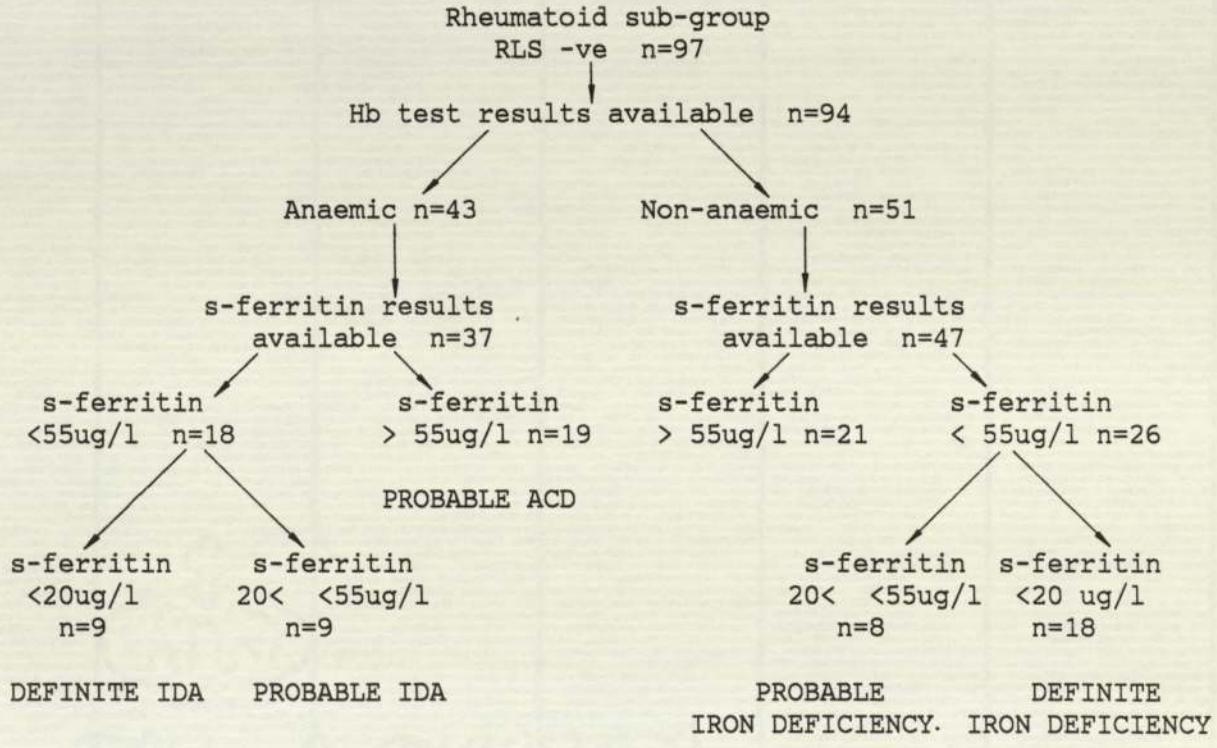


FIGURE 3.7: Classification of iron status in rheumatoid subjects without RLS based on s-ferritin values

Iron status	No of subjects affected (valid %) RLS +ve n=34	RLS -ve n=84
Definite IDA	10 (29.4%)	9 (10.7%)
Definite ID	6 (17.6%)	18 (21.4%)
Probable IDA	6 (17.6%)	10 (11.9%)
Probable ID	5 (14.7%)	9 (10.7%)
Probable ACD only	2 (5.9%)	18 (21.4%)
Others	5 (14.7%)	20 (23.8%)

TABLE 3.20: Incidence of iron deficiency and ACD in the rheumatoid sub-group based on Hb, s-ferritin and bone marrow iron stores

Definite iron deficiency with or without anaemia affected 47% of rheumatoid subjects with RLS and 32% of those without RLS. Though reduced iron stores were found in a greater proportion of those with RLS, this was not statistically significant ($p > 0.1$, Chi-square test with Yate's correction).

Employing the suggested lower limit for ferritin applicable to subjects with a chronic inflammatory condition (< 55ug/l), 79% of RLS +ve subjects and 55% of RLS -ve subjects showed some evidence of reduced iron stores. This difference was statistically significant ($p < 0.025$, Chi-square with Yate's correction).

Subjects with anaemia and s-ferritin >55ug/l were classified as having probable ACD. Other subjects in whom anaemia was associated with iron deficiency would, in view of the inflammation, almost certainly have had concurrent ACD. 5.9% RLS +ve and 21.4% RLS -ve subjects had probable ACD in the absence of iron deficiency; the difference not being significant ($p > 0.05$, Chi-square with Yate's correction).

Influence of s-iron and transferrin saturation

Values of s-iron and transferrin saturation (%) are both reduced in iron deficiency and ACD and were, as shown above, significantly lower overall in those with RLS. The relevence of s-iron or saturation below normal limits, due to what ever cause, was assessed in relation to RLS. The lower limit for s-iron was set at 11umol/l and 16% for transferrin saturation, as defined at the study hospital. 25/34 (74%) of subjects with RLS had a s-iron below normal limits compared with 44/75 (59%) of those without. Similarly for transferrin saturation, 23/34 (68%) and 38/75 (51%) respectively of those with and without RLS had values below normal limits. RLS was associated with a greater tendancy for low s-iron and transferrin saturation, but neither were statistically significant ($p > 0.2$ and 0.1 respectively).

3.1.5.8. Correlation between haematological indices and frequency and duration of RLS attacks

Analysis up to this point has focused on causal relationship between various factors and the incidence of RLS. One area of further investigation was to determine whether there was any correlation between frequency and duration of attacks and haematological parameters. Statistical analysis was by another non-parametric test, Kruskal-Wallis one-way analysis of variance.

Duration of RLS attack was independent of haematological parameters, while there was some correlation between s-iron, transferrin saturation, TIBC and frequency of attack.

3.1.5.9 Assessment of the effect of rheumatoid disease activity on incidence of RLS

Influence of disease serology

Serology (ie. the presence of IgM rheumatoid factor), determined by Latex and Rose Waaler tests was assessed in relation to RLS. The latex test is the more sensitive of the two tests, but less specific for rheumatoid

factor. 33/50 (66%) of RLS +ve subjects were Latex positive, compared with 65/87 (75%) of RLS -ve subjects. The Rose Waaler is the more sensitive test and the result is given in terms of IgM RF titre, a titre of 1/64 generally being recognised as 'sero-positive' [176]. 15/49 (31%) RLS +ve and 33/83 (40%) RLS -ve rheumatoid subjects had titres greater or equal to 1/64. Differences in frequency of sero-positive arthritis determined by both tests were not associated with RLS (p=0.372 and p=0.385 respectively, 2-tailed Chi-square test).

Influence of rheumatoid disease duration

Duration of rheumatoid disease from time of diagnosis ranged from less than a year to 35 years, mean 10.9 years, in those with RLS and from less than a year to 40 years, mean 9.25 in those without RLS, These were not significantly different (p=0.935; 2 tailed Mann-Whitney U test).

Influence of disease activity

Laboratory and clinical indices of disease activity were also compared in those subjects with RLS, during the previous month, or no RLS. These are summarised in Table 3.21

Variable		n	Mean value	Range	2-tailed Mann-Whitney U test probability	Significance
ESR	RLS +ve	38	40.6	3 - 80	0.582	NS
	RLS -ve	94	41.5	1 - 146		
CRP	RLS +ve	23	29.4	<4 - 129	0.794	NS
	RLS -ve	56	29.7	<4 - 100		
EMS	RLS +ve	22	88.5	0 - 360	0.220	NS
(mins)	RLS -ve	25	44.6	0 - all day		
RITCHIE	RLS +ve	22	11.3	0 - 26	0.257	NS
INDEX	RLS -ve	24	16.0	0 - 50		
GRIP	RLS +ve	22	94.3	51 - 212	0.207	NS
(mmHg)	RLS -ve	23	114.0	40 - 245		
PAIN	RLS +ve	22	2.6	0 - 9	0.537	NS
SCORE	RLS -ve	24	2.0	0 - 8		

TABLE 3.21: Summary of laboratory and metrology assessments in the rheumatoid sub-group

There was no overall significant difference in laboratory indices ESR or CRP, or in any of the clinical measures, EMS, Ritchie articular index, grip strength or pain score. The number of rheumatoid subjects who underwent metrological assessment was however limited.

In summary the incidence of RLS was found to be independent of rheumatoid disease duration, severity or activity.

Influence of rheumatoid drug therapy

Drug therapy was also investigated as a possible influential factor. 78% (31/40) RLS +ve and 77% (70/91) RLS -ve subjects were taking non-steroidal anti-inflammatory drugs. Second-line disease modifying therapy is prescribed, in general, for patients with persistantly active disease for at least six months, together with X-ray evidence of joint destruction. A significantly greater proportion of RLS +ve subjects (29/41 71%, compared with 43/90 48%) were found to be taking one or more of these agents ($p=0.014$; 2-tailed Chi-square test), but the distribution of sulphasalazine, parenteral gold, penicillamine and hydroxychloroquine were similar.

A surprisingly large number of patients were taking oral prednisolone; 35% (14/40) RLS +ve and 26% (24/91) RLS -ve; differences were not significant ($p=0.316$, 2-tailed Chi square test).

There was no evidence from the survey that any single agent used in the treatment of RA attributed in any way to the incidence of RLS.

3.2 RESULTS OF CLINICAL TRIAL OF ORAL IRON IN RLS

3.2.1 Study population

Fifteen subjects were recruited to the study during the period March 1986 - March 1987. The group comprised; 13 patients with rheumatoid arthritis, one female member of staff with osteoarthritis, and one otherwise healthy male. Full demographic data are given in Appendix A9 and summarised in Table 3.22

Sub-group	n	Sex ratio		Mean age (yrs)	Age range
		Female	Male		
Rheumatoid	13	11	2	57.9	35 - 80
Non-rheumatoid	2	1	1	53.5	49 - 58

TABLE 3.22: Summary of age and sex distribution of trial subjects

3.2.1.1 Rheumatoid trial sub-group (Subjects A - M)

Most (10/13) rheumatoid trial subjects were identified during the prevalence survey (see Section 3.1). The mean duration of joint disease amongst rheumatoid subjects was 11.6 years (range 2-30 years, median 6 years). Six members of the group had a positive Latex test, though on Rose Waaler test only one had a rheumatoid factor titre greater than 1/32.

With the exception of one individual, all were taking non-steroidal anti-inflammatory drugs, and nine of the thirteen were also on second-line disease modifying agents (sulphasalazine, penicillamine, gold, hydroxychloroquine). One was on long-term prednisolone. Seven subjects were taking additional analgesics in the form of paracetamol or co-proxamol and, one was taking dihydrocodeine. Two were taking benzodiazepines for night-time sedation. For complete drug histories see Appendix A9.

Only one rheumatoid subject had previously sought medical advice specifically relating to restless legs symptoms. She had been prescribed 'tranquillisers' on one occasion. On a further occasion oral iron was

prescribed for concurrent iron deficiency anaemia, following which she reported that RLS symptoms had disappeared.

3.2.1.2 Non-rheumatoid trial subjects (X & Y)

Both non-rheumatoid trial subjects were recruited by means other than the prevalence survey. The female member of staff volunteered to be included after hearing about the trial whilst working in the out-patient department, and the healthy male was referred by his general practitioner to a hospital neurologist specifically because of RLS symptoms.

Of the two non-rheumatoid subjects, one was on no regular medication, though admitted to taking garlic pills occasionally; the other with osteoarthritis who had previously had both hip joints replaced, was taking regular naproxen.

3.2.1.3 RLS symptoms in trial subjects

Subjects recruited to the study had 'classical' symptoms of RLS. All experienced dysaesthesia bilaterally between the knee and ankle. Seven subjects also complained of symptoms in the feet and in two discomfort also extended to the thighs. Symptoms occurred only when subjects were seated, predominantly in the evening, or on retiring to bed. All found a need to move the legs or to get up and walk around during an attack, which brought some relief. Five subjects reported that RLS symptoms interfered with sleep, and 11/15 described symptoms as 'extremely irritating'.

A history of RLS ranged from 6 months to 25 years. Two rheumatoid subjects reported that RLS had started prior to diagnosis of rheumatoid arthritis.

Based on initial interview prior to trial recruitment the mean number of attacks per week was 4 (range 2-7, median 2.5), and the estimated mean duration of attacks was 1.15 hours (range 0.3 - 3 hours).

3.2.1.4 Iron status of trial subjects

Haemoglobin, red blood cell and iron indices were assessed prior to the control phase of the trial. Results are summarised in Table 3.23. (For actual figures see Appendix A9). Where available results of recent bone marrow aspiration (ie. during 10 weeks prior to trial recruitment) were also collated.

Subject	Anaemic F Hb <11.4 M Hb <13.4	S-iron <11umol/l	S-ferritin <20ug/l	S-ferritin 20 - 55ug/l (RA only)	Bone marrow iron stores
Rheumatoid subjects (A - M)					
A	*	*	*		0
B	*	*			1
C			*		-
D	*	*	*		0
E				*	0
F	*	*			1
G		*	*		-
H	*	-	-		1
I	*			*	-
J	*	*	*		-
K				*	-
L		*	*		-
M					-
Non-rheumatoid subjects (X & Y)					
X					-
Y					-

* = Yes
\- = Not available
0 = Absent marrow iron stores
1 = Reduced marrow iron stores

TABLE 3.23: Summary of iron status in subjects at start of trial

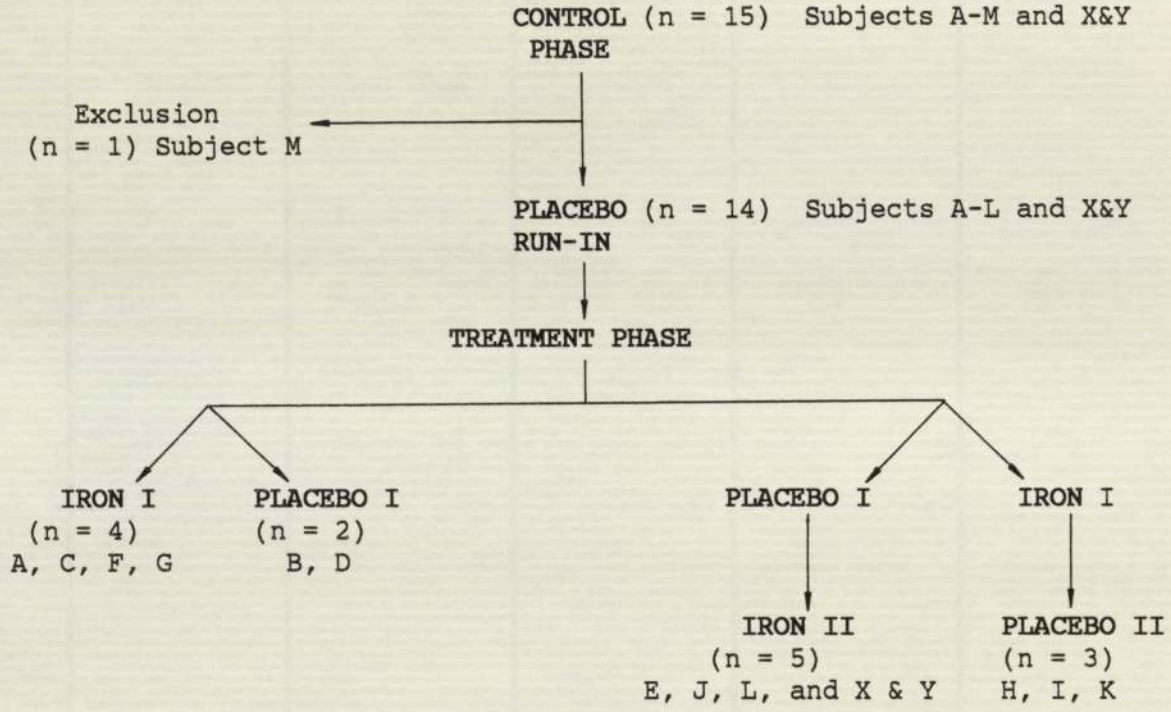
Seven rheumatoid subjects were anaemic with a s-ferritin <55ug and/or depleted marrow iron stores, indicating deficient iron stores. Four of the five non-anaemic rheumatoid subjects also had s-ferritin values indicative of iron deficiency. There was therefore evidence of iron deficiency in 11/12 rheumatoid subjects.

Haemoglobin, red cell parameters, and iron indices for the two

non-rheumatoid subjects were all in the normal range.

3.2.2 Treatment assignment

All 15 subjects were included in the control phase of the trial to assess baseline level of symptoms. Subject M, however, failed to meet the minimum level of attack frequency for the trial and was therefore excluded from the remainder of the trial. This subject was also excluded from trial analysis. Trial treatment allocation is shown in Figure 3.8.



(Subjects A-M rheumatoid; subjects X & Y non-rheumatoid)

FIGURE 3.8: Trial treatment allocation

Six rheumatoid subjects were randomised to either iron or placebo treatment according to the initial parallel between-patient trial design, the remainder received treatments in a cross-over fashion. Within patient comparison of iron and placebo medication was therefore possible in 10/12 rheumatoid and both non-rheumatoid subjects.

3.2.3 Evaluation of subject response to trial medication

Assessment of response to treatment was based on two types of subjective data; quantitative information on the number and duration of RLS attacks obtained from subject diary cards and, qualitative assessment of treatment phases by subjects according to five pre-set classifications.

Results were analysed to assess:

a) the effect of placebo tablets on RLS symptoms, in order to evaluate any 'placebo effect',

b) the efficacy of oral iron,

c) the duration of treatment required and length of any remission.

Results of rheumatoid and non-rheumatoid sub-groups were analysed seperately. Statistical analysis was performed using Student's t-test Chi-square test and Wilcoxon's rank sum test.

3.2.4 Trial results for the rheumatoid sub-group

Evaluation of response to treatment in rheumatoid subjects considered results from both between and within-patient arms of the trial.

3.2.4.1 Effect of iron and placebo on number of attacks of RLS

Individual results are detailed in Appendix A9. Group results are summarised in Table 3.24. The effect of treatment sequence is shown in Fig. 3.9.

Trial phase	CONTROL	PLACEBO RUN-IN	TREATMENT PHASES PLACEBO PHASE I	IRON	PLACEBO PHASE II	FOLLOW-UP
No. of subjects	12	12	5	10	3	8
Mean no. of attacks per subject	10.6	9.6	7.0	5.9	6.3	6.3
Range	4-18	2-18	2-13	0-15	0-10	0-15
Standard deviation	4.1	4.6	4.1	4.5	5.5	5.2
95% conf. limits	8.3-12.9	6.9-12.2	3.4-10.6	3.0-8.8	0-12.7	2.6-10.0

TABLE 3.24: Mean number of RLS attacks during trial phases

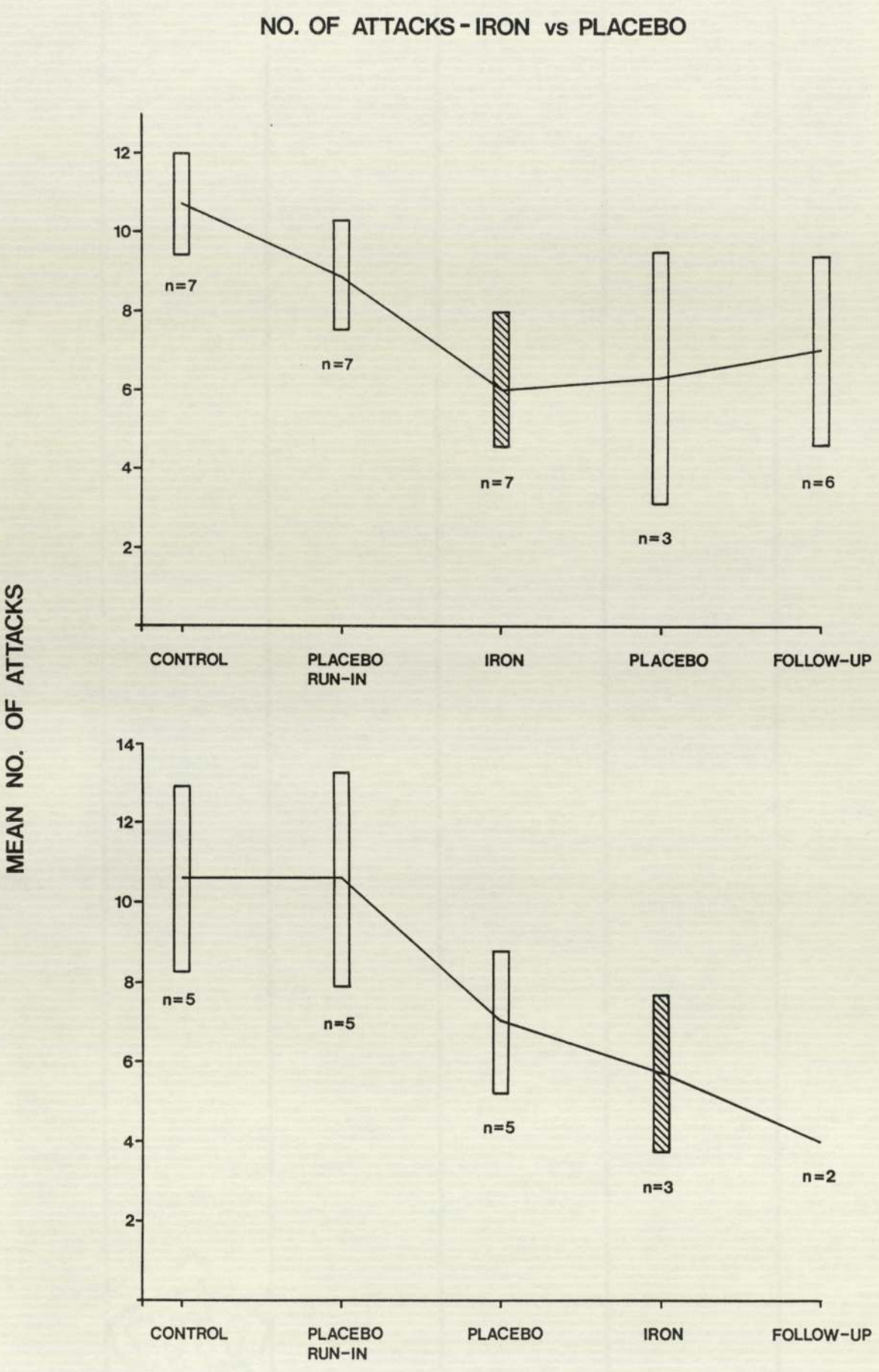


FIGURE 3.9: Graphs comparing mean number of attacks per four week treatment phase whilst on iron and placebo medication, according to treatment sequence
(Bars indicate standard error of the mean)

The effect of placebo tablets was assessed initially by comparing the mean number of attacks during the placebo run-in and control phases. Overall placebo was associated with a slight but insignificant reduction in the number of attacks ($p>0.2$, 2-tailed paired Student's t-test).

Five subjects were subsequently randomised to placebo immediately following the placebo run-in phase, denoted placebo phase I. Paired comparison with control and placebo run-in phases was performed to assess any period effect. Although not statistically significant ($p>0.05$, 2-tailed paired t-test), there was a 34% reduction in mean number of attacks.

Response to iron was compared in a similar manner. Group response during iron phase I was compared by pairing with control and placebo run-in phases. There was a 44% reduction in the mean number of attacks whilst on iron compared with that for the control phase, a significant difference ($p<0.05$, 2-tailed paired t-test). When compared with the placebo run-in phase this represented a 32% reduction, which was just outside statistical significance. ($p>0.05$, 2-tailed paired Student's t-test). On cross-over three further subjects were randomised to iron, denoted iron phase II. All three experienced a reduction in number of attacks compared with that associated with placebo and control phases.

Analysis so far has concentrated on the overall group response to treatment, the significance of which, was limited due to small recruitment numbers. Results of individual subjects show that infact 3/10 (A, G, I) had a remission in RLS symptoms within seven days of commencing iron. One of these subjects (G) was forced to discontinue iron treatment after only seven days due to a flare-up of arthritis. This will be discussed more fully in section 4.2.8. Of the seven other individuals who received iron, six experienced a greater than 25% reduction in number of attacks compared with the control phase. In total 9/10 subjects experienced >25% reduction in RLS attacks following iron therapy (see Table 3.25). In contrast only

4/12 had a similar improvement in symptoms following placebo. This difference was statistically significant ($p<0.025$, 2-tailed Chi-square test with Yate's correction)

	PLACEBO (best response) n =12	IRON n = 10
Complete remission	0 (0%)	3 (30%)
Partial remission *	4 (33%)	6 (60%)
No change **	7 (58%)	0 (0%)
Worsening	1 (8%)	1 (10%)

(* Partial remission defined as >25% reduction in number of attacks
** No change defined as <25% reduction in number of attacks)

TABLE 3.25: Response to placebo and iron compared with control phase

Eight rheumatoid subjects completed diary cards during the follow-up phase. Complete remission of symptoms lasted between one and five months in three subjects following iron treatment. Such a response did not occur with placebo. Of those who had a partial remission on iron this was maintained in two and worsened in three on follow-up.

3.2.4.2 Effect of iron and placebo on duration of RLS attacks

Subjects also recorded duration of each attack on diary cards, allowing the mean duration of attacks during each phase to be calculated for each subject. Individual results are detailed in Appendix A9. Results are summarised in Table 3.26 and shown in relation to treatment sequence in Figure 3.10.

There was no significant change in the mean attack duration associated with placebo either during run-in or treatment phase I ($p>0.2$ and $p>0.3$ respectively, 2-tailed paired t-test). Attack duration was similarly insignificantly affected by iron ($p>0.8$, 2-tailed paired t-test.)

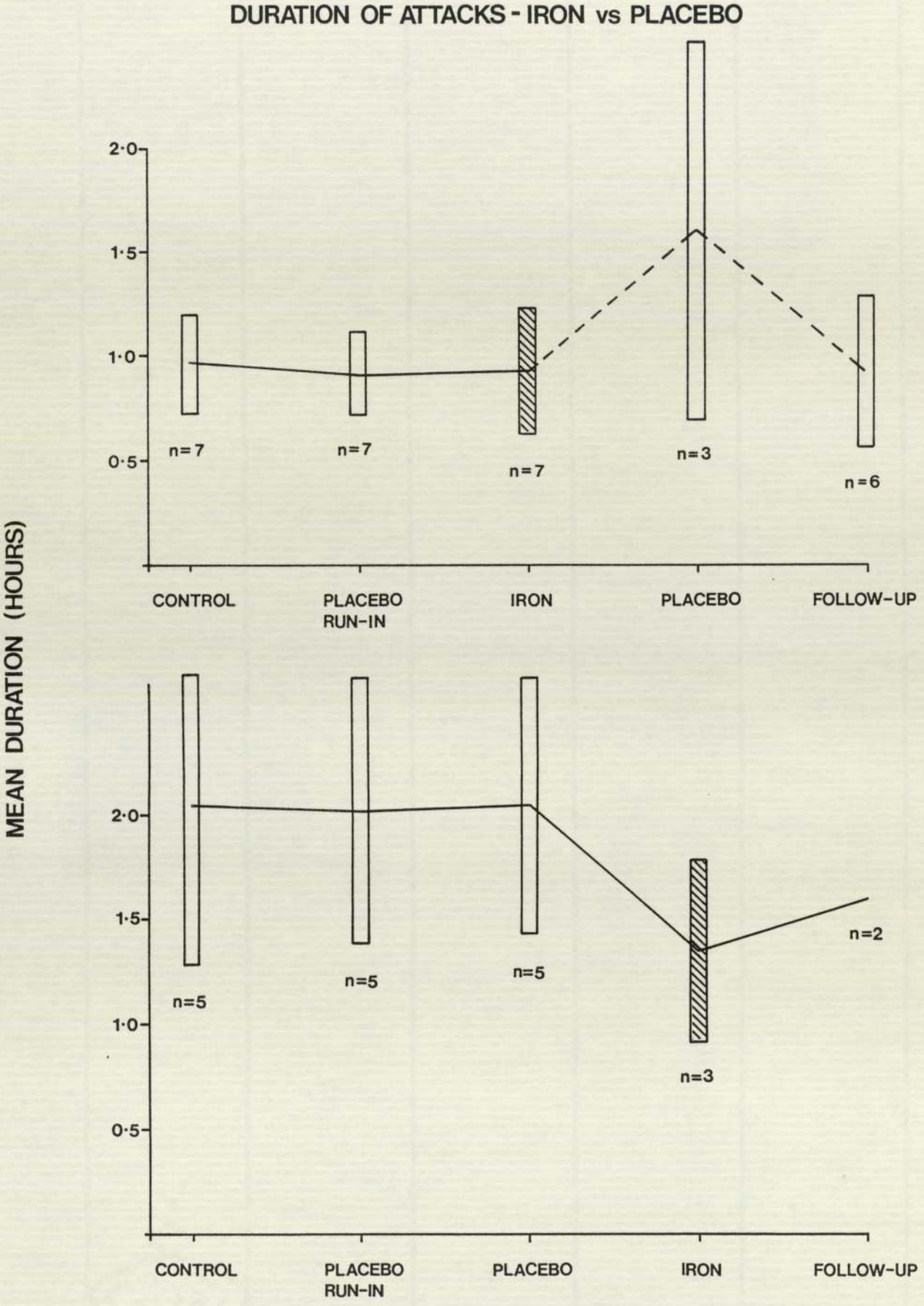


FIGURE 3.10: Graphs comparing mean duration of attacks per four week treatment phase whilst on iron and placebo medication, according to treatment sequence
(bars indicate standard error of mean)

Trial phase	CONTROL	PLACEBO RUN-IN	TREATMENT PHASES PLACEBO PHASE I	IRON	PLACEBO PHASE II	FOLLOW-UP
No. of subjects	12	12	10	10	3	8
Mean attack duration (hours)	1.4	1.3	1.0	1.0	1.6	1.1
Range	0.2-5.0	0.1-5.0	0.1-5.0	0-3.0	0-4.3	0-3.0
Standard deviation	1.3	1.3	0.7	0.9	2.4	1.0
95% conf. limits	0.7-2.1	0.6-2.1	0.6-1.4	0.5-1.6	-1-4.3	0.4-1.8

TABLE 3.26: Mean duration of attacks (hours) during treatment phases

3.2.4.3 Effect of iron and placebo on subjective assessment of RLS

Subjective assessment of symptoms was made at the end of each treatment period. This constituted ordered data and was analysed using a non-parametric test, Wilcoxon's rank sum test. The most favourable response that subjects had to placebo (prior to any iron) was compared with assessments for iron. Results are shown in Table 3.27 and graphically in Figure 3.11.

	PLACEBO (best response) n = 12	IRON n = 10
Cured	0 (0%)	3 (30%)
Much better	1 (8%)	3 (30%)
Slightly better	8 (67%)	3 (30%)
No change	2 (17%)	0 (0%)
Worse	1 (8%)	1 (10%)

TABLE 3.27: Subjective assessment of RLS following iron and placebo

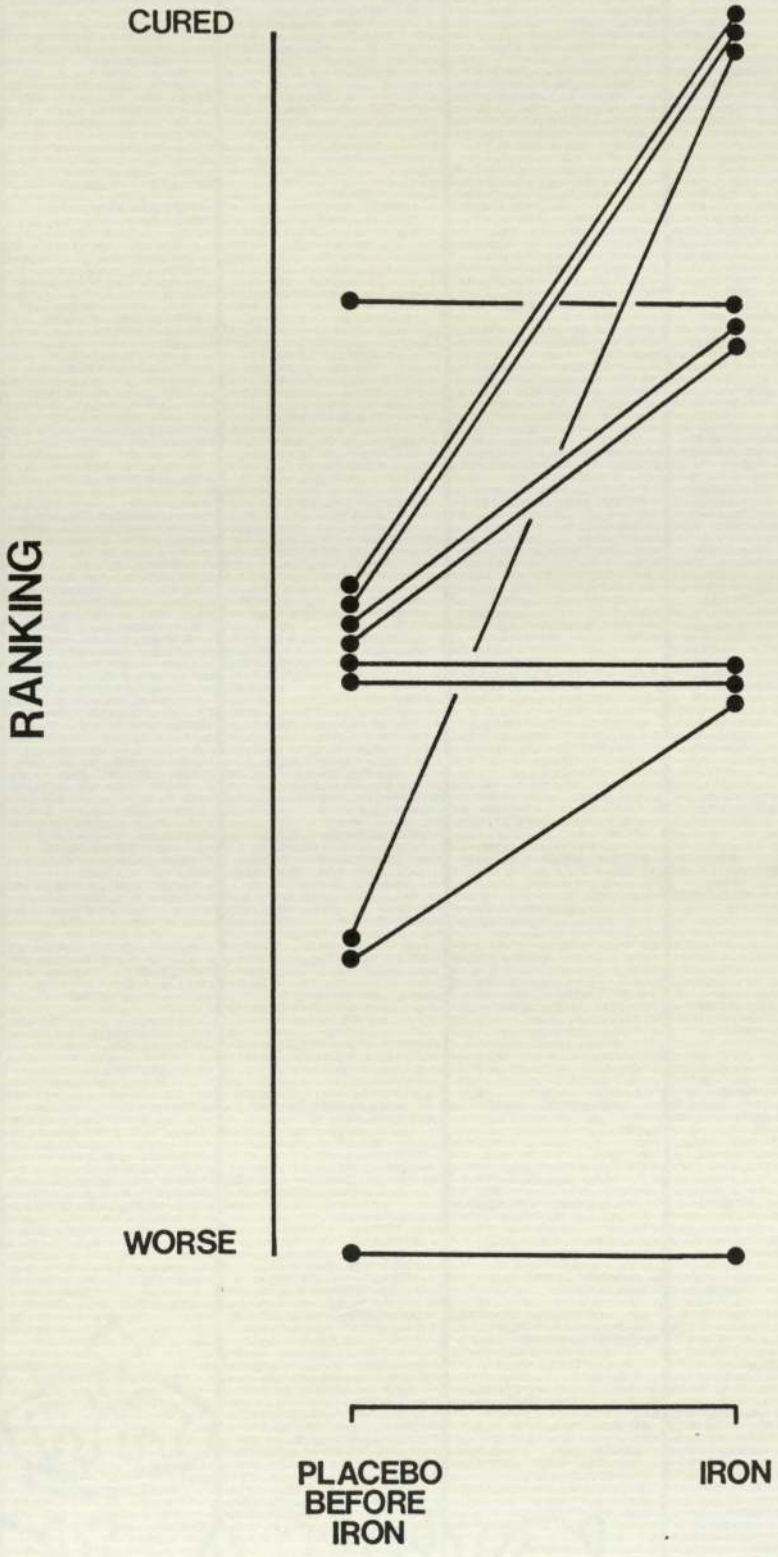


FIGURE 3.11: Comparison of subjective rankings of symptoms on placebo and iron in RA subjects

Six out of ten of rheumatoid subjects graded RLS symptoms as much better or cured following oral iron, compared with only 1/12 on placebo. In no case was iron graded worse than placebo. The slight difference between run-in placebo and subsequent placebo treatment seen in frequency data, was also apparent from subjective gradings. Even allowing for this, there was still a significant improvement in subjective assessment of symptoms following iron ($p<0.05$, 2 sample Wilcoxon's rank sum test).

Summary of response to trial medication in rheumatoid subjects

Oral iron was associated with an overall reduction in frequency of RLS attacks, which was also perceived as a subjective improvement in symptoms, both at a statistically significant level. At best placebo medication was associated with a small improvement in symptoms. This was probably a reflection of fluctuation in RLS symptoms. Three subjects experienced a remission in attacks after iron, a feature that did not occur in any subjects whilst on placebo. Duration of attack was not altered appreciably by iron or placebo medication.

3.2.4.4 Correlation of RLS with iron status and RA disease activity

Haematological indicators of iron status were measured at the beginning of each trial phase, together with ESR as a measure of disease activity. Values before and after oral iron were compared (see Table 3.28)

Variable	n	Mean (SD) Before Fe	After Fe	2-tailed paired t-test probability	Significance
Hb	10	11.4 (1.3)	12.0 (1.1)	p> 0.1	NS
s-iron	10	10.4 (7.1)	12.1 (7.4)	p> 0.1	NS
%sat.	9	13.3 (6.7)	15.3 (8.0)	p> 0.1	NS
TIBC	10	68.0 (14.2)	67.3 (10.1)	p> 0.5	NS
s-ferritin	9	19.2 (11.4)	32.4 (17.8)	p< 0.02	S
ESR	10	32.0 (19.1)	28.1 (15.1)	p> 0.1	NS

TABLE 3.28: Effect of iron on haematological variables in RA subjects

Administration of oral iron was associated in all subjects by a significant rise in s-ferritin ($p < 0.02$). This was not accompanied by an increase in ESR, indicating that the change in s-ferritin was a reflection of a true increase in iron stores. With the exception of one subject (pt. H) whose symptoms worsened with iron, frequency of attack was inversely proportional to s-ferritin. See Figure 3.12. Any changes in s-ferritin in subjects given placebo throughout the trial are difficult to interpret due to missing data.

Other indicators of iron status; Hb, s-iron, % transferrin saturation, and TIBC did not change significantly following treatment with oral iron, and did not correlate with frequency of attacks.

Frequency of attack was assessed in relation to ESR. There was no correlation between these two variables. This was in agreement with subjects' overall impression that RLS symptoms were not related to rheumatoid activity.

3.2.5 Trial results for the non-rheumatoid sub-group

The effect of iron and placebo on RLS symptoms were similarly compared in non-rheumatoid subjects. In view of the small numbers statistical analysis was inappropriate.

3.2.5.1 Effect of iron and placebo on number of RLS attacks

The number of attacks during each treatment phase for both non-rheumatoid individuals is shown in Figure 3.13. Neither oral iron or placebo medication were associated with an improvement in frequency of RLS.

3.2.5.2 Effect of iron and placebo on duration of RLS attacks

Similar representation of data regarding duration of attack is shown in Figure 3.14. Again there was no real change in duration of attacks with trial medication.

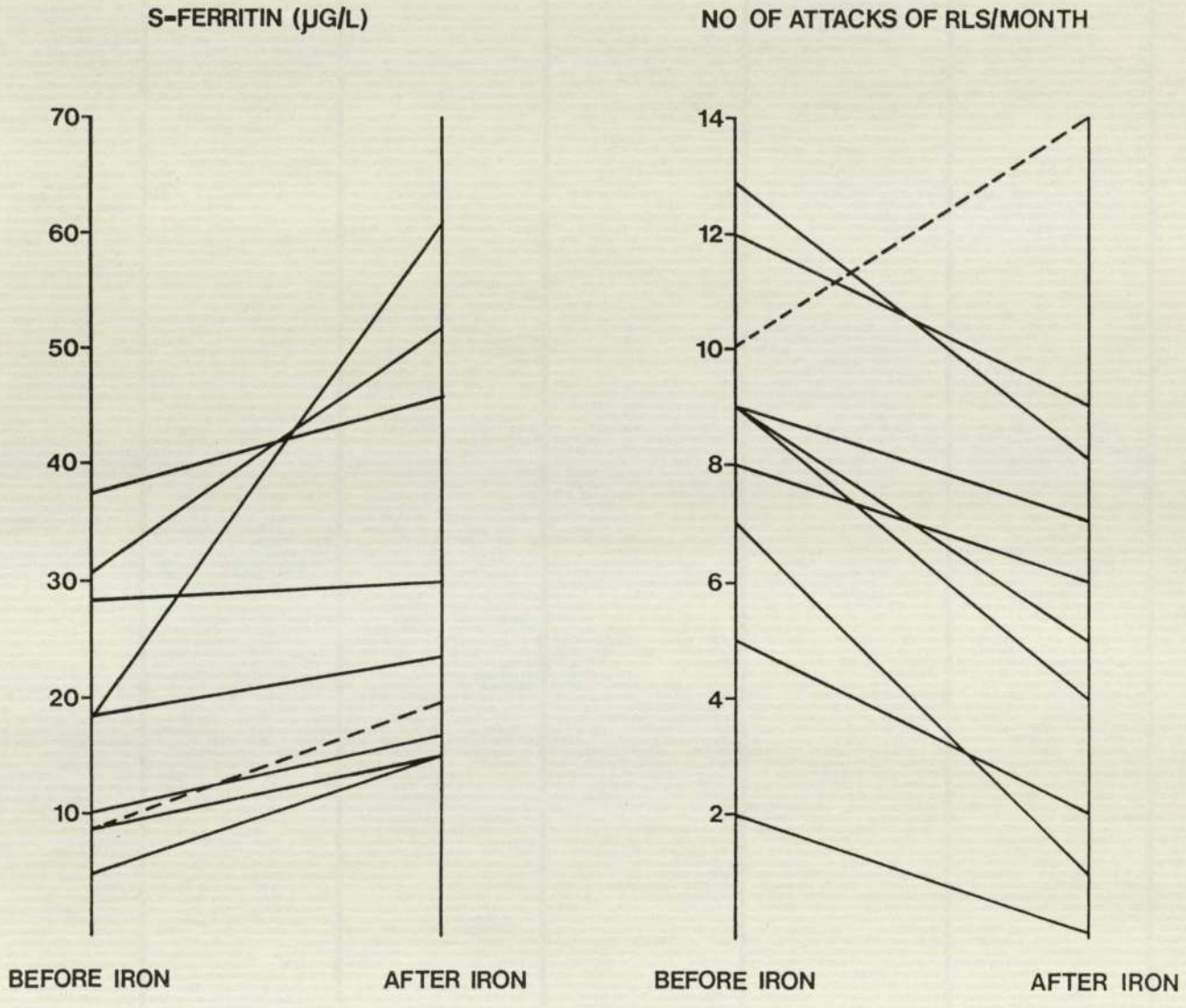


FIGURE 3.12: Correlation between increase in s-ferritin and reduction in attack frequency following iron in rheumatoid subjects

Subjects	Control	Trial phase Placebo run-in	Placebo I	Iron II	Follow-up
X	27	26	25	26	-
Y	12	7	11	10	-

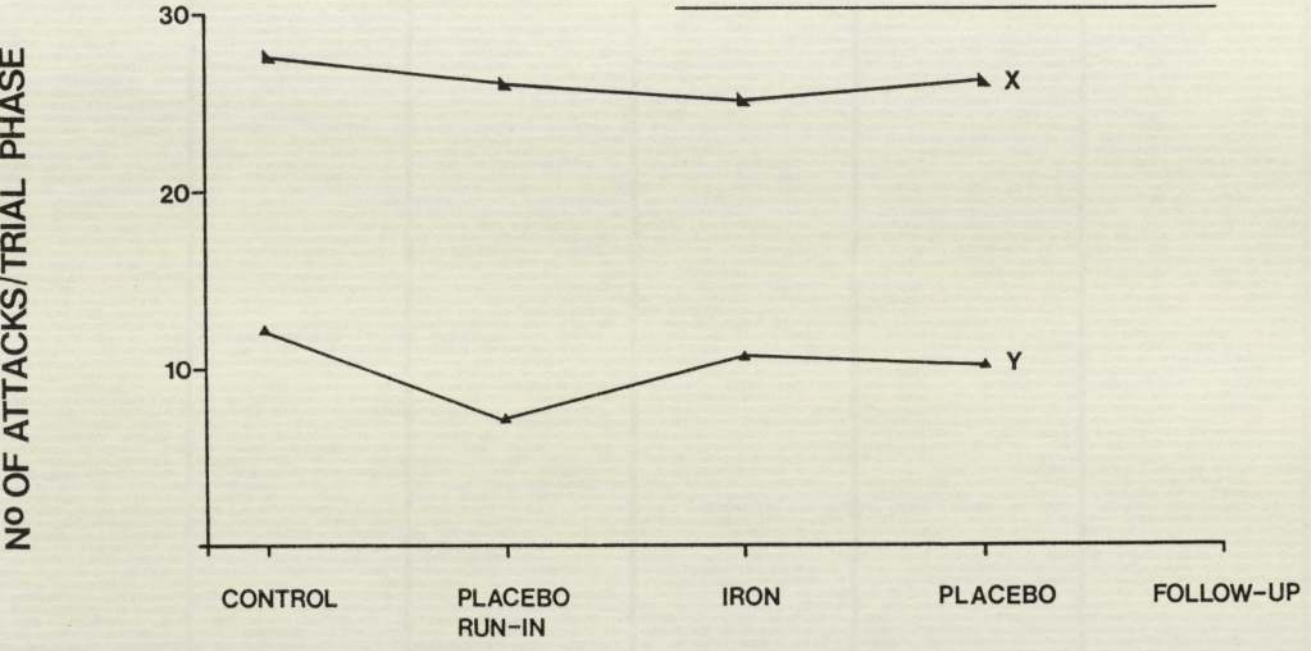


FIGURE 3.13: Individual subject plots of number of attacks of RLS during each trial phase in non-rheumatoid subjects.

Subjects	Control	Trial phase Placebo run-in	Placebo I	Iron II	Follow-up
X	1.5	1.5	1.4	1.5	-
Y	5.4	2.9	2.9	2.1	-

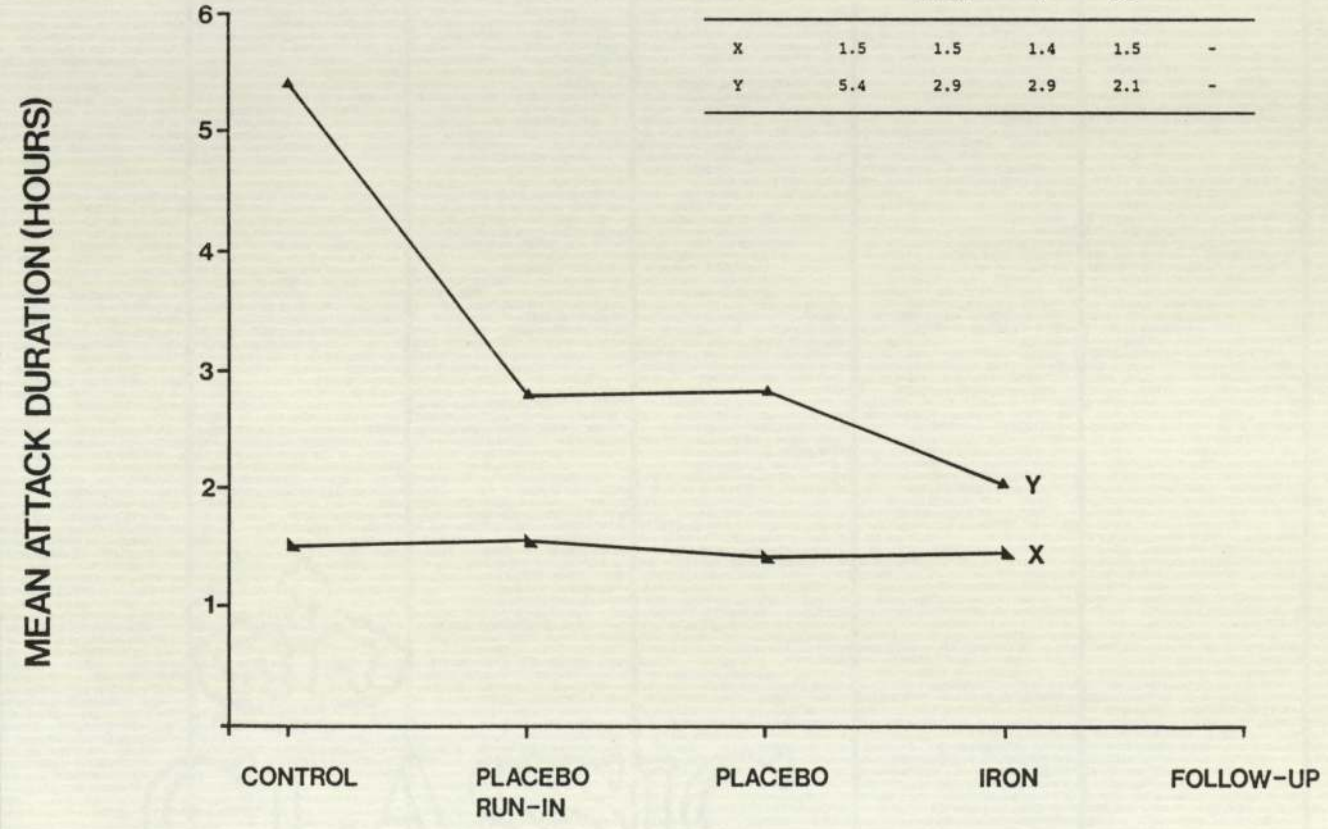


FIGURE 3.14: Individual subject plots of mean duration of attacks of RLS during each trial phase in non-rheumatoid subjects

3.2.5.3 Subjective assessment of iron and placebo on RLS

Subjective gradings are represented in Figure 3.15. Scores for subject X reflect the lack of objective change in symptom frequency or duration of attack. Pt. Y reported a subjective slight improvement following both placebo and iron medication, but this did not represent a major change in symptoms.

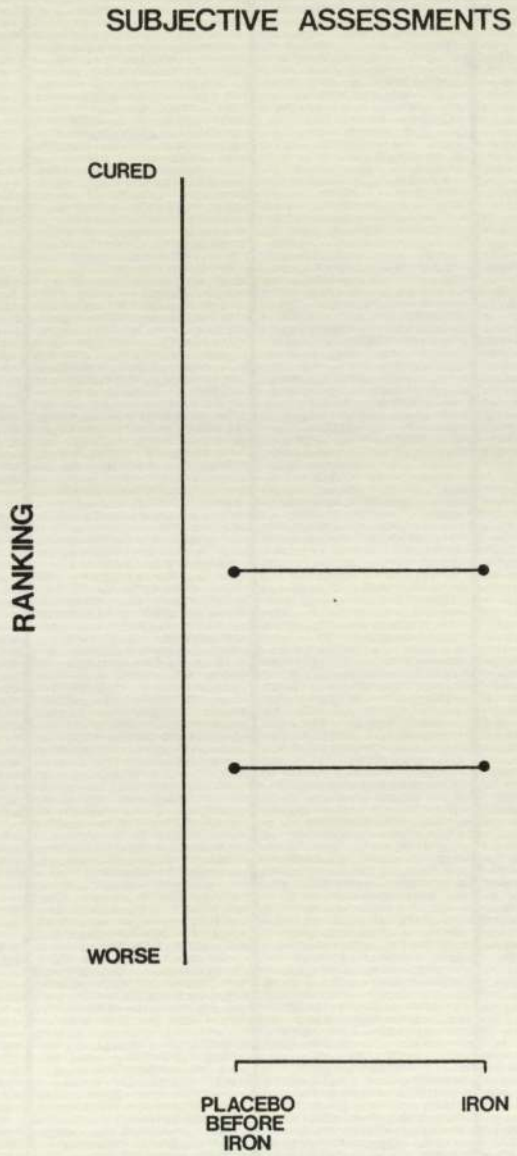


FIGURE 3.15: Comparison of subjective rankings of symptoms on placebo and iron in non-rheumatoid subjects

Hence in the two non-rheumatoid subjects studies, neither oral iron or placebo medication had any significant effect on quantitative or qualitative assessment of symptoms.

3.2.6 Evaluation of compliance

Patient compliance with trial medication was assessed by tablet count at the end of each treatment phase. The level of compliance was then calculated using the following formula:

$$\frac{\text{(No. of tablets that should have been taken - No. actually taken)}}{\text{No. that should have been taken}} \times 100$$

Mean compliance figures for each subject are given in Table 3.29.

	Subject	Mean compliance (%)
Rheumatoid subjects	A	69
	B	89
	C	-
	D	-
	E	98
	F	89
	G	82
	H	92
	I	95
	J	100
	K	105
	L	85
Non-rheumatoid subjects	X	98
	Y	83

Table 3.29: Mean figures showing % drug compliance for each subject

Overall a high level of compliance was achieved throughout the trial. Subject C failed to return any containers and therefore compliance could not be assessed, however the patient was considered reliable and it was felt that the treatment regime had been adhered to. Subject D also forgot to return containers but claimed to have taken the tablets as prescribed on 'most' days. A degree of non-compliance was suspected.

3.2.7 Assessment of adverse effects associated with trial medication

Information on adverse effects, attributed by subjects to trial medication, was recorded during clinic visits. This comprised of details offered by

patients voluntarily and that sought be direct questioning. Side-effects and frequency are listed in Table 3.30.

Information source	Placebo n = 14	Ferrous sulphate n = 12
Voluntary information	Abdominal discomfort (2) Constipation (1)	Abdominal discomfort (1) Constipation (2) Black stools (1) * Sweats (1) ** Joint 'flare-up' (2)
Direct questioning	Constipation (1) * Sweats (1)	Abdominal discomfort (1) Constipation (1) Black stools (2)
Total	5	11

* Same patient
** Rheumatoid subjects (no. 1,8)

TABLE 3.30: Comparison of adverse drug reactions associated with placebo and ferrous sulphate trial medication

10/14 trial subjects reported an adverse event in association with trial medication, the majority being of a minor nature. Constipation and abdominal discomfort were common to both iron and placebo tablets. The major adverse effect associated with iron was a flare-up in arthritis reported by two rheumatoid subjects (A & G). Erythrocyte sedimentation rate was measured before and after administration of iron in both patients, but did not reflect a significant change of inflammatory activity. An increase in Ritchie articular index and reduction in grip strength was recorded during metrological assessment of one subject (A).

3.3 LOW-DOSE IRON ABSORPTION TEST

Results of the low-dose iron absorption test were analysed firstly to verify the test as a means of differentiating between iron repletion and uncomplicated iron deficiency, secondly to assess iron absorption in subjects with rheumatoid arthritis and finally as a means of differentiating ACD and IDA.

3.3.1 Study population

The iron absorption test was performed on 38 subjects; 25 with rheumatoid arthritis, 6 with uncomplicated iron deficiency and 7 healthy iron replete volunteers.

Rheumatoid subjects were classified as having definite or classic rheumatoid arthritis [177] and were all in-patients with active disease at the time of the test. Subjects had all undergone recent bone marrow aspiration as part of routine investigation of iron deficiency. In accordance with bone marrow iron stores, subjects were divided into three groups; 9 with absent iron stores, 7 with reduced and a further nine with normal or increased iron stores. Fourteen of the rheumatoid subjects were anaemic (mean Hb 9.4, SD 1.2, range 7.9-11.1). Nineteen were hypoferraemic and 17 had reduced transferrin saturation. Disease activity in the three sub-groups, as indicated by ESR, did not differ significantly (one-way analysis of variance).

Of the six subjects with pure iron deficiency, three were found to have gastrointestinal bleeding, while deficiency in the others was thought to be dietary. Three of the six were anaemic (mean Hb 7.5, SD 1.7, range 5.7-9.0). None of the subjects with uncomplicated iron deficiency anaemia or with rheumatoid disease and absent marrow iron stores, had been commenced on iron supplements prior to the absorption test.

Healthy iron replete control subjects were all members of hospital staff and consequently the mean age of this group was significantly lower than

either the iron deficient or rheumatoid sub-groups.

Demographic and baseline haematological parameters for each subject are given in Appendix A10, and summarised in Table 3.31.

Parameter	RHEUMATOID SUBJECTS Bone marrow iron grading Absent n=9	Reduced n=7	Normal n=9	Iron deficient n=6	Iron replete n=7
Sex	9F	7F	5M:4F	1M:5F	2M:5F
Age (yrs)	59.3 (14.2)	54.1 (21.7)	59.3 (8.6)	47.2 (19.4)	27.1 (3.6)
range	39-40	22-79	53-79	27-75	23-33
Hb	10.0 (1.8)	10.0 (1.5)	12.4 (1.9)	10.4 (3.4)	14.3 (0.8)
MCV	73.2 (12.5)	77.8 (10.5)	86.4 (7.1)	73.5 (13.5)	89.9 (1.9)
s-iron T=0	6.6 (2.3)	10.1 (6.4)	12.5 (6.4)	5.2 (1.8)	22.6 (8.6)
s-ferritin	10.6 (4.8)	51.9 (45.8)	243.8 (282)	11.3 (8.5)	79.9 (52.2)
TIBC	79.9 (4.5)	58.0 (8.1)	59.2 (8.8)	75.0 (14.7)	61.8 (7.1)
%saturation	7.1 (2.7)	14.5 (7.2)	21.0 (9.0)	7.3 (3.1)	37.4 (12.7)
ESR	37.6 (23.4)	43.1 (28.3)	48.6 (34.9)	-	-

TABLE 3.31: Demographic and baseline haematological parameters of iron absorption test subjects (SD)

3.3.2 Iron absorption test procedure

Iron absorption tests were all commenced between 8.30 and 9am. Two rheumatoid subjects received prednisolone on the morning of the test; breakfast-time medication in the others was delayed until the test was completed.

The test was generally well tolerated. Several subjects complained of headache which was attributed to dehydration, despite encouragement to drink plenty of water.

Iron absorption test curves were drawn by plotting the change in s-iron,

compared with pre-test value, against time post dose. Curves were also drawn based on percentage change in s-iron, and these have been used in the thesis, to aid comparison. Discussion of differences in curves, however, concentrate on actual changes in s-iron.

3.3.4 Validation of method

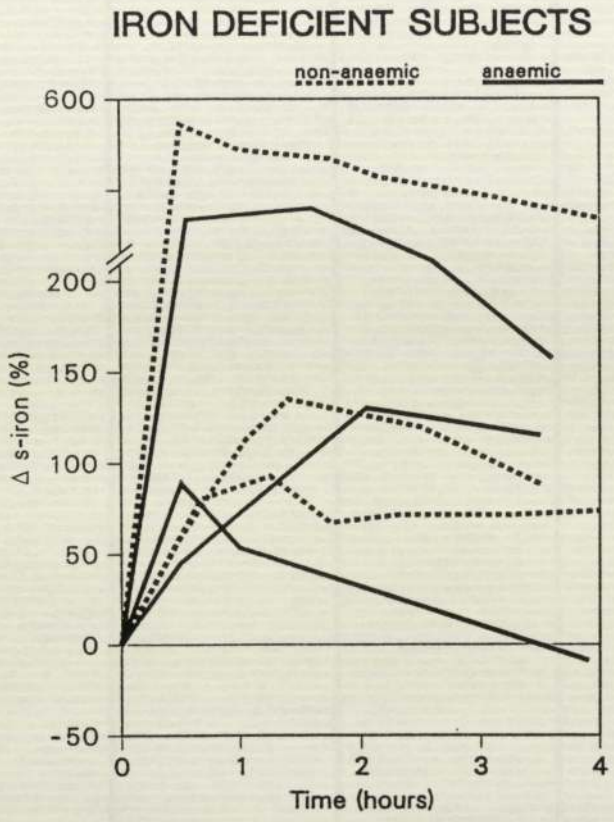
A control experiment was performed in which the test was done twice in a normal volunteer with and without oral iron. Administration of 10mg elemental iron produced significant changes when compared with the control ($p < 0.05$, unpaired Wilcoxon's rank sums test). Maximum variation in s-iron during the control test was 1.2umol/l, well within the same-day coefficient of variation [128].

3.3.5 Comparison of absorption curves in uncomplicated iron deficiency and iron repletion

Iron absorption test curves (% change in s-iron against time) for iron deficient and iron replete controls are shown in Figure 3.16. Actual serum iron values may be found in Appendix A10. Absorption curves for the two groups were noticeably different. Characteristics were compared with reference to the maximum height of curves and rate at which it was attained. See Table 3.32.

	Iron deficient n=6	Iron replete n=7
Max. increase in s-iron (umol/l)	10.9	3.4
(Range	4 - 19.3	0.8 - 8.9)
Time post dose of maximum increase (hours)	1.1	1.4
(Range	0.5 - 2.0	0.5 - 2.5)

TABLE 3.32: Maximum increase in s-iron and time taken iron deficient and iron replete volunteers



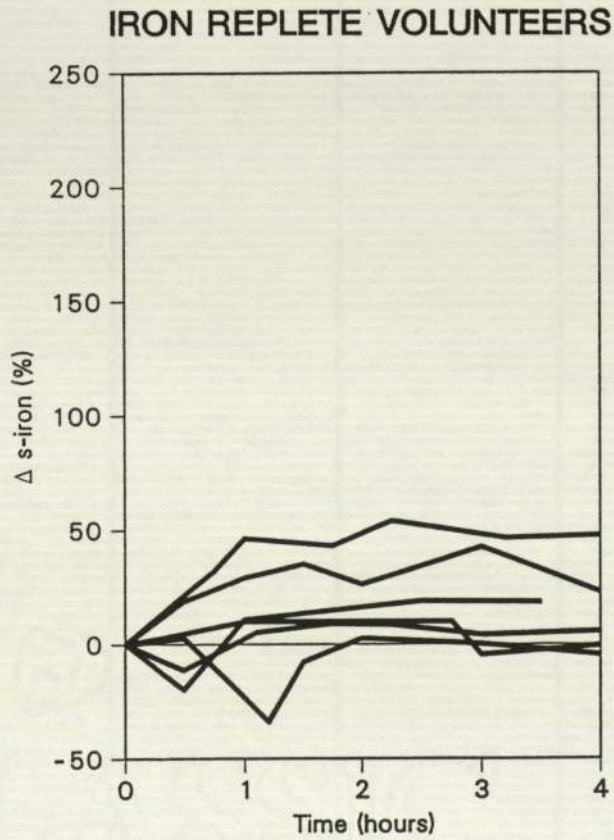


FIGURE 3.16: Iron absorption curves

The maximum increase in s-iron was significantly greater in iron deficiency compared with iron replete subjects ($p < 0.05$, unpaired Wilcoxon's rank sums test). The time taken to reach maximum increase was not, however significantly different. Increases in serum iron seen in the iron deficient sub-group were comparable with those reported by Crosby et al. [110]. Rate and extent of increase in s-iron appeared to be independent of the presence or severity of anaemia.

Mean curves for the two sub-groups are shown in Figure 3.17. Changes in s-iron were significantly different at sample times between 30 minutes and 3 hours ($p < 0.01$ at 30 mins, $p < 0.05$ at 1,1.5,2 and 3 hours, unpaired Wilcoxon's rank sums test). Levels of significance were higher when % figures were compared ($p < 0.01$ at 30 mins, 1,1.5,2, and 3 hours).

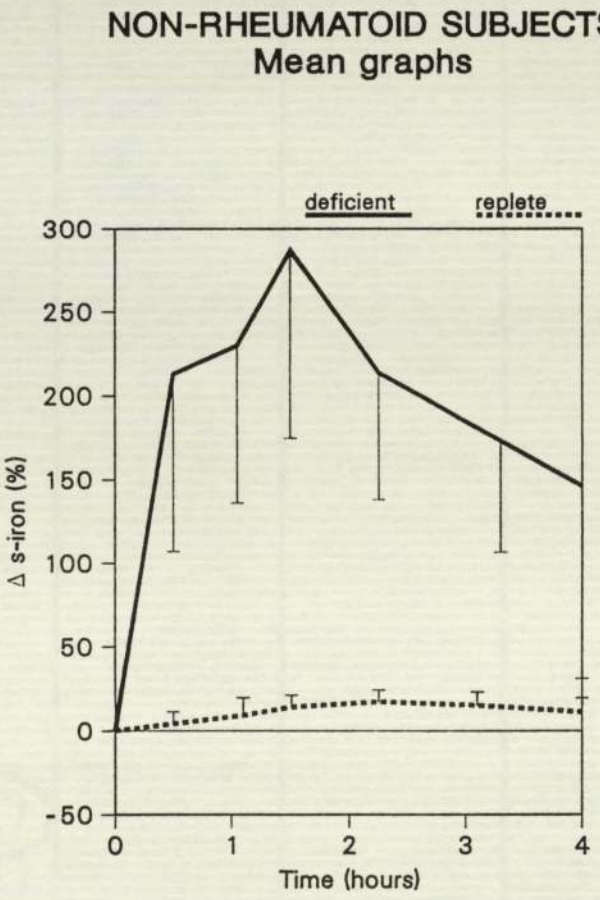


FIGURE 3.17: Comparison of mean iron absorption curves for iron deficient and iron replete subjects

Maximum discrimination between the two sub-groups occurred at 1 hour post dose, when the percentage change in s-iron was greater than 50% in all iron

deficient subjects. Changes were all less than 50% in healthy volunteers. See Figure 3.18.

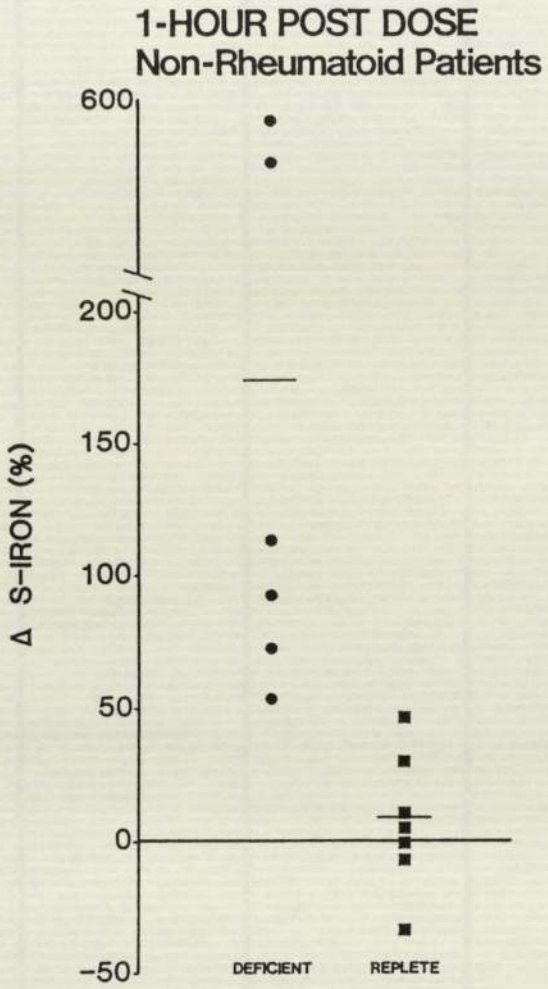


FIGURE 3.18: Maximum discrimination between changes in serum iron at 1 hour post-dose in iron deficiency and iron repletion

Results therefore confirm Crosby's original findings, demonstrating that the test may be used to differentiate between iron deficiency and iron repletion. The marked differences at 1 hour post-dose suggest that the test may be simplified to just two samples, pre-dose and 1 hour post-dose.

3.3.6 Comparison of iron absorption curves in rheumatoid sub-groups

Absorption test results for rheumatoid subjects may be found in Appendix A10 and individual curves are shown in Figure 3.19. Absorption curves for rheumatoid subjects with definite iron deficiency, indicated by absent iron stores, were strikingly different from those with normal iron stores. There was also a noticeable trend in four individuals with normal iron stores, for serum iron to decrease throughout the test. Two of these

subjects were also anaemic and hence diagnosed as having the anaemia of chronic disease.

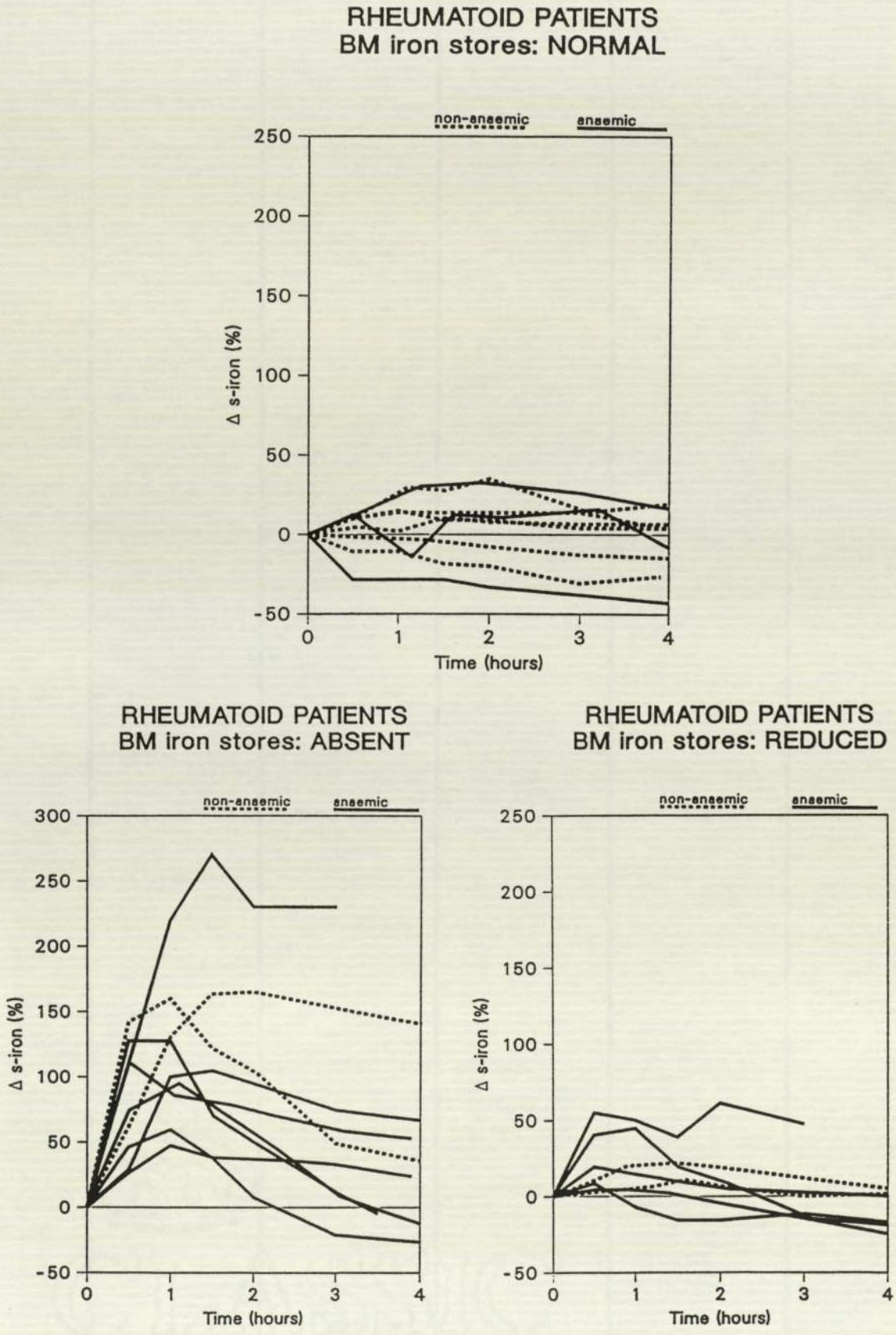


FIGURE 3.19: Iron absorption curves for rheumatoid subjects with absent, reduced and normal bone marrow iron stores

Greatest increases in s-iron occurred in those with absent iron stores. These were significantly higher than in subjects with normal or reduced marrow iron stores ($p < 0.01$, unpaired Wilcoxon's rank sums test). Peak changes seen in those with normal or reduced iron stores did not differ significantly. The time delay before maximum increase was not affected by iron status. See Table 3.33. Again there was no appreciable difference in the characteristics of absorption curves for those subjects with anaemia compared with those without.

	Bone marrow iron stores		
	Absent n=9	Reduced n=7	Normal n=5*
Max. increase in s-iron (umol/l)	6.9	2.2	3.1
(Range	3.1 - 19.0	0.4 - 4.9	0.9 - 6.7)
Time post dose of maximum increase (hours)	1.2	1.0	1.6
(Range	0.5 - 2.0	0.5 - 2.0	1.0 - 2.0

* excluding subjects with steady decrease in s-iron during test

TABLE 3.33: Maximum increase in s-iron and time taken in rheumatoid sub-groups according to iron status

Mean absorption curves for rheumatoid subjects are shown in Figure 3.20. Changes in s-iron were significantly different in individuals with absent iron stores at 30 minutes, 1 hour ($p < 0.01$) and 1.5 hours ($p < 0.05$) compared with those in subjects with depleted iron stores. Similarly, when compared with subjects with normal iron stores differences were significant at 30 minutes ($p < 0.01$), 1,1.5 and 2 hours ($p < 0.05$). There was no statistically significant difference at any sample time between absorption curves in iron replete or mildly deficient rheumatoid subjects.

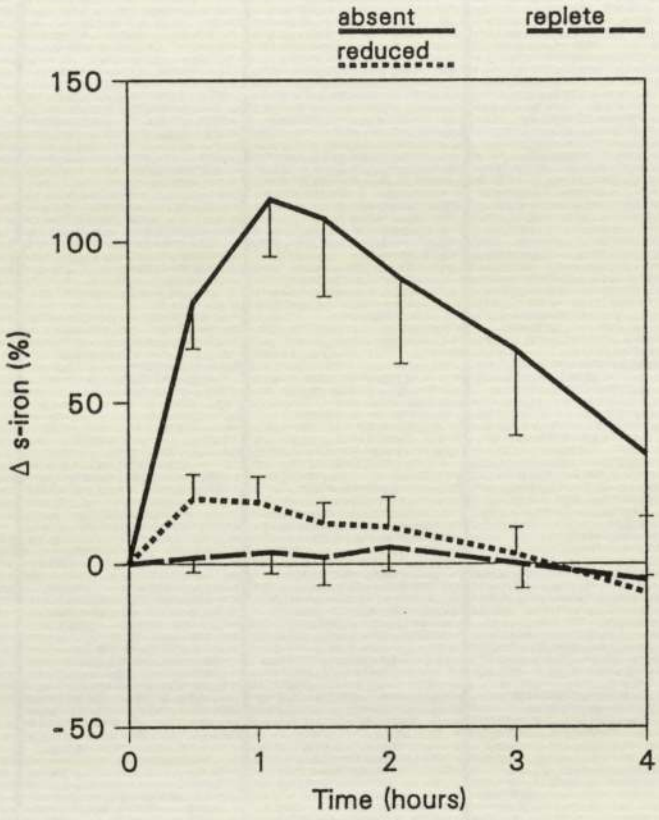


FIGURE 3.20: Comparison of mean iron absorption curves for rheumatoid subjects with absent, reduced and normal marrow iron stores

As in non-rheumatoid subjects, maximum discrimination between mean curves occurred at 1 hour post dose. See Figure 3.21. At this sampling time, eight out of nine subjects with absent iron stores had a change in s-iron greater than 50%. Changes were all less than this in those with reduced or normal iron stores. A decrease in s-iron at 1 hour was seen in four subjects who were not iron deficient and one out of six with reduced iron stores. Hence based on the small sample tested, a change in s-iron at 1 hour greater than 50% was a good indicator of iron deficiency, while a decrease in s-iron was indicative of normal iron stores.

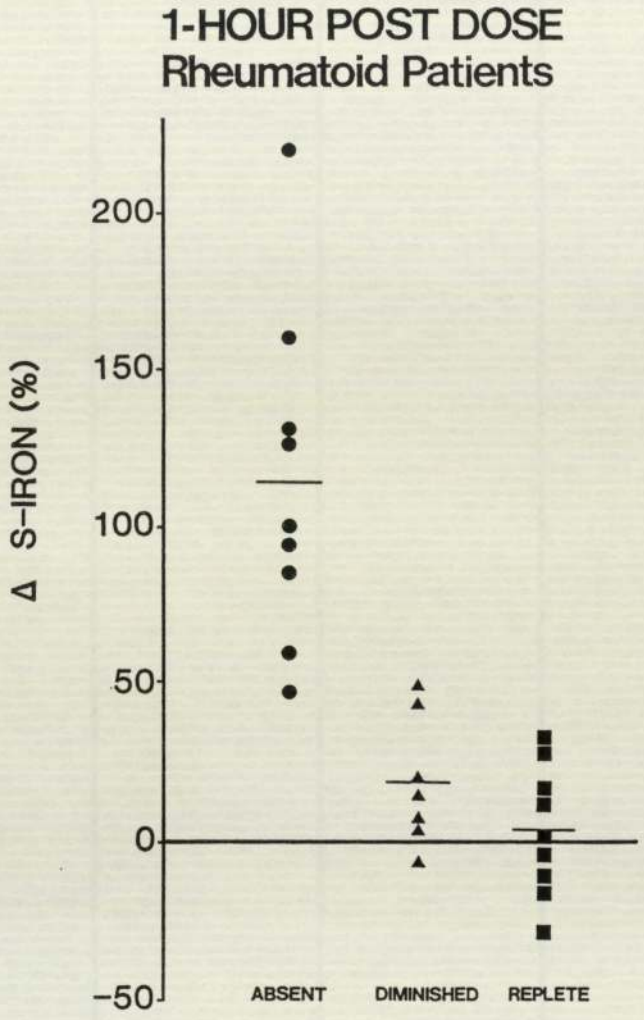


FIGURE 3.21: Maximum discrimination between changes in serum iron at 1 hour post-dose in rheumatoid subjects according to bone marrow iron stores

3.3.7 Effect of rheumatoid disease on iron absorption

The effect of rheumatoid disease on absorption of iron in subjects with concurrent iron deficiency was assessed. Increases in s-iron at 1 hour post-dose were compared between rheumatoid subjects, shown to have absent iron stores and non-rheumatoid subjects with uncomplicated iron deficiency. Sub-groups were well matched in terms of pre-test dose s-iron and s-ferritin values. Changes in s-iron were found to be not significantly different, suggesting that iron absorption in rheumatoid subjects with genuine iron deficiency is comparable with that in subjects without concurrent inflammatory disease.

There was no statistically significant difference in changes in s-iron at 1

hour between rheumatoid subjects with normal iron stores and iron replete volunteers. The trend, however,for s-iron to gradually decrease throughout the test in four of the rheumatoid subjects was not seen in any of the normal volunteers and suggested that iron absorption may have been reduced in the former subjects.

Using erythrocyte sedimentation rate as a measure of rheumatoid disease activity, the affect on iron absorption was assessed in rheumatoid sub-groups with normal and absent iron stores. ESR and changes in s-iron at 1 hour post-dose were weakly inversely correlated for both sub-groups (r= -0.320 and -0.225 respectively).

3.3.8 Differentiation of IDA and ACD in rheumatoid subjects

Of the 25 rheumatoid subjects included in the study, 14 were anaemic. The sensitivity of the low-dose iron absorption test in differentiating IDA from ACD was compared with three other suggested methods based on haematological parameters [71,138,69]. Results are shown in Table 3.34

Method	Absent n=7	Reduced n=5	Normal n=2	Total
s-ferritin < 20ug/l	7/7	1/5	2/2	10/14
s-ferritin < 55ug/l	7/7	2/5	2/2	11/14
s-ferritin <60ug/l and/or TIBC >55umol/l	7/7	4/5	2/2	13/14
Low-dose iron absorption test	6/7	0	2/2	8/14

TABLE 3.34: Comparison of different methods for differentiation of IDA and ACD

Based alone on a s-ferritin of less than 20ug/l, the recognised lower limit of normal, differentiation could be made successfully in 71% of anaemic subjects studied. All the subjects with absent iron stores did in fact have

s-ferritin values less than this limit.

If the lower limit of 55ug/l, suggested as being appropriate for rheumatoid disease [138], is employed then the sensitivity increased to 78%. Incorporating a limit of 55umol/l for total iron binding capacity, the sensitivity may be improved marginally.

The low-dose iron absorption test was found to accurately predict iron deficiency in 86% of subjects with definite iron deficiency anaemia, as indicated by absent bone marrow iron stores. Employing a 50% limit on the change in s-iron as being indicative of genuine iron deficiency, no subject with reduced or normal iron stores was incorrectly predicted as having absent iron stores. Relatively small subject numbers in the rheumatoid sub-group with reduced iron stores prevented clear discrimination from subjects with normal iron stores. Absorption curves did however tend to be less flattened in those with reduced iron stores. It was consequently not possible to accurately predict mild iron deficiency anaemia using the test. The two subjects with normal iron stores and concurrent anaemia, hence the anaemia of chronic disease, were predicted accurately based on a decrease in s-iron from pre-test values.

The results therefore show that none of the three methods proposed in differentiating IDA and ACD predict marrow iron stores accurately in all subjects. The low-dose iron absorption test was shown to be an efficient method of differentiating IDA and ACD, though based on the small subject numbers so far studied the test does not yet clearly predict mild iron deficiency.

4.0 DISCUSSION

This section follows the pattern of previous sections. Discussion relating to the three main areas of research; the prevalence survey, clinical trial and iron absorption test are dealt with individually, followed by an overall conclusion.

4.1 PREVALENCE SURVEY

4.1.1 Survey population

In undertaking any survey it is important that the sample population is representative of the total population, to ensure that findings are applicable to that population [178]. By selecting consecutive eligible patients presenting at hospital clinics with rheumatoid arthritis or iron deficiency it was hoped that the samples be random and representative of these populations as a whole. However, by recruiting hospital patients who perhaps tend to be more serious cases, or ones where the general practitioner has not been able to treat satisfactorily, it is possible that subjects were not truly representative.

One of the primary aims of the survey was to assess the prevalence of RLS in rheumatoid and iron deficient populations, and compare them with that of a healthy population. In order to make valid comparisons the control sample should be age and sex matched with the other populations.

The healthy sub-group included in the survey was matched in terms of sex ratio with both rheumatoid and iron deficient sub-groups. Age matching was not so satisfactory. The healthy sub-group was significantly younger than either the rheumatoid or iron deficient groups, though age ranges were infact similar. This was largely attributed to difficulty in finding 'healthy' elderly subjects. In an attempt to improve the age distribution, a day centre for the elderly (Fir Cones, Birmingham) was visited, but despite being well and mobile, most of the attenders were on medication for an underlying disease.

Interpretation of survey results was undertaken with these considerations in mind.

4.1.2 Prevalence and incidence of RLS

Both prevalence (number of subjects who had experienced RLS during the previous 12 months) and incidence (number of subjects who had had RLS during the month prior to interview) were determined for the three sub-groups studied. Prevalence figures allowed an assessment of the scale of the problem of RLS and comparison with a published study in rheumatoid population. By determining the incidence of RLS, comparison with most other published studies could be made, and also correlation with haematological parameters measured. In rheumatoid subjects it was also possible to assess the influence of disease activity and treatment.

4.1.2.1 Prevalence of RLS in healthy control group

The incidence of RLS among healthy individuals was low, affecting only 8.9% (± 6.3%). In a large study [2], Ekbom found the incidence to be 5.2% (26/500). The normal series from which this was derived composed hospital staff, friends and patients attending a surgical out-patient clinic with minor injuries or ailments.

The prevalence of RLS in the control group was 12.7% (±7.3%) and higher than the figure of 6% (4/70) for normal controls in a similar prevalence study [16].

4.1.2.2 Prevalence of RLS in rheumatoid arthritis

In contrast, the prevalence of RLS, or 'fidgets' as many refered to it, among hospital patients with rheumatoid arthritis was high at 36% (±7.6%) and significantly different from that amongst healthy controls.

This result is consistent with a similar, though smaller, prevalence survey in which 30% of hospital rheumatoid patients were found to have had RLS during the previous 12 months [16]. The prevalence of RLS in rheumatoid

arthritis remains, as far as is known, higher than that for any other disease state studied.

4.1.2.3 Prevalence of RLS in iron deficieny

Amongst patients referred to the hospital with iron deficiency the prevalence was also high affecting 25% (±14.2%). The incidence was found to be 19.4% (± 12.9%). Statistically, these figures were not significantly different from those of the healthy control population studied, but this was almost certainly due to the small sample size rather than there being no real difference.

In an equally small survey of subjects with serum iron concentration less than 10umol/l, Ekbom showed that 24% (12/49) had RLS [2]. No mention was made to the presence of anaemia or the cause of low s-iron, though iron deficiency was implied.

The survey therefore confirmed the high prevalence and incidence of RLS amongst subjects with rheumatoid arthritis and iron deficiency in comparison with a healthy population.

4.1.3 RLS symptoms, duration and frequency of attack

The survey demonstrated wide variation in RLS symptoms, whilst still conforming with diagnostic criteria. Only a small proportion of subjects in each sub-group experienced all the features that Ekbom defined as 'classical' (healthy 10%, rheumatoid 31.5%, iron deficiency 22%). Between one third and a half of subjects described a crawling sensation and 75 - 84% experienced the dysaesthesia between the knee and ankle. Phrases commonly used to describe the sensation included; 'crawling worms or insects', 'itching powder', 'running water'.

Ekbom identified a RLS sub-group where pain was a predominant feature and which was termed 'asthenia crurum dolorosa'[2]. Only 4/73 subjects of the survey population belonged to this category.

Ekbom stated that in most cases dysaesthesia was felt 'deep inside, in the muscles or bones'[14], and were rarely of a superficial nature. This was found to be the case in 70 - 100% of subjects, though some found the distinction difficult to make. Symptoms were bilateral in 63 - 86% of cases. There was no significant association between RLS and other leg problems, such as cramp, spontaneous jerks, or pins and needles.

Duration of attack ranged from a few minutes to several hours, duration and frequency tending to be greatest in the iron deficient sub-group. RLS symptoms amongst the healthy sub-group were in general milder, being less frequent and usually lasting less than an hour.

The main aggravating factor identified by 38% of subjects was that of a tiring day. Heat and cold had no consistent effect on symptoms. More than half of subjects in each sub-group did not associate RLS with any other specific factor.

Over 85% of RLS sufferers in each group found RLS either mildly or extremely irritating and, in over half of subjects, RLS interfered with sleep. Somewhat surprising, in view of this high level of inconvenience, was the low rate of referral to the medical profession, of only 12% overall. Many subjects were concerned about the response they were likely to get from doctors. Infact two-thirds of those who did venture to ask their doctor were given no explanation or treatment, supporting the notion that RLS is a poorly recognised condition amongst the medical profession.

4.1.4 Causal relationship between RLS, gender, age and family

4.1.4.1 Gender and RLS

In iron deficient and normal sub-groups there was no significant difference in the proportion of men and women with RLS compared with the sex ratio of each sub-group as a whole. In the rheumatoid sub-group the ratio of women to men with RLS was 12.5:1, which was significantly different to the 2.6:1

ratio for the sub-group as a whole. Thus among iron deficient and healthy individuals there was no predominance of either sex to developing RLS, while in the rheumatoid population RLS sufferers were almost five times more likely to be women.

Ekbom found no significant sex predominance, though severe cases were mostly found in women [2]. Other studies have found RLS more common in women [16,18], for example in one multi-centre G.P. study involving 174 patients with RLS, 70% were women [51]. The evidence therefore remains conflicting.

4.1.4.2 Age and RLS

RLS has been found to occur at any age [2], but predominantly affects the middle aged [51].

Among the total survey population the age of subjects with RLS ranged from 24 - 82 years. There was no statistically significant difference in age between those with RLS and those without, age distribution of those with RLS reflecting that of each sub-group. This was therefore perhaps at odds with previously published findings and may be due to small numbers of subjects, particularly in the iron deficient and healthy control group.

4.1.4.3 Hereditary factors and RLS

In this study 36% of subjects with RLS had a blood relative who also suffered with RLS, compared with only 10% of subjects without RLS. Thus there was a statistically significant association between family members and RLS.

Ekbom found that 32% (11/34) of subjects with severe RLS had near relatives with RLS, while there was a familial association in 63% (40/64) of mild cases [2]. In a study involving 5 generations of one family, RLS with myoclonus was found to be associated with autosomal dominant inheritance [22]. The current study therefore supports previous findings of a familial

association in a proportion of those with RLS.

4.1.5 Association between RLS and iron status

Prior to the start of this study previous workers had paid only cursory attention to the role of iron status as a possible influential factor in the pathogenesis of RLS.

Ekbom noted that 25% (19/77) of patients with RLS had decreased serum iron concentration [2], and it has also been observed that although RLS may occur in non-anaemic individuals, that those with anaemia experience worse symptoms [51]. In a primary medical text it is noted that the sudden onset of restless legs may be the first symptom of anaemia due to chronic blood loss [32].

Iron status was assessed in most survey subjects who had experienced RLS during the previous month and in those without RLS. In a proportion of healthy and rheumatoid subjects RLS was found to occur in the absence of either anaemia or haematological iron deficiency

In comparing haematological parameters, it was notable that for each sub-group mean values for haemaglobin, mean cell volume and haemoglobin, serum iron concentration and transferrin saturation were lower in those with RLS. In healthy and iron deficient sub-groups where subject numbers were low, statistical significance was not attained. Amongst rheumatoid subjects however, haemaglobin, mean cell haemoglobin, s-iron, transferrin saturation and s-ferritin were significantly lower.

The incidence of anaemia or iron deficiency within each sub-group was considered. None of the healthy individuals with RLS were anaemic or microcytic, though 2/7 had s-iron and transferrin saturation suggestive of iron deficiency.

All subjects within the iron deficient sub-group were anaemic. There was no significant difference in the severity of iron deficiency in those with or

without RLS when individual parameters were compared.

Investigation of the rheumatoid sub-group showed that there was no significant difference in numbers, with or without RLS, who were anaemic. Based on s-ferritin values there was also no significant difference in numbers of subjects who had definite iron deficiency with or without anaemia or those with the anaemia of chronic disease. Assuming a lower limit of normal for s-ferritin of 55ug/l and, where available, bone marrow assessment of iron stores to help distinguish those with probable iron deficiency, it was found that there was a significant association between RLS and iron deficiency. The overall incidence of iron deficiency in the rheumatoid sub-group was 53% (63/118) which is in keeping with literature values ranging from 30 - 70% [65,66,67,68,69].

Haematological parameters in this sub-group were considered in respect of frequency and duration of RLS attacks. Duration of attacks were found to be independent of iron status. Frequency of attack on the other hand was associated with significantly different s-iron, transferrin saturation and total iron binding capacity. Low ranking of s-iron and transferrin saturation, and high ranking of TIBC were associated with nightly attacks, while high ranking of s-iron and saturation and low ranking TIBC correlated with attacks occurring less than once a week.

4.1.6 Association of RLS and rheumatoid disease activity/treatment

There was no significant correlation between RLS and rheumatoid disease serology, duration or laboratory and clinical indicators of disease activity. This suggests that the high incidence of RLS in RA is unrelated to the underlying joint disease. Significantly more subjects with RLS were also taking disease modifying drugs for rheumatoid arthritis. No single agent was implicated however, although the exact mechanism of action of these drugs is not known, they are all thought to act as immunosuppressants [179].

In summary, RLS may occur in subjects with no obvious indication of iron deficiency. In conditions of iron deficiency and rheumatoid arthritis, where iron deficiency is a common extra-articular feature, the incidence of RLS is high. Investigation of a rheumatoid population suggests that there is an association between RLS and iron deficiency rather than altered iron status due to ACD. A combined effect can not, however, be ruled out. Frequency of attack was found to correlate with total iron binding capacity and, inversely with s-iron and transferrin saturation. Although these findings suggest a causal relationship between RLS and haematological iron deficiency this might only be a reflection of brain or tissue levels of iron. Investigation of such levels may reveal a more specific association between iron and restless legs syndrome.

4.2 TRIAL OF IRON IN THE TREATMENT OF RLS

The trial of iron in RLS was undertaken to assess in a properly controlled fashion the anecdotal observation of iron being of benefit in the treatment of RLS, in both iron deficient and non-iron deficient individuals. In the absence of an acknowledged standard treatment, iron was compared with placebo. Prior to the commencement of this study there had only been four published controlled studies of treatment in RLS.

4.2.1 Trial population

The trial group was mixed; 13 subjects with rheumatoid arthritis, one with osteoarthritis and one otherwise healthy individual. The recruitment drive focused primarily on the rheumatoid group since this population has the highest reported incidence of RLS. The main source of subjects was the prevalence survey being undertaken concurrently.

Recruitment of such a heterogenous trial population required seperate analysis of results since the effect of rheumatoid disease on outcome was not known.

4.2.2 Trial size

In undertaking any trial it is important that it is of adequate size [180,181]. A trial with only a small number of subjects carries considerable risk of producing a false negative result (or large Type II error). The feasibility of recruiting sufficient subjects for this study was therfore assessed prior to its commencement.

A variety of methods for calculating the number of subjects required have been described [182,183]. The method chosen was based on a Chi-square test and final outcome of success or failure[178]. In order to perform the calculation it was necessary to estimate the likely success rate on placebo (p_1) and decide on the rate of success on iron that one wished to detect as being different from placebo (p_2). In view of the considerable 'placebo effect' reported by previous studies [154], the estimated success rate was

set high at 25%. For iron to provide a useful treatment choice for RLS it was felt that it should at least halve the failure rate of placebo. It was also necessary to set acceptable limits on the risk of getting a false positive or false negative result, known respectively as type I and type II error. Type I error represents the significance level of the Chi-square test used and is conventionally set at 0.05. Type II error is the probability of not detecting a significant difference when there really is a difference of magnitude $p_1 - p_2$ and, is generally in the range 0.05 to 0.2. Choice of all of these values is clearly somewhat arbitrary but must be based on clinically relevant goals.

The required number of subjects on each treatment (n) is given by the following formula:

$$n = \frac{p_1 \times (100 - p_1) + p_2 \times (100 - p_2)}{(p_2 - p_1)^2} \times f(\text{Type I, Type II error})$$

where p1 = 25%
p2 = 65%
Type I error = 0.05
Type II error = 0.1
f(Type I, Type II error) = 10.6 [Table, 178]

Hence the number of subjects required on each treatment was calculated to be a minimum of 28.

Having calculated the number of subjects required it was then necessary to assess the likely accrual rate. The average annual number of patients with rheumatoid arthritis attending clinics at the base hospital was estimated to be approximately 500. Based on the previous reported prevalence of RLS in RA (30%) [16], and clinical impression (DRB) that approximately one third have classical symptoms, it was estimated that the required number of eligible subjects could be recruited in the time available using this population alone.

The actual recruitment of trial subjects (13 rheumatoid, 2 non-rheumatoid) was therefore unexpected and disappointingly low. Some of the main reasons

for this are highlighted by referring to the breakdown of RLS symptoms amongst the rheumatoid prevalence sub-group shown in Figure 4.1

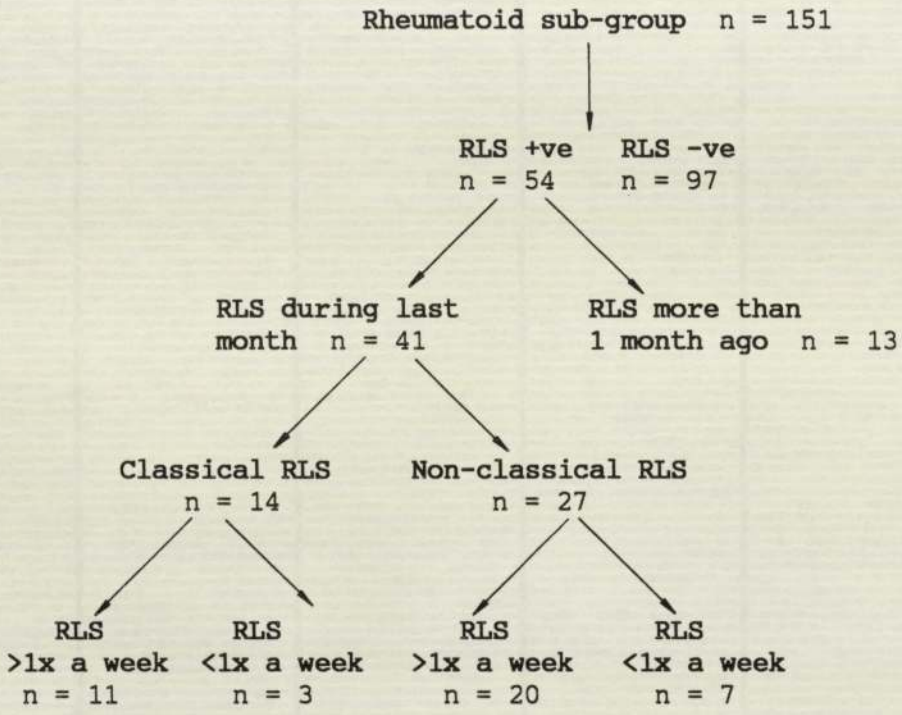


FIGURE 4.1: Breakdown of RLS symptoms in rheumatoid prevalence sub-group

Firstly, the estimated number of patients with definite RA seen annually was possibly an over-estimate since many of those with chronic progressive disease attended clinics more frequently than anticipated. During the 18 month period in which the prevalence survey was conducted, clinics were attended once or twice each week and still only 151 rheumatoid subjects were interviewed.

Secondly, strict entry criteria restricted recruitment to those with classical symptoms occurring at least once a week. While 26% of rheumatoid subjects with RLS had classical symptoms, which did not differ too greatly from the assumed incidence, fewer experienced RLS at least once a week.

The low numbers of non-rheumatoid subjects is partly a reflection of the low incidence of RLS in healthy individuals and the fact that symptoms tend to be mild. Amongst the seven healthy individuals with RLS identified

during the prevalence survey, one had classical symptoms, but again less frequently than the trial protocol required.

While limiting recruitment numbers, such strict entry criteria were thought essential in view of the lack of clear diagnostic criteria and severity of RLS symptoms detailed in previous studies. It was hoped that this would aid comparison with any other studies published in the future.

Despite the small number of subjects included in the trial, recruitment was nevertheless comparable with most other controlled studies (See Table 4.1). There have only been two much more extensive trials, both of which were large multicentre studies [51, 61], and one of these included subjects with a variety of leg symptoms [61].

4.2.3 Trial design

Selection of a suitable design for the trial presented a number of difficulties. The initial between-patient design, in which each patient was randomised to one treatment only, was chosen for a number of reasons. Such a design makes no assumptions as to whether treatment is likely to be symptomatic or curative. It provided a method of making the study double-blind thereby reducing investigator bias. This design was also thought to be appropriate in terms based on estimated recruitment figures and numbers required to detect a significant outcome. The single blind placebo run-in phase was added because of concern about placebo effect and due to the subjective nature of the condition and methods of evaluating response.

The main problem with use of between-patient design for a trial of treatment in RLS is the large inter-patient variability in symptoms and severity, and comparatively large number of subjects required.

Six months into the trial the design was modified to a within-patient cross-over design, a widely used method in other RLS trials (see Table

4.1). Whilst an interim change in design not common practice, a crossover design offered several advantages, the main one being that each patient could act as his own control allowing more precise comparison of the two treatments. This approach also halved the number of subjects required. By retaining the placebo run-in period the method ensured that in the event of complete remission occurring whilst on iron, the same patient would also have previously been on placebo, thus reducing the likelyhood of the response being due to spontaneous remission. Amendment of design had the disadvantage of extending the total trial duration from four to five months and complicating trial analysis.

4.2.4 Efficacy of iron in treatment of RLS

Ten rheumatoid subjects were randomised to iron; 90% experienced complete remission or greater than 25% reduction in frequency of symptoms, and 60% graded symptoms as 'cured' or 'much better'. Reduction in mean number of attacks was statistically significant compared with the control phase. Iron did not affect duration of attack, suggesting an all or nothing effect.

In those who experienced complete remission in symptoms, the response was rapid, occurring within one week of starting iron treatment. This is consistent with the 'rapid' rate of response mentioned in earlier anecdotal reports [18, 58]. Norlander observed complete recovery within a day of one to three iron injections. In an unpublished open study also in rheumatoid patients, workers found symptoms relieved within 24 hours following intramuscular injection of iron dextran in one patient, and within seven days following oral ferrous sulphate (200mg t.d.s.) in four further patients [62]. Such a rapid rate of response suggests a non-haematological mechanism of action. Those in whom symptoms were alleviated, remained symptom free for one to five months after discontinuation of oral iron.

Oral iron failed to have a significant effect on either frequency or

duration of symptoms in non-rheumatoid subjects. In view of the size of the sub-group, which was obviously very limited, no firm conclusions can be made.

4.2.5 Influence of iron status on response of RLS to oral iron

All rheumatoid subjects who completed the trial had evidence of iron deficiency, either with or without concurrent anaemia. This is consistent with the observation that increased severity of RLS is associated with iron deficiency [18], and greater tendency towards iron deficiency in those with frequent attacks as seen in the prevalence survey.

All three subjects who responded dramatically had s-ferritin less than 20 ug/l prior to treatment, a clear indication of reduced iron stores. Although iron therapy produced some changes in iron indices and haemoglobin, response to iron was not associated with correction of iron deficiency. This was obviously unlikely to occur in the subject who discontinued treatment after only seven days.

In the non-rheumatoid subjects it was notable that iron had a negligible effect and that neither of these individuals were iron deficient. Though the sample was small, this contrasts with previously reported findings of success in treating RLS with iron even in those who were not known to be iron deficient [18].

4.2.6 Assessment of the 'placebo effect'

The 'placebo effect' may be defined as 'the change in the patient's condition that is attributable to the symbolic import of the healing intervention, rather than to specific pharmacological or physiological effects' [184]. It has been suggested that as many as 30 - 40% of patients can be effectively treated by placebo [184]. Placebos may not only be effective in subjective conditions but also in those monitored with objective measurements such as hypertension [178,185], angina pectoris [186], peptic ulcer disease [187] and diabetes mellitus [184]. Factors

affecting the 'placebo effect' include the beliefs and expectations of both patient and physician [188], and the doctor-patient relationship [178]. By including a placebo in a randomised trial one is able to help eliminate placebo effect from the therapeutic comparison. During the course of the current trial subjects were seen at the most clinic visits by both the investigator and supervising consultant, thus reducing the variation in doctor influence.

When employing placebos in a trial they should ideally be identical in all respects to the active preparation, except that the active drug is absent. Those used in this study were identical in colour, texture, shape and size. There was one short coming, that of the characteristic of iron to stain faeces black. The possibility of using a poorly absorbed ferric salt as placebo was briefly considered. This was however discounted since potential reduction to the ferrous salt could not be excluded and the fact that the capacity for absorption is increased in iron deficiency.

All trial subjects received placebo during the trial. No individual achieved complete remission whilst on placebo medication. Four out of twelve rheumatoid subjects experienced a greater than 25% reduction in frequency of attacks and one graded symptoms as 'much better'. Placebo was therfore associated in some subjects with a small improvement in symptoms confirming that the 'placebo effect' requires consideration in such a subjective condition as RLS.

4.2.7 Patient compliance

Compliance with prescribed medication was assessed during the trial by tablet count. This is a frequently used method [189], being relatively quick, simple and having been shown to correlate with other methods of measuring compliance [190]. It should be recognised that removal of doses does not automatically guarrentee correct administration.

Alternative methods of assessing compliance include measurement of drug and metabolite levels in blood or urine [189]. However, since iron is a physiological element this was not feasible. Another alternative is incorporation of tracer or marker substances into the dosage form, such as isonicotinic acid, isoniazid [191], riboflavin [192], or sodium bromide [190]. This approach was not pursued due to its associated formulation problems and potential toxicity.

Based on tablet count, compliance amongst trial subjects was extremely good, with a mean figure of 90%, ranging from 69 -105%. This is comparable with compliance during a clinical trial of aspirin and naproxen (90%) [193], and study of different theophylline preparations [194]. It was better overall than the generally recognised fact that 30% of patients in most studies fail to follow advice [195]. Over compliance in one subject demonstrated the possible disadvantage in supplying patients with an overage.

4.2.8 Side effects to trial medication

The two main adverse effects of trial medication were minor gastrointestinal discomfort and constipation. These are recognised as being associated with both oral iron and placebo tablets [153]. Of note, however, was the apparent flare-up in rheumatoid disease reported by two of the ten rheumatoid subjects, coinciding with administration of iron.

Iron dextran injections have been observed to exacerbate joint symptoms in rheumatoid patients, generally occurring after a delay of approximately 24 hours and lasting for about seven days [196,197,198,199]. Lack of published reports of similar exacerbations with other iron preparations led to the suggestion that dextran, rather than iron might be responsible for joint symptoms. A mechanism involving induction of reticuloendothelial cell blockade leading to immune complex synovitis has been suggested [200]. However, in a recent study, synovial flare following total-dose iron

dextran infusion was closley monitored and findings suggested a mechanism involving iron-promoted oxidant stress [199].

Only one case was located in the literature of oral iron being associated with an exacerbation of joint symptoms. This involved a woman with mild erosive rheumatoid arthritis in whom ingestion of ferrous sulphate (200mg t.d.s.) was associated on the third day with a clinical flare-up of arthritis [201]. This was characterised by an increase in articular index, morning stiffness, subjective pain score and decreased grip strength. Similar changes in articular index and grip strength were observed in subject A. Also, as in the documented case, values of erythrocyte sedimentation rate were not significantly altered. In view of the lack of similar reports, this type of reaction to oral iron would appear to be rare. The hypothesis of iron causing free radical formation as a potential mechanism in inflammatory joint disease may be relevant here [136].

4.2.9 Controlled trials in the treatment of RLS

There remain less than a dozen published controlled studies evaluating drug therapy for the treatment of RLS. See Table 4.1.

During the last few years clinical investigation has concentrated on further assessment of levodopa. Combined results of three double-blind crossover placebo controlled trials reported levodopa to provide significant improvement in symptoms in 36/39 patients studied [45, 202, 203]. Additional evidence of benefit in 13/15 patients is also provided by two recent open studies [205, 206]. Doses employed ranged from 50 - 200mg taken at night, usually as a single dose. In some patients relief is short-lived requiring a second dose to be taken during the night [203].

There has been further interest in the role of narcotic analgesics for RLS, though as yet unconfirmed by controlled studies. Sandyk et al. reported on six patients in whom oral narcotic analgesics (codeine and oxycodone)

Drug	No. of subjects	Daily dose	Trial design	Ref.
Pre - Nov 1985:				
Carbamazepine	6	200 - 600mg	Crossover	50
Carbamazepine	174*	100 - 300mg	Parallel	51
Clonazepam	6	1mg o.n.	Crossover	54
Oxerutins	596*	250mg q.d.s.	Parallel	61
Post - Nov 1985:				
Clonazepam	6	0.5mg q.d.s.	Crossover	55
Madopar^{R}	20	50 - 100mg o.n.	Crossover	45
Madopar^{R}	13	200mg o.n.	Crossover	202
Madopar^{R}	6	100mg o.n.	Crossover	203
Clonidine	20	0.075mg b.d.	Parallel	204

* = multi-centre studies
Madopar^{R} = levodopa combined with benserazide a peripheral decarboxylase inhibitor
Crossover = each patient receives placebo and active drug in random order
Parallel = each patient randomised to placebo or active drug

TABLE 4.1: Published placebo controlled trials of treatment for RLS

alone, or in combination with a benzodiazepine brought long-lasting relief [207]. Another recent controlled trial showed benefit of low dose clonidine [204], previously only suggested by an anecdotal reports [49,208].

Evaluation of treatment response has for the most part relied on subjective patient assessment of dysaesthesic symptoms. This was also the case in the current trial, though quantitative information relating to the frequency and duration of attacks was collected in an attempt to make it less subjective. Recent studies have, however, sought to find objective methods to complement patient assessments. Objectivity has arisen from the discovery that dysaesthesic symptoms of RLS are associated with involuntary movements. Whilst awake approximately 50% [209] of patients with RLS

experience "dyskinesias while awake" [22, 210]. These are discrete stereotypic leg movements which may be myoclonic (ie. <0.25sec). The presence of involuntary movements in subjects with RLS was briefly assessed as part of the prevalence survey. Only 6/47 rheumatoid and 1/7 healthy subjects with RLS claimed to experience this feature, while none of the seven iron deficient subjects were affected. During the night RLS has been associated with periodic movements during sleep (PMS) [7, 211] in about 80% of patients [209]. PMS is a motor parasomnia which occurs during stages 1 and 2 of rapid eye movement sleep. It is usually asymptomatic, though it may lead to insomnia or day-time drowsiness [30]. PMS involves dorsiflexion of the foot with flexion of the leg at the knee and hip lasting 0.5 to 6 seconds, occurring every 20 to 40 seconds [203]. It has been suggested that RLS and PMS may be two clinical manifestations of the same CNS dysfunction [7].

PMS and "dyskinesias while awake" may be readily quantified by polysomnography, thus providing an indirect objective assessment of drugs in RLS [209]. Techniques used include electromyography (EMG) from anterior tibialis muscles [206], videotape monitoring and electroencephalography (EEG) to quantify time spent in the various stages of sleep. In a recent study [203] workers used a suggested immobilisation test (SIT). This required patients to sit motionless on a bed, with legs outstretched and eyes open, for 30 minutes. Each time they experienced dysaesthesia or an urge to move the legs they pressed a button. At the same time EMG recordings were made to indicate leg movements. The test provides the first objective method to quantify RLS, but needs refinement since not all patients experience movements during the test. So far only a few studies have used these methods but they may provide a useful solution to reliance on totally subjective assessments.

All the drugs so far evaluated in clinical trials require treatment to be taken regularly, since they only provide symptomatic relief. The current

trial is the only one known to have assessed oral iron in RLS. It has an advantage over other trial drugs in that remission was found to persist after iron was discontinued. Though other trial medications were apparently well tolerated in most patients, in general, side-effect profiles of these drugs are more serious than that of iron supplementation. The use of narcotic analgesics is unlikely to become first choice in RLS due to dependency with chronic administration.

4.2.10 Possible role of iron in the pathophysiology of RLS

This study has confirmed the association of iron deficiency with RLS, which was found to be a predisposing factor, rather than the primary cause. Oral iron was effective in relieving symptoms in most subjects, in some cases resulting in complete remission. The rate of response and iron indices of those so affected suggest a non-haematological mechanism of action.

Current hypotheses regarding the aetiology of RLS, and related PMS and akathisia continue to concentrate on a central rather than peripheral origin for both sensory and motor pathology. Response of RLS to agents known to affect neurotransmitters has lead to speculation that the condition may be due to under-activity of GABA-ergic, serotonergic, dopaminergic or endogenous opiate systems, or over-activity of the adrenergic system [209].

There is mounting evidence of involvement of the dopamine systems, in view of the relatively high incidence of RLS in Parkinson's disease [14], the deleterious effect of dopamine antagonists [14,44], the effectiveness of levodopa [45,202,203,205,206] and association of RLS with neuroleptic-induced akathisia [38,39]. The distribution of non-haem iron in the brain of the rat and human has been shown to parallel that of dopamine [202]. Iron is also necessary for tyrosine hydroxylase, the rate-limiting enzyme, in the synthesis of dopamine [89] and is an integral part of the dopamine D_2 receptor [41]. In rats with iron deficiency there is a decrease

in formation or change in response of post-synaptic D_2 receptors. The susceptibility of patients with iron deficiency to develop RLS may therefore be related to disturbance of dopamine formation and dopamine receptor dysfunction [202]. Akathisia has also been associated with lowered serum iron [40], though discussion on the relevance of this to dopamine pathways and the pathophysiology of akathisia has been conflicting [212,213,214].

The reported effectiveness of narcotic analgesics in RLS and antagonistic effect of naloxone [46] support the hypothesis that the endogenous opiate system may be involved. Divalent metal ions, eg. Fe^{2+}, have also been shown to be associated with opiate receptor sites. It has been suggested that the effects of iron deficiency on receptors, here too, might be involved in the pathophysiology of RLS [215]. Interaction between dopamine and opiate systems have been shown to occur; acute administration of opioids causing an increase in release of striatal dopamine, while neuroleptics have been shown to produce supersensitivity of striatal opioid receptors. It is therefore possible that administration of narcotic analgesics may ameliorate RLS by enhancing the activity of the dopaminergic system in the striatum and nigrostriatal neurones [207].

In determining the pathophysiology of RLS it is necessary for the mechanism to account for the nocturnal nature of symptoms and temporary relief on walking or moving the legs. If iron is indeed shown to be intimately involved in the pathophysiology of RLS, then the evening trough in serum iron level may be relevant. The irresistible need to move the legs and relief on movement may to achieve a higher level of dopamine function [202]. However, in the absence of any definite proof, the pathophysiology of RLS remains speculative.

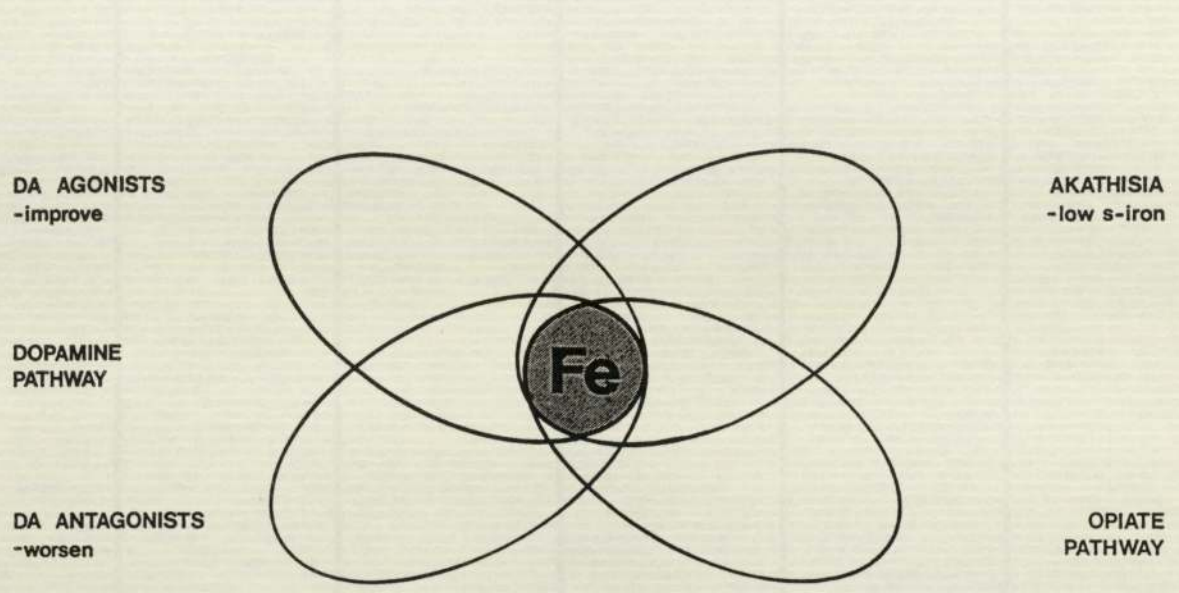


FIGURE 4.2: Possible role of iron in the pathophysiology of RLS

4.2.11 Recommendations for management and further investigation of RLS

Findings from the prevalence study and clinical trial together with the recent literature allow a number of recommendations to be made with regard to diagnosis and treatment, and further investigation of RLS.

Diagnosis and treatment of RLS

Diagnosis of RLS should ideally be preceded by needle EMG and nerve conduction studies to exclude peripheral neuropathy. These procedures should perhaps be repeated at night, since this is when symptoms usually occur. Drug-induced causes of RLS should be excluded. These include dopamine antagonists, caffeine and barbiturate withdrawal. Iron status should be assessed, firstly since RLS may be an early sign of iron deficiency requiring appropriate investigation and treatment, and secondly, because trial results suggest that administration of oral iron supplements in iron deficient subjects may relieve RLS. Based on the current trial,

administration of iron to non-iron deficient individuals can not be recommended though, in the absence of iron overload, is unlikely to be detrimental. In the absence of iron deficiency, levodopa is currently considered to be the treatment of choice. This should be administered as Madopar^{R} or Sinemet^{R}, starting at 50mg as a single night-time dose. Other alternatives are clonazepam, carbamazepine, clonidine or codeine.

Further investigation of treatment and pathogenesis of RLS

Establishing a recognised objective method of assessing RLS is essential for standardisation of further epidemiological studies and clinical trials of drug therapy for this condition. Current methods rely heavily on the association of RLS with PMS and "dyskinesias while awake" which have not been found to occur in all subjects and have not been shown conclusively to have the same aetiology as RLS. Further experimentation with the suggested immobilisation test is required to determine the ideal duration, patient position and environmental conditions to increase the sensitivity of the test.

Clinical trials have generally involved small subject numbers. Since RLS is a comparatively rare condition, further studies should take the form of well organised multi-centre trials with involvement of local general practitioners, who are more likely to learn about RLS symptoms from their patients than hospital specialists. In particular, the findings of the current pilot trial should be followed up by a large scale study looking at different patient populations with or without concurrent iron deficiency. A parallel trial design should be considered, providing treatment groups are closely matched for RLS severity, since iron may alter the underlying disease condition, thus making crossover comparison difficult to interpret. In view of the rapid response demonstrated, the treatment period could usefully be reduced from four weeks to two.

Further verification of the role of neurotransmitters and iron-dependent

enzymes in RLS and associated PMS, dyskinesias while awake and neuroleptic-induced akathisia is needed. Methods including cerebrospinal fluid studies and positron emission tomography using receptor ligands may be useful [209].

4.3 A LOW-DOSE IRON ABSORPTION TEST

In 1984 a paper by Crosby et al [110] reported on an iron tolerance test in which a physiological dose of non-radioactive iron was successfully used to differentiate mild iron deficiency from iron repletion in otherwise healthy volunteers. Intrigue in the apparent sensitivity of the test, prompted the current investigation of the method, in particular its application to two of the dilemmas of iron metabolism in rheumatoid disease. The test was employed as a means of assessing iron absorption in rheumatoid arthritis and as a possible method of differentiating iron deficiency anaemia and the anaemia of chronic disease.

4.3.1 Differentiation of uncomplicated iron deficiency from iron repletion

Using a test dose of 10mg elemental iron, the method was initially performed in subjects with uncomplicated iron deficiency and healthy iron replete volunteers. In accordance with Crosby's findings iron absorption curves were distinctly different for these two groups. Serum iron measurements were taken over a 4 hour period, values in iron deficient subjects showing an impressive rise after one hour. The maximum increase occurred earlier than in Crosby's study, which reported peak levels between two and three hours post-dose. This disparity may be related to dosage form used. The current study used an aqueous solution of ferrous sulphate whereas the previous study used powdered ferrous sulphate or fumarate. Absorption of the powder may therefore have been delayed due to dissolution time.

The iron deficient subjects studied included three in whom deficiency had progressed to anaemia, the other three being merely iron deficient. There was no apparent difference in absorption curves between those with anaemia and those without. This contrasts with the original study [110] in which serum iron returned toward the initial level more quickly in those with iron deficiency anaemia than those who were iron deficient but not anaemic.

Similar differences were also reported in earlier studies using large doses of iron, the rise in serum iron being greatest in subjects with iron deficiency but no anaemia [79]. In both instances it has been suggested that differences were due to the slower rate of clearance of serum iron in non-anaemic individuals, compared with anaemic subjects, where iron is rapidly removed from the plasma for erythropoesis. Small subject numbers in the iron deficient sub-group studied may have contributed to this not being detected.

4.3.2 A simplified low-dose iron absorption test

Maximum discrimination between iron deficient and iron replete individuals occurred at 1 hour after the test dose. A rise in serum iron greater than 50% at this time was highly selective for iron deficiency. This suggests that the test may be simplified to just one sample at time 0 and another one hour later. In uncomplicated iron deficiency this simplified test provides an alternative measure of iron deficiency to serum ferritin. This may be of benefit particularly in centres or poorer countries that are unable to afford assay kits and necessary equipment for ferritin determination. At the time of this research the cost of assay materials, excluding equipment or personnel was £1.60 per ferritin measurement, compared with only 3-4p for serum iron determination.

4.3.3 Value of the low-dose iron absorption test.

The current study has demonstrated that the low-dose iron absorption test was sufficiently sensitive to detect iron deficiency when other haematological indices, such as total iron binding capacity, mean cell volume and haemoglobin concentration were still normal.

Earlier criticism of iron absorption tests which employed large doses of elemental iron does not therefore appear to be applicable to low-dose methods. The major assumption with this type of absorption test is that the post-administration rise in serum iron is a reflection of the total

quantity of iron absorbed. Critics of the method have pointed out that other factors such as the rate of absorption, the pre-administration serum iron concentration, total iron binding capacity and rate of clearance from plasma by the bone marrow and body tissues are also likely to contribute to the height and shape of iron absorption curves. Whilst this is undoubtedly true, results show that absorption of iron and subsequent change in serum iron are primarily a reflection of iron status. Recently other workers have also shown the method to be a powerful tool for investigating the physiological differences in body iron stores of normal subjects [216].

4.3.4 Further investigation of the low-dose iron absorption test

The results based on the relatively small number of subjects studied are encouraging. For wide acceptance of such a method further large scale studies are required. In particular, the value of the simplified test should be investigated more thoroughly in a prospective study to determine the true sensitivity of the 50% change in serum iron as a diagnostic indicator of iron deficiency.

The low-dose method currently provides a qualitative assessment of iron absorption. For the method to be approved as a means of assessing extent of iron absorption it should be done in parallel with the whole body counting method, widely regarded as the method of choice for measuring iron absorption [79]. This technique involves administration of a radio-labelled test dose (^{59}Fe) and measurement of whole body radioactivity one to five hours later to determine the total dose of radioactivity. Iron retention is assessed by a further count approximately 14 days later, when all unabsorbed radioactivity has been excreted. The final count is then corrected for radioactive decay. By assessing the correlation of the two tests it may be possible to develop the low-dose test as a semi-quantitative non-radioactive method of determining absorption characteristics of new iron compounds and formulations and also dietary products. This would have obvious advantages in subsequently being able to

avoid exposure to radioisotopes and the need for expensive isotope counting equipment. A further application requiring investigation is that of malabsorption. The test may provide a simple method of testing this in subjects failing to respond to oral iron supplements.

4.3.5 Effect of active rheumatoid arthritis on iron absorption

As far as is known this is the first time the low-dose iron absorption test has been examined in subjects with a medical condition other than uncomplicated iron deficiency. The method was used firstly as a qualitative means of assessing iron absorption in rheumatoid arthritis. This is clinically relevant since it has been suggested that impaired intestinal absorption may be one of the mechanisms involved in the pathogenesis of the anaemia of chronic disease [111] and may then influence response to iron supplements in subjects with concurrent iron deficiency anaemia.

As in those free from inflammatory disease, iron absorption curves were distinctly different in rheumatoid subjects with normal or depleted bone marrow iron stores from those with absent iron. Absorption curves were similar for rheumatoid and non-rheumatoid subjects with definite iron deficiency and comparable pre-test s-iron and s-ferritin values. At 1 hour post-dose, identified as the time when maximum differences between sub-groups occurred, rises in s-iron were not significantly different. In contrast iron absorption in almost half the rheumatoid subjects with normal or increased iron stores was depressed compared with curves of iron replete volunteers. In the former subjects, s-iron values were found to steadily decrease throughout the test. Failure of s-iron values to increase did not correlate significantly with ESR, as a measure of disease activity. It was however noted that s-ferritin values in these subjects were high ranging from 172 to 953ug/l, despite concurrent hypoferraemia.

Results therefore suggest that iron absorption is depressed to a certain extent in subjects with normal iron stores, but in those with concurrent

genuine iron deficiency, absorption is increased to the same extent as in uncomplicated iron deficiency.

Various authors have published studies on iron absorption in rheumatoid arthritis, with conflicting findings. Early studies [111,113,114,217] were based on radioiron balance, a technique which involves oral administration of a measured quantity of ^{59}Fe and collection of all faeces over the following 7 to 10 days. Since there is no appreciable excretion of labelled iron, either in the urine or gastrointestinal tract, the iron absorption may be calculated by subtracting the amount recovered in faeces from the dose administered. The precision of this method has however been frequently questioned due to errors incurred from incomplete faecal collections and the fact that the radioiron absorption is often very low, thus magnifying any error in counting [79]. When making comparisons with normal and iron deficient controls, authors of these early studies did not distinguish between rheumatoid subjects with normal or depleted iron stores. This makes comparison with the current study particularly difficult. Three of these studies [111,113,217] concluded that iron absorption was reduced in RA when compared with normal iron replete controls and subjects with uncomplicated iron deficiency. One study [114], however, found no evidence of impaired absorption when compared with equally immobile controls.

Radioiron balance has largely been replaced by more accurate whole body counting as a means of assessing iron absorption. Boddy and Will [118] compared absorption in 15 rheumatoid subjects with normal controls and found no significant difference in iron absorption between RA patients with normochromic, normocytic anaemia and the control group. When compared with iron deficient controls, rheumatoid patients with hypochromic anaemia had increased absorption but not to the same extent as those with iron deficiency anaemia not suffering from RA. Weber et al [117] also found absorption in nine rheumatoid subjects with iron deficiency to be reduced

compared with six with uncomplicated iron deficiency. In a recent sophisticated study the method used distinguished between 'mucosal uptake', 'mucosal transfer' and total retention [218]. In patients with RA and normal bone marrow iron stores, uptake did not differ significantly from normal controls, whilst mucosal transfer was lower. Conversely, iron deficient rheumatoid subjects had lower uptake than in subjects with uncomplicated iron deficiency but similar mucosal transfer. Iron retention was decreased in rheumatoid subjects with and without stainable bone marrow iron, compared with normal and iron deficient controls.

The findings of the low-dose absorption test are most closely in agreement with a recent study by Benn et al [219] in which iron absorption was decreased in all 11 patients with normal iron stores and maximally increased in RA subjects with iron deficiency anaemia, to a similar extent as in uncomplicated iron deficiency. These results and the current research therefore suggest that in rheumatoid subjects with iron deficiency anaemia, administration of oral iron will correct anaemia as far as iron deficiency is the cause.

The decline in serum iron throughout the test in some rheumatoid subjects investigated may be indicative of reduced iron absorption. Alternatively it may be a reflection of the hypoferraemic state and associated sequestration of iron by tissue macrophages and the synovial membrane, that occurs in inflammation [134]. Similar iron with-holding mechanisms occur in neoplastic disease and infection [220]. Even a single period of fever may cause a rapid decline in serum iron and increased serum ferritin, starting during the incubation phase [221]. It has been suggested that hypoferraemia may be an attempt to deprive potential pathogens of an essential growth factor [80].

Although the current research supports some published findings, further investigation using larger groups of subjects is required, taking care to

match groups in terms of disease activity and iron status.

4.3.6 Differentiation of IDA and ACD in rheumatoid arthritis using the low-dose iron absorption test

The other application of the low-dose iron absorption test that was assessed was as a means of distinguishing IDA and ACD. This currently poses a sizeable problem since almost all rheumatoid subjects with active disease have ACD [219] and up to 70% [69] may also have concurrent IDA. The distinction is important clinically to avoid overlooking potentially curable sources of bleeding and unnecessary use of iron supplements which, as was demonstrated in the clinical trial (see Section 3.2.7), may aggravate joint symptoms. Bone marrow aspiration and assessment of reticuloendothelial iron stores is currently the only accurate way of making this differentiation.

Iron absorption using the low-dose test was assessed in 25 rheumatoid subjects categorised according to bone marrow iron stores. Significant differences were seen in the four hour absorption curves between rheumatoid subjects with absent and normal or slightly reduced iron stores. Of more importance practically, it was found that distinction between those with absent iron stores and the others could be made based on just two blood samples, one at time 0 and another at 1 hour post-dose. Iron stores in eight out of nine subjects were correctly predicted as being absent, based on a 50% increase in serum iron. Using this limit all the other subjects were correctly defined as having normal or slightly reduced iron stores. It was not possible, using the results so far collected, to accurately differentiate between those with reduced and normal iron stores. It should however be pointed out that even using bone marrow aspiration, this distinction is often difficult to make and prone to subjective interpretation by the operator.

Of the 25 rheumatoid subjects studied, fourteen were in fact anaemic. Based on changes in serum iron at 1 hour, the type of anaemia was correctly

diagnosed in 57% of subjects. This rather low figure was a result of the poor differentiation between subjects with slightly reduced and normal iron stores. 88% of subjects with definite iron deficiency anaemia or ACD were, however, predicted correctly. The predictive value of other parameters suggested as being useful in differentiating IDA and ACD was also assessed. It was interesting to note that all rheumatoid subjects with absent iron stores had serum ferritin values less than 20ug/l. A serum ferritin value of less than 55ug/l had a predictive value of 80%, but like the iron absorption test did not always differentiate between reduced and normal iron stores.

The search by other workers for an alternative means of overcoming this diagnostic dilemma has continued, primarily concentrating on haematological parameters. In one study a nomogram of serum ferritin with ESR was suggested as an accurate predictor of iron deficiency, but findings were not confirmed in a subsequent study [223]. The same workers also compared blood values in 79 rheumatoid subjects divided on the basis of marrow iron stores. Iron status failed to be predicted accurately by red blood cell indices or serum iron. In an earlier study Hansen et al showed in patients with anaemia and rheumatoid arthritis, a serum ferritin below 60ug/l to be indicative of iron deficiency [139]. In a more recent study they investigated the response to iron in relation to pre-treatment serum ferritin [222] and found that in 87% of subjects with a ferritin value less than 60ug/l haemoglobin increased. In another recent study, red cell ferritin was assessed but found to have no value in predicting response to iron, and hence iron status [224]. The evidence regarding the value of these tests in differentiating IDA and ACD remains conflicting.

The results of the low-dose iron absorption test as a means of differentiating IDA and ACD are encouraging, but the true potential of the test needs clarification. The method is simple to perform, well tolerated

and inexpensive. Compared with bone marrow aspiration, it has the advantage of providing an objective measurement and does not require specialist medical skills in technique or interpretation. In addition, although sternal bone marrow aspiration is a routine medical procedure, there is a small risk of puncturing major blood vessels [99].

In order to fully evaluate the potential role of the low-dose iron absorption test it is necessary to increase patient numbers, focusing particularly on those who are anaemic, with normal or reduced bone marrow iron stores. Work should concentrate on the simplified test method since this was found to be as sensitive as the full duration test and is more likely to accepted in medical practice. A long-term follow-up study should also be undertaken to evaluate the efficacy of the iron absorption test in predicting response to oral iron therapy.

4.4 CONCLUSIONS

Prevalence survey

1. The survey showed the prevalence of RLS in iron deficiency (25%) and rheumatoid arthritis (36%) to be high, compared with that of a normal healthy population (12.7%).

2. Sufferers presented with a wide variation in symptoms, frequency and duration of RLS attacks. 'Classical' RLS occurred in 31.5% rheumatoid, 22% iron deficient and 10% healthy individuals.

3. Over 85% of subjects were inconvenienced by symptoms, though many felt inhibited in seeking advice from a doctor. In those who did the response was often unhelpful.

4. RLS was not found to consistently predominate in any particular sex or age. Familial association was significant.

5. RLS may occur in the absence of iron deficiency or anaemia. In rheumatoid arthritis there was a significant association between iron deficiency and RLS, as opposed to the anaemia of chronic disease. There was an inverse relationship between iron deficiency and frequency of RLS attacks. Findings indicate that haematological iron deficiency is a predisposing factor rather than the main causative issue in RLS.

6. RLS did not correlate with rheumatoid disease activity or specific anti-inflammatory or disease modifying drug therapy.

Clinical trial of iron in RLS

1. Treatment with oral iron was associated with a significant improvement in RLS in 9/10 subjects with rheumatoid arthritis and concurrent iron deficiency.

2. 3/10 rheumatoid subjects experienced complete remission after as little as seven days treatment with iron, lasting for between one and five months. Such a response was not seen with placebo.

3. Speed of response and persisting iron deficiency suggest a non-haematological mechanism of action.

4. Oral iron was not found to be effective in healthy subjects without concurrent iron deficiency, though the treatment group was limited.

5. The 'placebo effect' was observed in a proportion of subjects, emphasizing the need for double-blind placebo controlled trials.

6. Compliance with trial medication was good and adverse effects generally minor. Exacerbation of joint disease was associated with iron therapy in two rheumatoid subjects. This is a rarely reported effect with oral iron and supports the suggested role of iron in the pathophysiology of rheumatoid disease.

The low-dose iron absorption test

1. The low-dose iron absorption test was found to provide a sensitive method of differentiating iron deficiency from iron repletion in subjects without inflammatory disease. Peak rise in serum iron was independent of the presence or severity of anaemia.

2. Maximum discrimination between iron deficient and iron replete sub-groups was found to occur at 1 hour post-dose allowing the test to be simplified.

3. In rheumatoid disease the test was found to differentiate anaemia due to 'absent' iron stores from that due to the anaemia of chronic disease. Further investigation is required to assess the method as a means of differentiating normal and slightly reduced marrow iron stores and hence providing an alternative method to bone marrow aspiration in the differentiation of IDA and ACD.

4. The results suggest that iron absorption is depressed in rheumatoid subjects with normal iron stores, but in those with concurrent genuine iron deficiency, absorption is increased to the same extent as in uncomplicated iron deficiency.

5. The low-dose iron absorption test is simple, safe and inexpensive, providing an objective assessment of iron stores.

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APPENDIX A1

DIAGNOSTIC CRITERIA FOR RESTLESS LEGS SYNDROME

Subjects included in the Prevalence Survey and Clinical Trial were diagnosed as having Restless Legs Syndrome based on the following criteria, based on the detailed description by Ekbom [2].

1. Discomfort in the legs.

2. Symptoms occurring at rest.

3. Discomfort associated with an irresistable need to move the legs which provides some relief.

Subjects were defined as having 'classical' symptoms if the following features were present:

1. Discomfort of a crawling nature, affecting the area between the knee and ankle, and sensed to be coming from deep within the limb, rather than superficially.

2. Symptoms occurring bilaterally.

Exclusion Criteria

1. Neurological disorders.

2. Varicose veins.

3. Leg ulcers.

4. Parkinson's disease.

5. Concurrent psychotic medication.

APPENDIX A2

DIAGNOSTIC CRITERIA FOR RHEUMATOID ARTHRITIS

Rheumatoid subjects included in the study had **definite** or **classic** rheumatoid arthritis in accordance with criteria laid down by the **American Rheumatism Association** [177], as shown below.

1. Morning stiffness (1 hour or more).
2. Pain on motion or tenderness in at least one joint.
3. Swelling of at least one joint.
4. Swelling of at least one other joint.
5. Symmetrical joint swelling.
6. Subcutaneous nodules.
7. X-ray changes typical of rheumatoid arthritis.
8. Positive test for rheumatoid factor.
9. Poor mucin clot of synovial fluid.
10. Characteristic histologic changes in the synovial membrane.
11. Characteristic histopathology of rheumatoid nodules.

Criteria 1 - 5 must be present for at least six weeks.

For diagnosis of definite RA at least five of the criteria must be met, and for classic RA at least seven of the above criteria.

APPENDIX A3

DIAGNOSTIC CRITERIA FOR IRON DEFICIENCY

Criteria listed below are those used in diagnosis of iron deficiency and iron deficiency anaemia during the research.

Iron deficiency

1. Where results of bone marrow assessment of iron stores were available, subjects reported to have iron stores graded as 0 (absent) or 1 (reduced) were considered to be indicative of iron deficiency.

2. In the absence of inflammatory disease, a serum ferritin value of 20ug/l or less was indicative of iron deficiency in both male and female subjects.

3. In normal healthy volunteers, when results of serum ferritin determination were unavailable, iron deficiency was diagnosed in those with serum iron less than 11umol/l, transferrin saturation less than 16% and total iron binding capacity greater than 70umol/l.

Iron deficiency anaemia

1. In accordance with local hospital limits, female subjects with a haemaglobin value less than or equal to 11.2g/dl and in male subjects of 13.2g/dl or less were defined as anaemic.

2. Iron deficiency anaemia is a microcytic anaemia, indicated by mean cell volume of less than 80fl.

3. Iron deficiency as indicated by parameters above.

APPENDIX A4

QUESTIONNAIRE TO ASSESS THE PREVALENCE OF RESTLESS LEGS SYNDROME (RLS)

I am a pharmacist at Selly Oak Hospital doing a research project with some of the doctors at the hospital. We're interested in strange sensations that some people experience in their legs. I should be most grateful if you would allow me to ask you some questions (about your arthritis), the medicines that you take and any strange feelings that you experience in your legs. It won't take more than about 10 minutes. All the information collected will be strictly confidential.

Thank you.

Demographic Data Name Interview date _ _/_ _/_ _ Hospital Reg No. _ _ _ _ _ _ (if applicable)		Variable/ Column/ Coding
1. Population sub-group		SUBGP [1]
Rheumatoid		1
Healthy		2
Iron deficient		3
2. Case number		CASE [2 - 4]
Rheumatoid with RLS	1 - 499	— — —
Rheumatoid without RLS	500 - 999	
Healthy with RLS	1 - 499	
Healthy without RLS	500 - 999	
Iron deficient with RLS	1 - 499	
Iron deficient without RLS	500 - 999	
3. Coding card		CC [5]
Sheet 1		1
Sheet 2		2
4. Age		AGE [6 - 7]
Age in completed years		— —

5. Sex	SEX [8]
Female	1
Male	2
6. Diagnosis	DISEAS [9 - 10]
Healthy	01
Sero-negative rheumatoid arthritis	02
Sero-positive rheumatoid arthritis	03
Other rheumatoid diagnosis	04
Iron deficiency anaemia	06
7. RLS Diagnosis	EKBOMS [13]
RLS Negative	1
RLS Positive	2
Start of questionaire	
8. (RHEUMATOID SUBJECTS ONLY) How long have you suffered with arthritis ?	DURATN [11 -12]
Duration in years	-- --
Less than 1 year	88
9. I would now like to ask you some questions about any medicines that you take.	
Firstly, are you taking any iron preparations at the moment, or during the past 12 months ?	IRON [14]
No	1
Currently taking an iron preparation	2
Taken iron during the past 12 months	3
10. Are you taking any painkillers regularly ?	ANALGE [15]
No	1
Paracetamol	2
Aspirin	3
Compound analgesics (eg. co-proxamol)	4
Opioid analgesics	5
Yes, but not specified	6

11. Are you taking a non-steroidal-anti-inflammatory drug ?	NSAIDS [16]
No	1
Indomethacin	2
Naproxen	3
Ibuprofen	4
Diclofenac	5
Piroxicam	6
Sulindac	7
Other (please specify)	8
12. Are you taking any 'second-line' drugs ?	SECOND [17]
No	1
Sulphasalazine	2
Gold	3
Penicillamine	4
Hydroxychloroquine	5
Other (please specify)	6
13. Are you taking prednisolone tablets ?	STEROD [18]
No	1
Yes	2
14. Are you taking any other medicines regularly that your doctor has prescribed or which you have bought from a chemist shop ?	OTHER 1 & 2 [19 - 24]
No	1
Yes (please specify)	
15. Have you ever had funny feelings in your legs; a feeling of restlessness or a crawling sensation that comes on when you are sitting down or in bed; that makes you want to keep moving your legs in order to relieve the feeling ?	
Broadly, Yes — go to Q.16 and 17 etc	
No — go to Q.16, then Q.31 then finish	

16. Have you ever heard of any of your family of friends complaining of such a feeling ?	RELFAM [25]
No	1
Blood relative	2
Non-blood relative	3
Friend	4
Family and friends	5
Family member, unspecified	6
17. Would you use any of the following descriptions for the feeling you get in your legs ?	FEEL [26]
a. Crawling sensation	Incl. a 2
b. An irritation	g + h 3
c. Aching feeling	b - h 4
d. Pain	i 5
e. Like pins and needles	
f. A burning sensation	
g. Restlessness	
h. An indescribable feeling	
i. Other (please specify)	
No RLS	1
18. Is the sensation on the skin surface or deep inside ?	DEPTH [27]
Deep	2
Skin surface	3
No RLS	1
19. Where abouts do you feel it ?	SITE [28]
Between knee and ankle	2
Between knee and ankle, and another area of leg	3
Feet only	4
Ankles, knees or thighs only	5
Arms	6
Arms and legs	7
No RLS	1
20. Are both legs affected ?	BILAT [29]
One leg only	2
Both legs	3
No RLS	1

21. When does the sensation occur during the day ?	WHEN [30]
In the evening only, when sitting	2
Bed-time only	3
During the night	4
Evening and during the night	5
During day-time and evening when sitting	6
Other (please specify)	7
No RLS	1
22. How long does each episode last for ?	TIME [31]
Only couple of minutes	2
Less than 30 minutes	3
Between 30 minutes and 1 hour	4
More than 1 hour	5
No RLS	1
23. Which of the following (if any) help to relieve the sensation ?	RELEF1 [32]
Moving the legs	2
Walking around	3
Moving the legs and walking	4
No RLS	1
24. Do any of the following help at all ?	RELEF2 [33]
Heat	2
Cold	3
Analgesics	4
Rubbing the legs	5
Hanging legs out of bed	6
Hot drink	7
Nothing else specific	8
No RLS	1
25. How often does the sensation in your legs interfere with sleep at night ?	SLEEP [34]
Not at all	2
Occasionally	3
Frequently	4
No RLS	1

26. How often do you have RLS ?	FREQ [35]
Every night	2
2-3 times a week	3
Once a week	4
Less than once a week	5
Less than once a month	6
Other (please specify)	7
No RLS	1

27. When did you last experience RLS ?	LAST [36]
During the last month	2
More than 1 month ago, but less than 3 months	3
Between 3 and 12 months ago	4
More than 1 year ago	5
No RLS	1

28. Do any of the following tend to bring on RLS ?	EXACER [37]
Tiring day	2
Exercise	3
Heat	4
Damp weather	5
Other (please specify)	7
Nothing specific	8
No RLS	1

29. For how long have you had a problem with RLS ?	STARTD [38 - 39]
Duration in years	-- --
Less than 12 months	66
No RLS	88

(RHEUMATOID SUBJECTS ONLY)	CORREL
30. Do you find that the leg sensations get worse when your arthritis is particularly bad ?	[40]
Correlation	2
No correlation	3
Don't know	4
No RA	5
No RLS	1

31. Do you regularly experience any of the following in your legs ?	LEGS [41]
No	1
Cramp	2
Spontaneous jerking movements	3
Pins and needles	4
Other (please specify)	5
INCONV	
32. How would you classify the level of inconvenience caused by RLS ?	[42]
	2
Accepted as normal	3
Mildly irritating	4
Extremely irritating	
No RLS	1
	GP
33. Have you ever mentioned your RLS to a doctor ?	[43]
No	
If yes, which of the following were you given ?	
Iron	3
Clonazepam	4
Diazepam	5
Explanation only	6
No explanation or treatment	7
Other (please specify)	8
No RLS	1

RHEUMATOID SUBJECTS ONLY

Objective and subjective indicators of disease activity from metrology assessment

1. Early morning stiffness (mins)	EMS [44 - 46] — — —
2. Ritchie articular index	RITCHI [47 - 48] — — —
3. McKonkey score	MCKON [49 - 51] — — —
4. Average grip strength (left and right)	AVGRIP [52 - 54] — — —
5. Pain score (1-10 visual analogue score)	PAIN [55 - 57] — . —

Biochemical and haematological indicators of disease activity

1. Erythrocyte sedimentation rate (mm/1st hour)	ESR [11 - 13] — — —
2. C- reactive protein (mg/l)	CRP [58 - 60]
Value (if greater than 4mg/l)	— — —
Less than 4mg/l	777
3. Rheumatoid factor	LATEX [61]
Latex positive	1
Latex negative	2

4. Rose Waaler titre	RWAAL [62]
Less than 1/32	1
1/32	2
1/62	3
1/128	4
1/256	5
1/512	6
> 1/1024	7

ASSESSMENT OF IRON STATUS - HAEMATOLOGICAL INDICIES

1. Haemoglobin (g/l)	HB [63 - 66] — — . — —
2. Serum iron concentration (umol/l)	FE [67 - 70] — — . — —
3. Transferrin saturation (%)	SATN [71 - 74] — — . — —
4. Total iron binding capacity (umol/l)	TIBC [75 - 78] — — . — —
5. Serum ferritin concentration (ug/l)	FERRIT [6 - 10] — — — . — —
6. Red blood count ($x10^9$/l)	RBC [14 - 17] — — . — —
7. Platelet count ($x10^9$/l)	PLTS [18 - 20] — — —
8. White blood count ($x10^9$/l)	WBC [21 - 25] — — . — — —
9. Mean cell volume (fl)	MCV [26 - 30] — — — . — —

10. Packed cell volume	PCV [31 - 35] .
11. Mean cell haemoglobin (pg)	MCH [36 - 39] .
12. Results of bone marrow assessment of iron stores (if done)	BM [40]
Absent iron stores	0
Depleted iron stores	1
Normal iron stores	2
Increased iron stores	3

TRANSCRIPT OF AN INTERVIEW WITH A PATIENT WITH RLS

The transcript that follows is that of an interview with a 79 year old lady with emphysema requiring intranasal oxygen virtually 24 hours a day. She was not interviewed as part of the prevalence survey, but was referred by a medical physician, knowing of the work being undertaken within the hospital. The interview lasted approximately 10 minutes.

Interviewer indicated by letter I, and patient by letter P.

I: Can you describe the sensation you get in your legs?

P: 'Well, it's like little ants running up and down my legs and you just can't sit still.... you move your legs
..that's the best way I can describe it.. like something running up your legs continuously'

I: Is it painful?

P: 'Well no it isn't so much painful as something...
..you think this will drive me silly..
..it's not a pain'

I: Does it ache at all?

P: 'Well I don't think it does really. It's just a sensation....but it's horrible'

I: Where abouts in your legs do you get it?

P: 'Mostly from the knees downwards --- and knees.'

I: Does it go into your feet at all?

P: 'Yes, but I don't get it in my hands, it's usually in my legs.'

I: Do you feel as though the ants are on the surface of your legs or are they inside your legs?

P: 'That's rather difficult. I don't really know. Under the skin certainly.'

I: Are both your legs affected?

P: 'Yes, always both legs.'

I: When does the restlessness come on?

P: 'Not any special sort of day.
When I was younger, it just used to come --- it used to be there.'

I: Does it happen any time of day, or in the evening, or at night?

P: 'It can happen any time of the day but during the day I suppose you can walk about ... do things to try to get ...
It's worse when you sit down ... to see a T V programme...
They say I've got this breathing complaint, part of it is stress,so relax . Well, how can you relax when you've got ants running up and down your legs?'
'In other words, it's a vicious circle, I can't relax because my legs won't relax, although I'm tired and it isn't necessarily when I'm out of breath either. It just happens.'

I: Does it happen when you're in bed at night?

P: 'Yes it does. The other night I woke up and oh dear,
I moved my legs to try to get them on to something cold, thinking that might help. You just shuffle about and move them but nothing seems to take off.
I imagine it's a bit like Chinese torture because you think - I'll go mad if it doesn't stop.'

I: Is there anything you find that helps it to go away?

P: I've tried walking about (but of course I can't now because I can't walk about).
I've tried having a bath.
I've tried everything. I don't think anything helps, not really.
If it's going to be there, it's going to be there....'

I: Does anything help it to go away, does walking around?

P: 'It might help it to go away. Do something else to take your mind off it. Mind you, it's a bit difficult to forget. The very fact that you're moving your legs, you feel you're doing something for it. You're not really but you feel as though you are.'

I: When it comes on, how long does it last?

P: 'It can last anything up to two to three hours sometimes. Or about twenty minutes. But when I was in hospital it was lasting all through the night ... and you see ... they wouldn't give me sleeping tablets in the hospital because they say it's bad for you when you've got a chest complaint. I always say ... well my doctor gives me a sleeping tablet. He says lying awake all night gasping is not doing you any good.
I was at my wits end my legs were so bad. I couldn't sleep. Lying in bed didn't help and getting up didn't help.'

I: Does it normally interfere with your sleeping ?

P: 'Well yes, I think it does. Of course you're asking me now. It's been nearly two months since it really bothered me. You know you forget until it comes again.'

I: How long have you had it. When did it start ?

P: 'I can tell you exactly. Forty two years ago. I was six months pregnant with my first baby and I couldn't think what on earth it was. I used to get out of bed and go and get in a very cold bed in the next bedroom. It was enough to give me pneumonia ... but that's what I used to do.
Eventually I went to the doctor. He said, well you are pregnant ... and you are likely to get all sorts of things aren't you...
But I'm not pregnant now, I'm seventy nine and I'm still getting it.'

I: Over the years, have you asked your G P about it ?

P: 'Yes and no. I've got no response from any G P really. I don't think it's their fault. It's me not being able to describe it properly or perhaps they don't know it exists ... when you think of all the horrible things that do exist ... well, it's trivial ... but it isn't ....'

I: How long have you had the problem with your breathing ?

P: 'About five years I think ... it's been worse since 1983.'

APPENDIX A5

NORMAL LIMITS FOR HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS

Haematological parameters

Red blood count (RBC) x 10^{12}/l	Male	4.2 - 5.7
	Female	3.8 - 5.1
Haemoglobin (Hb) g/dl	Male	13.2 - 17.0
	Female	11.2 - 15.0
Packed cell volume (PCV) l/l	Male	0.4 - 0.5
	Female	0.35 - 0.45
Mean cell volume (MCV) fl		80 - 98
Mean cell haemoglobin (MCH) pg	Male	28 - 34
	Female	27 - 33
Serum iron (s-iron) umol/l		11 - 30
Total iron binding capacity (TIBC) umol/l		45 - 70
Transferrin saturation (saturation) %		> 15%
Serum ferritin (s-ferritin) ug/l		> 20
Erythrocyte sedimentation rate (ESR) mm/1st hour	Male	0 - 12
	Female	0 - 19

Biochemical parameters

C-reactive protein (CRP) mg/l	< 4
Rose Waaler titre	< 1/32

APPENDIX A6

PROTOCOL FOR CLINICAL TRIAL

CLINICAL TRIAL TO ASSESS THE EFFICACY OF ORAL FERROUS SULPHATE vs PLACEBO IN THE TREATMENT OF EKBOM'S SYNDROME IN PATIENTS WITH RHEUMATOID ARTHRITIS

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AF/December/85 (revised Dec'86)

INTRODUCTION

Ekbom's syndrome (Restless legs syndrome) RL, is an unpleasant, often distressing lower limb dysaestnesia. It is felt symmetrically, deep within the lower limbs, usually between the knee and ankle. It occurs at times of inactivity, characteristically in the evening when sitting down or on retiring to bed. Although poorly documented in the literature, studies carried out have reported prevalence figures of 2.5 to 15% in a normal population. RL has been treated with a variety of drugs; most recently clonazepam and carbamazepine have been used. Although effective these drugs do not provide a cure and must be used continuously.

The cause of RL is not known. Clinical examination, nerve conduction studies and electromyography on affected subjects have been normal. However certain medical conditions have been reported to be associated with an increased incidence of RL; pure iron deficiency, pregnancy, uraemia, gastrectomy, anaemia and recently rheumatoid arthritis. All these conditions are characterised by the fact that they may either lead to pure iron deficiency or to redistribution of iron, with increased reticuloendothelial iron stores and the anaemia of chronic disease. There have also been a number of anecdotal reports of success using oral iron to treat RL.

It was with these facts in mind that it was decided to formally assess the effectiveness of oral iron by means of a placebo controlled trial. Ferrous sulphate tablets were chosen as the source of iron for the trial on the basis that they are; comparatively well absorbed, associated with few side-effects, readily available and cheap. It was decided to conform with current medical practice and therefore use the standard recommended (B.N.F.) dose of 200mg t.d.s.

AIMS OF STUDY

1. To compare the efficacy of oral ferrous sulphate with placebo in the treatment of Ekbom's syndrome.

2. To determine the duration of treatment required to obtain relief.

3. To follow-up the length of time patients remain symptom free.

4. To relate iron status (and activity of rheumatoid arthritis) to occurrence of symptoms, in a hope that it may provide mechanistic clues as to the cause of Ekbom's syndrome.

SELECTION OF PATIENTS

Admission criteria:

1. Male and female patients aged 18 years or older.

2. Patients under the care of Selly Oak Hospital diagnosed as having rheumatoid arthritis (to be extended to include patients with other diagnoses).

3. Patients diagnosed as having Ekbom's syndrome, and having experienced symptoms at least ONCE A WEEK during the previous month.

4. Patients who have given consent to participate and co-operate in the trial.

Exclusion criteria:

1. Patients already on iron therapy.

2. Patients symptomatic of iron deficiency, requiring active treatment, and therefore not suitable for placebo medication.

3. Patients with recent gastro-intestinal disease.

4. Patients with thalassaemia or sideroblastic anaemia and therefore at risk of iron overload.

5. Patients taking phenothiazine-type drugs and therefore at risk of developing akathisia which could be confused with Ekbom s syndrome.

6. Patients with polyneuropathy.

STUDY DESIGN

The study will take the form of a double blind, randomised, cross-over, placebo controlled trial. Efficacy of treatment will be assessed by means of diary cards on which patients record occurrence and duration of symptoms.

It is estimated that the trial will continue for 12 months, starting in March 1986.

There will be 4 phases to the study:

CONTROL PHASE (Weeks -3 - 0)

Patients diagnosed as having Ekbom's syndrome will be asked to monitor occurrence of symptoms using a diary card, for at least ONE MONTH. This is necessary in order to determine a base line occurrence of symptoms with which the effect of treatment can be compared. They will continue with their usual medication. At the end of this phase suitable patients will formally enter the trial.

PLACEBO RUN-IN PHASE (Weeks 1 - 4)

All patients will receive placebo tablets (1 tablet t.d.s.) for ONE MONTH and will be asked to monitor symptoms using a diary card. This phase is necessary to overcome the placebo effect that has been found to be significant in previous trials involving Ekbom's syndrome.

TREATMENT PHASE (weeks 5 - 8 & 9 - 12)

This phase will have a cross-over design.
Patients will be randomised to receive either:

Ferrous sulphate tablets followed by placebo tablets

or Placebo tablets followed by ferrous sulphate tablets

This part of the trial will be triple blind; neither the patient, prescribing doctor or assessing pharmacist will know which medication has been given. Treatment with each type of tablets will continue for ONE MONTH. Patients will be asked to continue completing diary cards.

FOLLOW-UP PHASE (Weeks 9 - 12)

Patients will be followed up to assess duration of response.

See Diagram 1 for schematic representation of trial design.

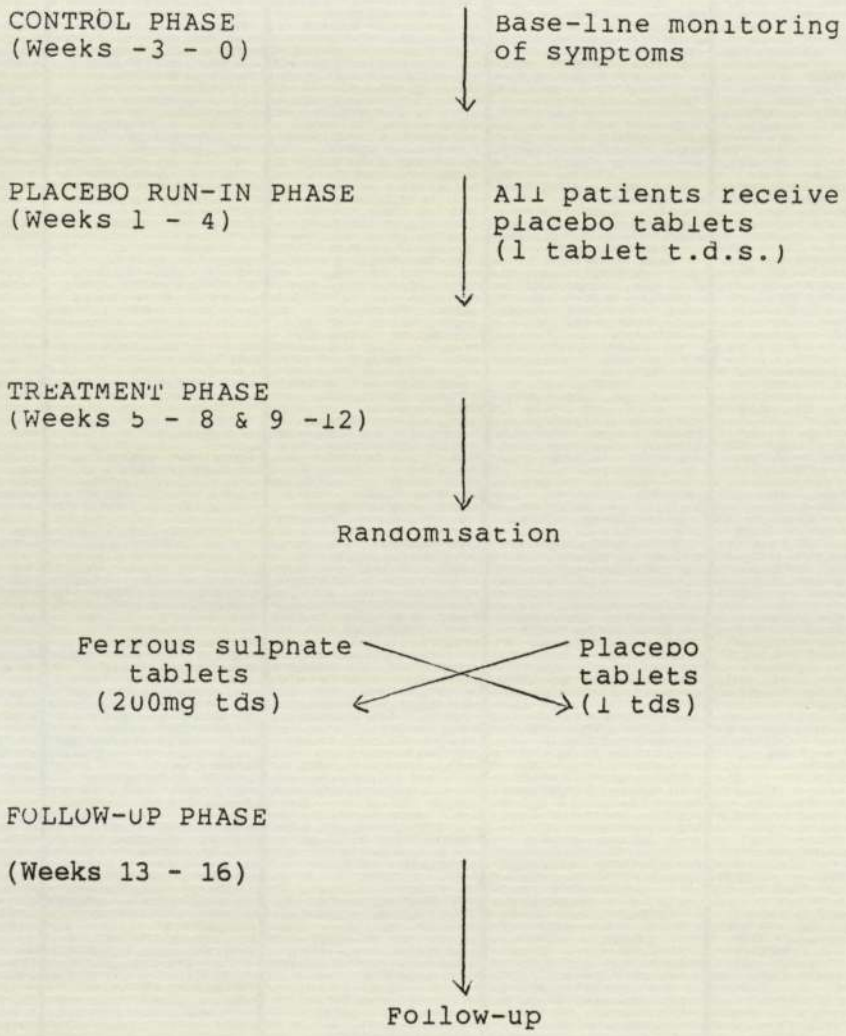


Diagram 1 : SCHEMATIC REPRESENTATION OF TRIAL DESIGN

MEDICATION, PRESENTATION and ADMINISTRATION

Medication will take the form of:

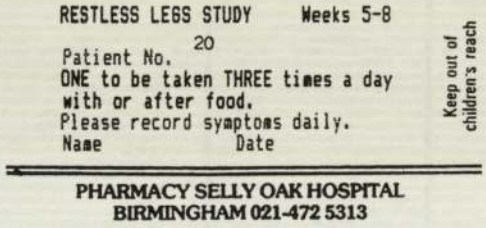
Ferrous sulphate 200mg tablets
White, sugar coated

Placebo tablets
White, sugar coated,
matched in size and shape

Containers

Tablets will be packed in white opaque plastic bottles with broad screw-top closures to aid opening. Each bottle will contain 100 tablets ie. surficient for one month plus a few extra to assess compliance. Three bottles will be dispensed for each patient, labelled with Patient No.; one for the PLACEBO RUN-IN labelled 'Weeks 1 - 4' and two for the TREATMENT PHASE labelled 'Weeks 5 - 8' and 'Weeks 9-12 .

Directions



Special instructions

1. In order to minimise side-effects from the ferrous sulphate, patients will be advised to take tablets with or after food and with a drink of water.

2. Patients on concurrent penicillamine will be instructed to take their penicillamine first thing in the morning and not to take the first dose of trial tablets until lunch-time. This will help prevent the iron interfering with penicillamine absorption.

3. Similarly, patients on antacids (often as a result of NSAID gastro-intestinal side-effects) will be instructed to take iron separately from the antacid.

DISPENSING

Trial medication will be prescibed by Rheumatology doctors and dispensed by Anne Fonseca in the out-patient clinic.

CONCOMITANT DRUG THERAPY

Patients will be instructed to continue with all other medication as before.

CLINICAL ASSESSMENT

All patients will be seen by Dr D. Blake in the Rheumatology Out-patient Clinic.

WEEK -3 - Initial assessment

1. Patients will be assessed for symptoms of restless legs by interview using a prepared questionnaire, to be undertaken by the investigators.

2. Iron status will be assessed by means of a blood sample; undertaken by the Out-patient Haematology department.

3. Patients will be asked to keep a record of symptoms during the following 4 weeks on a diary card.

WEEK 1 - Consent and commencement of placebo phase

1. Diary cards for 'weeks -3 - 0 will be collected and assessed.

2. Patients suitable for selection into the trial will have their legs examined to exclude any neurological abnormalities.

3. Suitable patients will be given written and verbal explanation of the trial and those agreeable will be asked to give written consent.

4. Consented patients will be examined to assess severity of their rheumatoid arthritis.

5. Iron status will be reassessed.

6. Patients will receive treatment bottle for 'weeks 1 - 4'.

7. Patients will be asked to continue filling in the diary card, and to bring it to the next appointment.

WEEK 5 - Commencement of treatment phase

1. Diary cards for 'Weeks 1 - 4' will be collected.

2. Remaining tablets will be collected and counted for assessment of compliance.

3. Patients will be asked about overall symptoms of RL and response to the tablets.

4. Side-effects of trial tablets will be monitored from voluntary patient information and that elicited by direct questioning. ('Have the tablets disagreed with you in any way?).

5. Iron status will be reassessed.

6. Patients will receive the treatment bottle for 'weeks 5 - 8', according to randomisation and stratification.

7. Patients will be asked to continue recording symptoms on the diary card and to bring it to the next appointment.

WEEK 9 - Second part of treatment phase.

1. Repeat as for Week 5, with patient receiving treatment for Weeks 9 - 12.

WEEK 12 - End of trial medication

1. Diary cards for Weeks 9 - 12 will be collected.

2. Remaining tablets will be collected and counted for compliance.

3. Patients will be asked about overall symptoms of RL and response to the tablets.

4. Side-effects to the trial tablets will be recorded as before.

5. Iron status will be reassessed.

6. A further diary card will be given to assess recurrence of symptoms; to be brought to the next clinic appointment.

ETHICAL CONSIDERATIONS

The study will be conducted in accordance with the provisions of the Declaration of Helsinki. The nature of the study will be fully explained to all patients prior to entry and their consent obtained. Patients will be informed of their right to withdraw at any time.

Local approval of the study has been obtained from the South Birmingham Health Authority Ethical Committee.

DATA COLLECTION

Anne Fonseca will be responsible for collection, collation and analysis of trial data.

Patient Information.

TREATMENT OF RESTLESS LEGS WITH FERROUS SULPHATE.

´Restless legs´ (also known as Ekbom´s syndrome) is a common complaint, affecting 1 in 20 of the population. It is not a serious condition but causes considerable discomfort and loss of sleep in some people. Several drugs have been used in its treatment but, although effective, they ´control´ rather than treat the condition, and can be associated with unpleasant side-effects.

Work done at Selly Oak Hospital has shown that patients with rheumatoid arthritis are particularly prone to this condititon and, that a short course of iron tablets may help relieve symptoms of restless legs. Iron is a common dietary component. It is given in the form of ferrous sulphate tablets to treat restless legs. At the dosage used it rarely causes any side-effects, apart from a mild stomach upset in a few people.

This treatment has been successful in a number of patients but we should like to evaluate it fully in an organised study. As a sufferer of restless legs we believe that this may relieve your symptoms and by taking part in this study it may subsequently enable us to treat other patients.

What would you be asked to do?

1. To take a course of tablets for approximately 8 weeks. Some patients will receive ferrous sulphate tablets, whilst others will have inactive ´dummy´ tablets. This allows us to assess how effective the iron tablets are.

2. Record episodes of restless legs on a diary card.

3. To give a blood sample when you come to your usual clinic appointment; you probably do this routinely already.

Patients who are given inactive ´dummy´ tablets during the study will be given the opportunity of having active treatment afterwards.

Please feel free to discuss any points that you are unsure about with Dr Blake.

Patient consent form

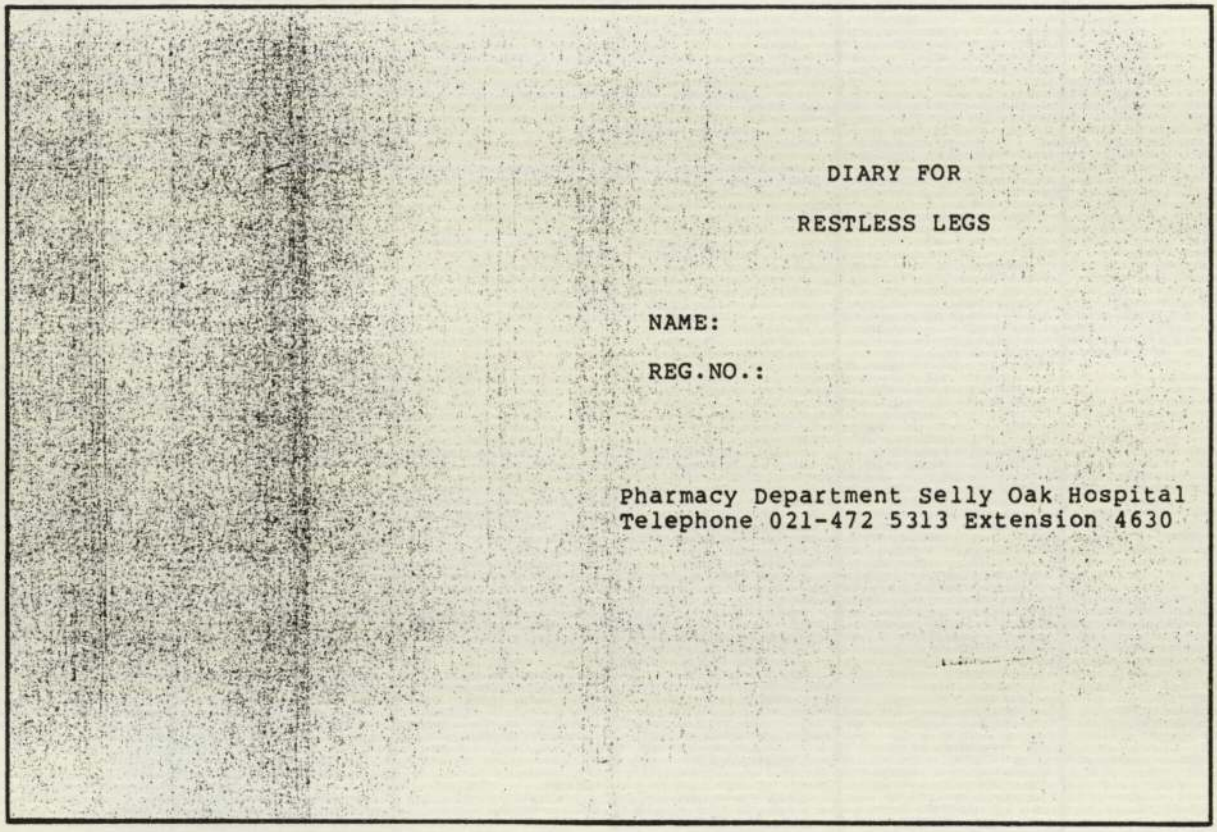
TREATMENT OF RESTLESS LEGS WITH ORAL FERROUS SULPHATE.

I .....Reg. No. .....
have received written and verbal explanation of the nature of this study and freely give my consent to participate.
I understand that I may withdraw from the trial at any time without explanation.

Signed ..... Date.....

Investigator .....

DIARY CARD ISSUED TO TRIAL SUBJECTS TO RECORD RLS SYMPTOMS



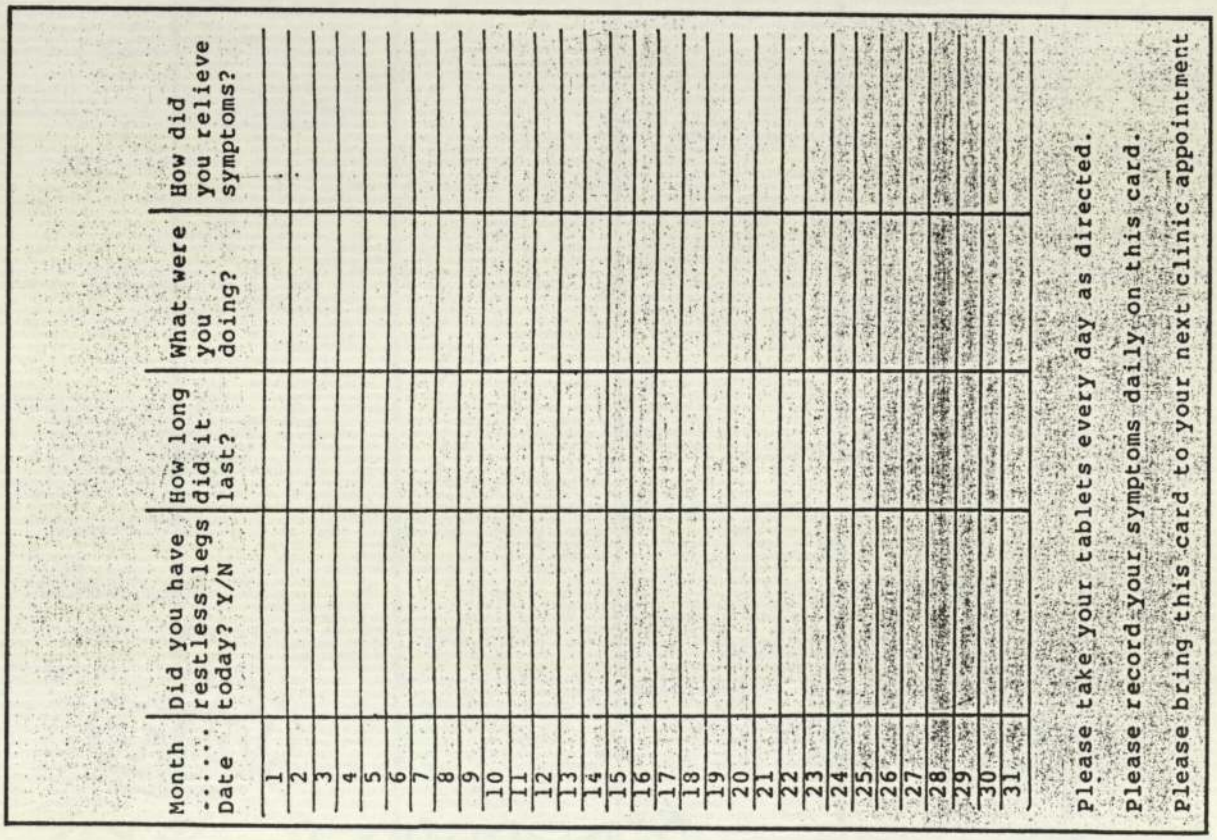
DIARY FOR

RESTLESS LEGS

NAME:

REG.NO.:

Pharmacy Department Selly Oak Hospital
Telephone 021-472 5313 Extension 4630



Month ..... Date	Did you have restless legs today? Y/N	How long did it last?	What were you doing?	How did you relieve symptoms?
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				
26				
27				
28				
29				
30				
31				

Please take your tablets every day as directed.

Please record your symptoms daily on this card.

Please bring this card to your next clinic appointment

APPENDIX A7

PROTOCOL FOR LOW-DOSE IRON ABSORPTION TEST

Preparation of patients

1. Patients can eat, drink and have their drugs administered as normal the day BEFORE the test.

2. On the day of the test patients should be STARVED FROM MIDNIGHT.
   They MAY DRINK WATER as required.

3. Where possible, drugs known to interfere with iron absorption (eg. antacids, tetracyclines, penicillamine), or to enhance it (eg. ascorbic acid) should be avoided. This should always be confirmed with the doctor.

Original test procedure

1. In order to minimise the influence of diurnal variation the test is commenced between 8.30-9.30am.
   To avoid unnecessary anxiety from repeated venepuncture a butterfly needle or venflon should be used, and kept patent with Hepsal.

2. At time 0, patients should have a blood sample taken (1 x 5ml fresh 1 x 10ml clotted) for measurement of baseline indices (full blood count, Hb, MCV, serum iron, TIBC, %saturation, serum ferritin).

3. The test dose of iron (10mg elemental iron) is administered as a freshly prepared solution of ferrous sulphate. This may be followed by a drink of water.

4. Blood samples (1 x 5ml clotted) should be taken to assess serum iron at 0.5, 1, 1.5, 2, 3, and 4 hours post dose.

5. Patients will then be able to eat and drink normally.

Revised 'Shortened' test procedure

1. Preparation of patient as above.

2. Baseline blood samples as above.

3. Blood samples (1 x 5ml clotted) should be taken at 30 and 60 mins post dose.

REMEMBER to label blood tubes and specimen bag with sample times. Also mark specimen bag 'IRON ABSORPTION TEST'

APPENDIX A8

LISTING OF THE SPSS[x] DATALIST CREATED TO HANDLE PREVALENCE SURVEY DATA.

```
File handle testdata/name = 'testdata.dat'
File handle rlsfile/name = 'rlsfile.sf'

Data list file = testdata records = 2
                 /1 SUBGP 1  CASE 2-4  CC1 5  AGE 6-7 DISEAS 9-10
                 DURATN 11-12  EKBOMS 13  IRON 14  ANALGE 15 NSAIDS 16
                 SECOND 17  STEROD 18  H2ANTA 19  BENZOD 20 OTHER1 21-22
                 OTHER2 23-24  RELFAM 25  FEEL 26  DEPTH 27 SITE 28
                 BILAT 29  WHEN 30  TIME 31  RELEF1 32 RELEF2 33  SLEEP 34
                 FREQ 35  LAST 36  EXACER 37  STARTD 38-39 CORREL 40
                 LEGS 41  INCONV 42  GP 43  EMS 44-46 RITCHI 47-48
                 MCKON 49-51  AVGRIP 52-54  PAIN 55-57  CRP 58-60 LATEX 61
                 RWAAL 62  HB 63-66 (1)  FE 67-70 (1)  SATN 71-74 (1)
                 TIBC 75-78 (1)
                 /2 CC2 5  FERRIT 6-10 (1)  ESR 11-13  RBC 14-17 (2)
                 PLTS 18-20  WBC 21-25 (2)  MCV 26-30 (1) PCV 31-35 (3)
                 MCH 36-39 (1)  BM 40

MISSING VALUES   AGE (99)/ SEX (9)/ DISEAS (99)/ DURATN (77,99)/
                 EKBOMS TO BENZOD (9)/ OTHER1 (99)/ OTHER2 (99)/
                 RELFAM TO EXACER (9)/ STARTD (99)/ CORREL TO GP (9)/
                 EMS, MCKON, AVGRIP, CRP (777,888,999)/ RITCHI (88,99)/
                 PAIN (8.8, 9.9)/ LATEX (8,9)/ RWAAL (8,9)/
                 HB TO TIBC (99.9)/ FERRIT (999.9)/ ESR (999)/ RBC (9.99)/
                 PLTS (999)/ WBC (99.99)/ MCV (999.9)/ PCV (9.999)/
                 MCH (99.9)/ BM (9)

VAR LABELS       DURATN Duration of disease in years/
                 IRON   Iron therapy/
                 ANALGE Analgesics/
                 SECOND Second line agents/
                 STEROD Oral steroids/
                 H2ANTA H2 blockers/
                 BENZOD Benzodiazepines/
                 OTHER1 Other drugs/
                 OTHER2 Other drugs/
                 RELFAM Family or friends with RLS/
                 FEEL   Description of sensation/
                 DEPTH  Depth of sensation/
                 SITE   Area affected/
                 BILAT  Symmetry of RLS/
                 WHEN   Time when symptoms occur/
                 TIME   Duration of attack/
                 RELEF1 Relieving factors/
                 RELEF2 Relieving factors/
                 SLEEP  Interference with sleep/
                 FREQ   Frequency of attacks/
                 LAST   Last symptoms/
                 EXACER Exacerbating factors/
                 STARTD How long had RLS/
                 CORREL Correlation with RA/
                 LEGS   Other leg symptoms/
                 INCONV Level of inconvenience/
                 GP     Doctor referral/
                 EMS    Early morning stiffness/
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                RITCHI Ritchie articular index/
                MCKON  McKonkey score/
                AVGRIP Grip strength/
                PAIN   Pain score/
                CRP    C-reactive protein/
                LATEX  Rheumatoid factor/
                RWAAL  Rose Waaler titre/
                HB     Haemoglobin/
                FE     Serum iron/
                SATN   % Saturation/
                TIBC   Total iron binding capacity/
                FERRIT Serum ferritin/
                ESR    Erythrocyte sedimentation rate/
                RBC    Red blood count/
                PLTS   Platelet count/
                WBC    White blood count/
                MCV    Mean cell volume/
                PCV    Packed cell volume/
                MCH    Mean cell haemoglobin/
                BM     Bone marrow iron stores

VALUE LABELS    SUBGP  (1) Rheumatoid arthritis
                       (2) Normal healthy
                       (3) Iron deficient/

                SEX    (1) Female
                       (2) Male/

                DISEAS (01) Healthy
                       (02) Sero-negative RA
                       (03) Sero-positive RA
                       (04) Other RA
                       (05) Osteoarthritis
                       (06) Iron deficiency/

                DURATN (88) Less than one year/

                EKBOMS (1) Negative
                       (2) Positive/

                IRON   (1) No
                       (2) On iron
                       (3) Iron in last year

                ANALGE (1) None
                       (2) Paracetamol
                       (3) Aspirin
                       (4) Co-preps
                       (5) Opiates
                       (6) Unspecified/

                NSAIDS (1) None
                       (2) Indomethacin
                       (3) Naproxen
                       (4) Ibuprofen
                       (5) Diclofenac
                       (6) Piroxicam
                       (7) Sulindac
                       (8) Other/
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SECOND (1) None
(2) Sulphasalazine
(3) Gold
(4) Penicillamine
(5) Hydroxychloroquine
(6) Other/

STEROD (1) None
(2) Yes/

H2ANTA (1) None
(2) Ranitidine
(3) Cimetidine/

BENZOD (1) None
(2) Hypnotics
(3) Diazepam
(4) Other
(5) Lorazepam/

OTHER1, OTHER2 (1) None (2)
(2) Amitriptyline
(3) Other antidepressants
(4) Thyroxine
(5) Diuretics
(6) Antihypertensives
(7) Quinine
(8) Glibenclamide
(9) Anti-emetic
(10) Carbamazepine
(11) Sodium valproate
(12) Antacid
(13) Hormones
(14) Ossopan
(15) Hydroxychloroquine
(16) Antihistamine
(17) ACTH
(18) Theophylline
(19) Tamoxifen
(20) Paroven
(21) Warfarin
(22) Vitamins
(23) Timolol
(24) Oral contraceptives
(25) Clomiphene
(27) Folic acid
(28) Chlorambucil
(29) Cinnarizine
(30) Inhaler/

RELFAM (1) No
(2) Blood relative
(3) Non-blood relative
(4) Friend
(5) Both
(6) Unspecified/

FEEL (1) No Ekboms
(2) Classical
(3) Indescribable
(4) Other discomfort

(5) Other/

DEPTH (1) No Ekboms
(2) Deep
(3) Skin
(8) Don't know/

SITE (1) No Ekboms
(2) Calves only
(3) Calves and other
(4) Feet only
(5) Other area
(6) Arms
(7) Arms and legs/

BILAT (1) No Ekboms
(2) Unilat
(3) Bilat
(8) Don't know/

WHEN (1) No Ekboms
(2) PM sitting
(3) bed-time
(4) Night
(5) PM and night (6) am and pm
(6) AM and PM
(7) Other
(8) Don't know/

TIME (1) No Ekboms
(2) Mins
(3) Up to 30 mins
(4) Up to 1 hour
(5) Over 1 hour
(8) Unspecified/

RELEF1 (1) No Ekboms
(2) Move legs
(3) Walk
(4) Move and walk
(8) Unspecified/

RELEF2 (1) No Ekboms
(2) Heat
(3) Cold
(4) Analgesics
(5) Rub legs
(6) Hang legs
(7) Hot drink
(8) Nil else/

SLEEP (1) No Ekboms
(2) Never
(3) Occasionally
(4) Frequently
(8) Don't know/

FREQ (1) No Ekboms
(2) Every night
(3) 2-3 a week
(4) Once a week

(5) Less than weekly
(6) Less than monthly
(7) Other/

LAST (1) No Ekboms
(2) Last month
(3) Last 3 months
(4) Last year
(5) Over a year/

EXACER (1) No Ekboms
(2) Tired
(3) Exercise
(4) Heat
(5) Damp
(7) Other/
(8) Unspecified/

STARTD(88) No Ekboms
(44) Before RA
(55) Since RA
(66) Less than year
(77) Don't know/

CORREL (1) No Ekboms
(2) Correlation
(3) No correlation
(4) Don't know
(5) Not RA/

LEGS (1) None
(2) Cramp
(3) Jerking
(4) Pins and needles
(5) Others/

INCONV (1) No Ekboms
(2) Normal
(3) Mildly annoying
(4) Very irritating/

GP (1) No Ekboms
(2) No
(3) Iron
(4) Clonazepam
(5) Diazepam
(6) Explanation
(7) No explanation Tx
(8) Other

CRP (777) Less than 4

LATEX (1) Negative
(2) Positive
(8) Other

RWAAL (1) Less than 1/32
(2) 1/32
(3) 1/64
(4) 1/128
(5) 1/256

(6) 1/512
(7) 1/1024/

BM (0) Absent
(1) Depleted
(2) Normal
(3) Increased

FINISH

DATA FILE FOR PREVALENCE SURVEY RESULTS

Rheumatoid subjects without RLS

14-Mar-89 SPSS-X RELEASE 3.1 FOR VAX/VMS
11:58:58 ASTON UNIVERSITY on KIRK:: VMS V4.7

Page

31-Mar-89 SPSS-X RELEASE 3.1 FOR VAX/VMS
10:45:34 ASTON UNIVERSITY on KIRK::

CASE	CC1	AGE	SEX	DISEAS	DURATN	EKBOMS	IRON	ANALGE	NSAIDS	SECOND	STEROD	H2ANTA	BENZOD	OTHER1	OTHER2	RELFAM	FEEL	DEPTH	SITE	BILAT	WHEN	TIME	RELEF1	RELEF2	SLEEP	FREQ	LAST	EXACER	STARTD	CORREL	LEGS	INCONV	OP	EMS	RITCHI	MCKON	AVGRIP	PAIN	CRP	LATEX	RWAAL	HB	FE	SATN	TIBC	FERRIT	ESR	RBC	PLTS	WBC	MCV	PCV	MCH	DM
501	1	56	1	2	6	1	1	9	8	3	2	1	1	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	9	1	1	999	99	999	999	10	777	1	1	13.8	99.9	99.9	99.9	243.0	30	5.04	338	8.35	84.8	.428	27.4	9
502	1	56	2	3	8	1	1	5	8	1	1	2	3	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	2	1	1	60	28	85	76	2	4	9	9	15.3	16.8	32.7	51.3	83.2	30	4.91	313	8.64	87.6	.430	31.2	2
503	1	75	1	99	4	1	1	9	9	9	9	9	9	99	99	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	999	99	999	999	10	999	9	9	99.9	99.9	99.9	99.9	999.9	999	9.99	999	99.99	999.9	9.999	99.9	9
504	1	60	2	2	8	1	1	3	2	2	1	1	1	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	999	99	999	999	10	777	1	1	12.9	8.7	14.1	61.9	14.9	28	4.68	309	7.73	88.0	.412	27.5	9
505	1	62	1	3	4	1	1	9	7	1	1	1	1	1	1	4	1	1	1	1	1	1	1	1	1	1	1	1	88	1	4	1	1	0	0	85	124	0	777	2	1	12.4	6.1	8.3	73.6	13.9	10	5.47	298	6.38	73.5	.402	22.7	9
506	1	46	1	3	22	1	1	4	3	5	2	1	2	10	13	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	5	1	1	999	99	999	999	10	999	2	9	14.6	99.9	99.9	99.9	70.2	18	4.58	311	14.50	95.0	.435	32.0	9
507	1	66	1	3	9	1	3	9	9	9	9	9	9	99	99	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	999	99	999	999	10	999	2	7	13.9	13.8	19.7	70.1	88.7	74	4.76	349	5.23	84.4	.402	29.3	9
508	1	72	1	2	3	1	1	5	3	4	2	1	1	14	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	2	1	1	999	99	999	999	10	777	1	1	13.3	12.8	17.3	73.8	24.1	22	4.62	466	9.09	91.0	.420	28.8	9
509	1	65	2	3	20	1	1	4	1	1	2	1	1	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	999	99	999	999	10	999	2	4	14.7	10.6	13.2	80.5	63.3	17	5.18	999	10.00	80.0	.420	28.3	2
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524	1	62	1	2	4	1	3	4	1	3	1	1	1	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	9	1	1	999	99	999	999	10	999	1	1	14.9	99.9	99.9	99.9	43.7	20	5.16	405	8.21	87.9	.454	28.9	9
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547	1	79	1	2	38	1	2	2	3	1	1	1	1	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	9	1	1	999	99	999	999	10	36	2	1	9.7	6.5	9.4	59.3	47.8	11	4.12	230	8.95	68.8	.284	23.6	2
548	1	77	2	2	21	1	1	4	4	1	2	1	1	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	3	10	90	170	0	30	1	1	13.2	13.0	21.1	61.5	55.8	62	4.18	368	11.81	90.3	.378	31.5	9
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559	1	47	2	3	5	1	1	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	2	1	1	999	99	999	999	10	13	2	5	15.0	7.6	14.9	51.0	63.0	10	5.14	321	7.94	87.7	.446	29.5	9
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563	1	52	1	2	14	1	1	9	2	1	1	1	1	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	0	99	999	999	10	999	1	1	99.9	99.9	99.9	99.9	999.9	999	9.99	999	99.99	999.9	9.999	99.9	9
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566	1	76	1	2	6	1	1	2	1	1	1	1	2	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	2	1	1	999	99	999	999	10	60	1	1	10.5	99.9	99.9	99.9	999.9	100	3.50	534	6.99	91.5	.296	30.9	9
567	1	77	2	3	8	1	1	4	1	1	1	1	1	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	999	99	999	999	10	777	2	1	10.7	99.9	99.9	99.9	999.9	53	4.55	352	9.17	76.8	.349	23.6	9
568	1	51	2	3	10	1	1	9	3	4	1	1	1	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	2	1	1	777	99	999	999	10	999	2	3	14.6	99.9	99.9	99.9	45.6	33	4.95	504	9.45	89.4	.443	29.4	9
569	1	71	2	3	3	1	1	9	3	4	1	2	1	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	9	1	1	3	6	70	121	1	999	2	9	15.2	20.8	27.5	75.5	7.6	10	5.00	999	11.36	89.7	.448	30.3	9
570	1	62	1	3	35	1	1	2	5	4	1	1	1	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	999	99	999	999	10	999	2	3	12.6	21.3	32.8	65.0	29.3	12	4.00	366	8.28	91.6	.366	31.5	9
571	1	53	1	2	8	1	1	4	2	1	1	1	1	6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	2	1	1	999	99	999	999	10	999	1	1	9.6	4.9	9.0	54.4	86.7	66	4.22	501	6.24	70.7	.298	22.7	9
572	1	59	1	3	30	1	1	9	6	4	1	1	1	6	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	999	99	999	999	10	999	9	9	14.6	16.1	25.3	63.7	45.3	32	4.91	290	7.73	87.2	.428	29.8	9
573	1	32	2	3	4	1	1	9	9	4	1	1	1	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	999	99	999	999	10	65	2	1	11.5	7.1	14.6	48.6	89.0	97	4.19	631	14.91	81.8	.343	27.5	9
574	1	39	1	3	2	1	1	9	5	2	1	1	1	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	9	1	1	999	99	999	999	10	777	2	1	12.1	17.2	27.9	61.6	23.4	32	4.14	232	7.36	87.6	.362	29.3	1
575	1	40	2	2	7	1	1	4	1	4	1	1	1	21	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	9	1	1	999	99	999	999	10	999	1	1	14.7	99.9	99.9	99.9	21.8	31	4.88	251	8.02	87.5	.427	30.2	9
576	1	49	2	2	88	1	1	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	4	1	1	999	99	999	999	10	100	2	1	11.2	5.6	12.0	46.5	330.0	85	3.94	508	10.24	86.4	.340	28.4	9
577	1	65	1	3	3	1	1	4	3	1	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	999	99	999	999	10	999	2	1	12.1	7.4	9.3	79.9	178.0	115	4.37	430	10.05	80.6	.352	27.7	2
578	1	58	1	3	15	1	1	4	2	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	3	1	1	999	99	999	999	10	84	2	4	10.8	9.2	16.1	57.1	44.7	58	3.69	354	10.67	85.4	.315	29.2	9
579	1	62	2	3	5	1	1	9	5	4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	999	99	999	999	10	43	2	2	13.4	11.2	18.8	59.5	28.1	18	4.31	373	12.27	84.4	.406	27.8	9
580	1	52	2	3	88	1	1	4	2	1	1	1	1	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	20	11	65	98	0	88	2	4	14.1	11.0	24.0	45.8	370.7	64	4.51	528	8.94	88.9	.401	31.1	3
581	1	60	1	99	99	1	9	9	9	9	9	9	9	99	99	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	3	1	1	999	99	999	999	10	999	9	9	99.9	99.9	99.9	99.9	999.9	999	9.99	999	99.99	999.9	9.999	99.9	9
582	1	70	2	3	7	1	1	9	3	4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	999	99	999	999	10	999	9	9	13.8	18.6	19.8	94.0	63.3	4	4.77	325	8.74	86.3	.412	28.9	9
583	1	46	1	3	10	1	1	9	2	2	1	1	1	6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	999	99	999	999	10	999	2	5	11.6	8.4	14.3	58.7	16.4	55	4.18	381	7.09	86.3	.361	27.7	9
584	1	77	1	3	8	1	1	9	5	9	1	1	1	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	999	99	999	999	10	999	2	1	9.4	5.2	8.2	63.4	30.0	35	4.34	426	14.23	69.2	.291	21.3	1
585	1	55	2	3	2	1	1	2	1	1	1	2	1	5	12	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	9	1	1	999	99	999	999	10	777	2	3	15.3	99.9	99.9	99.9	999.9	2	9.99	458	10.29	87.6	.476	28.1	9
586	1	45	1	3	7	1	3	2	6	4	1	1	1	1	1	4	1	1	1	1	1	1	1	1	1	1	1	1	88	1	5	1	1	999	99	999	999	10	999	9	9	11.8	10.0	19.6	51.1	32.9	25	4.30	379	12.19	85.8	.369	27.5	9
587	1	55	1	2	5	1	1	9	7	1	2	1	1	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	9	1	1	30	24	100	82	2	999	1	1	13.4	7.1	11.0	64.6	76.8	40	5.41	392	11.60	79.1	.430	24.8	1
588	1	75	1	3	99	1	2	9	3	1	2	1	1	4	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	9	1	1	999	99	999	999	10	999	2	9	11.4	8.0	12.0	66.6	40.5	43	4.37	608	11.81	82.1	.359	26.1	9
589	1	64	2	3	20	1	1	4	2	3	1	2	2	6	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	999	99	999	999	10	999	2	1	10.5	10.1	15.9	63.7	83.4	68	3.54	350	6.06	89.9	.318	29.8	9
590	1	70	1	2	6	1	1	9	2	2	1	1	1	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	60	2	55	120	2	4	1	1	9.0	5.0	6.0	82.7	3.9	29	4.16	576	9.20	65.0	.270	21.5	0
591	1	56	2	2	5	1	1	4	3	4	1	3	1	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	9	1	1	999	99	999	999	10	38	1	1	13.6	7.8	15.4	50.7	105.0	60	4.85	395	7.45	86.6	.420	28.1	2
592	1	65	2	3	5	1	1	4	1	4	1	1	2	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	9	1	1	999	99	999	999	10	999	2	3	15.0	99.9	99.9	99.9	209.0	23	5.12	231	6.68	88.0	.451	29.2	9
593	1	41	1	4	32	1	1	2	2	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	3	1	1	5	15	100	96	4	25	9	9	11.7	7.1	14.0	50.6	78.1	67	4.41	466	10.16	82.7	.365	26.6	9
594	1	44	2	2	30	1	1	9	1	1	1	1	1	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	999	99	999	999	10	777	1	1	14.0	99.9	99.9	99.9	999.9	14	4.64	261	8.97	89.4	.415	30.2	9
595	1	57	1	3	99	1	1	4	5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	4	1	1	999	99	999	999	10	999	2	5	11.1	8.4	12.9	65.2	245.0	112	3.87	335	7.00	83.4	.342	28.7	9
596	1	60	1	3	4	1	1	4	1	1	1	1	1	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	9	1	1	999	99	999	999	10	52	2	1	12.0	7.8	10.4	74.8	32.8	93	4.07	343	10.97	88.0	.358	29.4	9
597	1	60	2	2	88	1	1	1	1	1	1	1	1	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	3	2	65	142	0	999	1	1	13.3	10.0	19.8	50.6	219.4	40	4.65	999	6.60	83.0	.390	28.5	3

mber of cases read: 97 Number of cases listed: 97

umber of cases read: 97 Number of cases listed: 97

Rheumatoid subjects with RLS

14-Mar-89 SPSS-X RELEASE 3.1 FOR VAX/VMS
11:59:04 ASTON UNIVERSITY on KIRK:: VMS V4.7 Page

14-Mar-89 SPSS-X RELEASE 3.1 FOR VAX/VMS
11:59:07 ASTON UNIVERSITY on KIRK::

CASE	CC1	AGE	SEX	DISEAS	DURATN	EKBOMS	IRON	ANALGE	NSAIDS	SECOND	STEROD	H2ANTA	BENZOD	OTHER1	OTHER2	RELFAM	FEEL	DEPTH	SITE	BILAT	WHEN	TIME	RELEF1	RELEF2	SLEEP	FREQ	LAST	EXACER	STARTD	CORREL	LEGS	INCONV	OP	EMS	RITCHI	MCKON	AVGRIP	PAIN	CRP	LATEX	RWAAL	HB	FE	SATN	TIBC	FERRIT	ESR	RBC	PLTS	WBC	MCV	PCV	MCH	BM
1	1	60	1	3	6	2	3	9	7	3	2	1	1	2	1	9	3	2	2	3	3	4	4	8	4	3	2	8	4	3	3	4	2	300	13	110	108	6	70	2	7	10.2	5.2	7.2	72.4	28.5	80	4.86	765	12.48	63.4	.308	21.0	1
2	1	64	1	3	6	2	1	4	7	4	1	1	1	1	1	9	2	2	2	3	3	5	4	5	3	5	3	8	2	4	2	4	2	360	23	90	49	4	111	2	4	10.9	4.3	8.6	50.0	172.0	121	4.10	691	8.03	84.1	.345	26.6	3
3	1	45	1	3	3	2	1	9	2	5	1	1	1	1	1	9	4	9	3	3	2	4	3	5	2	2	2	8	2	3	2	3	2	0	9	65	68	4	777	2	2	11.0	6.0	8.3	72.4	3.8	12	4.81	322	7.68	71.2	.342	22.8	9
4	1	62	1	3	2	2	3	5	3	3	2	1	1	1	1	9	3	8	9	3	5	4	8	6	4	3	2	8	2	9	1	3	2	60	7	75	109	0	72	2	7	10.0	4.6	6.1	76.0	64.5	49	4.85	410	7.16	62.9	.305	20.6	0
5	1	48	1	2	88	2	1	4	2	2	1	1	1	1	1	9	3	8	3	3	3	8	2	8	9	5	2	8	66	9	1	3	9	210	6	90	66	2	56	2	1	9.4	4.4	9.0	50.2	53.9	80	3.83	457	6.67	76.4	.290	24.7	2
6	1	65	1	3	35	2	1	4	1	1	2	1	1	1	1	9	2	2	3	3	5	4	4	8	3	5	2	2	3	9	3	4	3	999	99	999	999	10	15	2	1	11.8	6.5	11.0	58.9	180.9	65	4.48	221	5.08	79.0	.354	26.4	1
7	1	46	1	2	14	2	3	9	3	3	1	1	1	1	1	9	9	2	2	3	9	9	9	9	9	9	4	9	99	9	9	9	9	999	99	999	999	10	21	1	1	13.1	14.3	27.9	51.2	65.1	23	4.76	304	6.98	85.8	.408	27.4	9
8	1	45	1	3	15	2	1	9	7	2	2	1	1	2	1	1	2	3	3	2	5	3	3	8	4	2	2	2	66	2	1	3	2	0	8	70	106	0	777	2	1	10.5	7.1	9.9	72.0	9.2	26	4.95	361	9.50	65.8	.326	21.2	0
9	1	44	1	2	7	2	1	9	6	4	1	1	1	1	1	9	4	2	3	3	5	5	4	8	3	5	2	2	99	3	1	4	9	0	23	100	63	2	999	1	1	12.3	11.7	19.0	61.7	40.7	25	4.29	369	5.35	89.8	.385	28.7	9
10	1	57	1	3	7	2	1	9	2	3	2	2	1	5	7	9	2	2	2	3	5	5	4	7	4	3	2	8	55	2	2	3	2	30	23	100	52	7	41	2	1	12.5	8.7	13.2	66.0	22.0	45	4.91	477	13.50	999.9	9.999	99.9	2
11	1	70	1	3	3	2	1	2	4	2	1	1	1	1	1	6	2	2	2	3	5	4	4	8	3	9	3	2	77	3	1	3	2	120	5	90	59	5	58	2	6	11.9	8.3	12.9	64.5	117.0	57	5.20	653	12.91	74.3	.387	22.9	9
12	1	32	1	2	12	2	1	9	8	4	2	2	1	1	1	9	2	2	3	3	5	2	2	8	2	5	2	2	6	2	3	3	2	999	99	999	999	10	999	1	1	12.1	99.9	99.9	99.9	999.9	32	3.62	354	11.07	96.3	.349	33.4	9
13	1	55	1	3	8	2	1	9	3	2	1	1	1	1	1	9	2	3	4	3	3	4	2	8	4	2	2	8	66	3	1	9	9	2	8	90	60	4	999	2	1	12.9	7.5	10.9	69.0	6.4	46	4.77	450	7.91	86.4	.412	27.0	9
14	1	55	1	2	25	2	3	4	6	2	1	1	2	1	1	9	2	2	3	3	6	5	4	8	3	3	2	8	15	3	1	4	2	999	99	999	999	10	999	1	1	11.6	7.8	10.7	73.1	69.5	70	4.68	616	8.82	77.0	.360	24.8	1
15	1	64	1	3	10	2	1	9	2	1	1	1	1	1	1	9	9	9	9	9	9	9	9	9	9	9	9	9	99	9	9	9	9	30	7	100	62	8	96	2	1	6.7	2.9	8.0	36.3	201.9	109	3.02	415	5.74	70.4	.212	22.1	9
16	1	49	1	99	18	2	1	3	1	1	2	1	1	1	1	9	3	9	3	3	6	3	3	8	2	3	2	2	99	4	2	3	2	15	0	85	142	2	999	9	9	99.9	99.9	99.9	99.9	999.9	999	9.99	999	99.99	999.9	9.999	99.9	9
17	1	51	1	3	30	2	3	2	6	1	1	1	1	8	1	6	3	9	3	3	5	3	3	7	3	6	4	2	55	9	1	9	9	180	27	55	105	0	999	2	1	9.9	4.5	6.2	72.2	10.3	75	4.95	383	8.63	65.7	.325	19.9	1
18	1	54	1	2	3	2	3	2	3	2	1	1	1	1	1	6	2	2	3	3	5	5	4	8	3	5	2	8	44	4	1	3	2	180	13	75	74	3	47	1	1	12.3	7.4	15.9	46.6	23.5	55	4.13	476	8.89	999.9	.355	29.7	9
19	1	67	2	4	4	2	1	9	2	4	1	1	5	1	1	1	2	3	2	3	5	5	2	8	4	3	2	2	55	2	1	4	2	999	99	999	999	10	999	1	1	14.1	21.6	29.7	72.7	19.5	5	5.00	295	8.32	90.1	.450	28.1	9
20	1	82	2	3	25	2	1	9	3	4	2	1	1	1	1	9	2	3	2	3	4	3	4	8	3	3	2	8	55	9	3	3	2	120	99	999	999	10	777	2	5	12.9	99.9	99.9	99.9	999.9	65	4.40	406	12.24	87.7	.385	29.3	9
21	1	51	1	3	20	2	1	5	1	6	2	1	1	9	1	9	2	2	7	3	3	2	3	5	2	3	2	5	2	9	2	4	2	999	99	999	999	10	999	2	9	13.3	99.9	99.9	99.9	999.9	5	4.99	368	8.75	85.6	.427	26.7	9
22	1	34	1	2	4	2	1	1	3	4	1	1	1	1	1	1	3	9	3	3	2	2	4	8	2	4	2	2	77	9	2	2	9	0	1	90	212	0	999	1	1	12.4	2.9	6.3	45.7	1.0	12	4.81	332	9.71	79.4	.381	25.8	9
23	1	68	1	2	25	2	1	9	2	1	2	1	2	1	1	9	2	2	7	3	5	3	2	8	2	2	2	8	4	3	1	4	7	999	99	999	999	10	999	1	1	7.0	4.0	4.6	86.9	4.4	43	4.19	535	11.03	55.9	.234	16.8	0
24	1	53	1	2	30	2	1	1	3	4	1	1	2	10	11	9	2	2	2	2	6	2	2	8	2	3	2	2	1	3	5	3	2	999	99	999	999	10	999	1	1	12.2	11.3	13.0	86.7	16.1	3	4.64	407	12.28	82.3	.382	26.4	9
25	1	59	1	3	6	2	1	2	1	2	1	1	1	12	1	2	4	3	3	2	2	2	2	8	2	5	9	8	99	9	2	2	9	20	12	100	69	5	999	2	5	14.1	7.8	13.2	59.1	83.2	12	5.02	413	10.40	87.5	.439	28.1	9
26	1	74	1	2	15	2	1	9	4	1	1	1	1	1	1	9	5	2	7	2	5	2	3	8	2	5	2	8	55	3	2	3	9	999	99	999	999	10	777	1	1	10.2	13.4	21.0	63.7	31.9	20	3.33	418	8.99	90.9	.303	30.7	9
27	1	77	1	2	7	2	1	2	1	4	2	1	1	4	1	9	3	9	2	3	2	5	3	8	2	3	2	8	99	9	9	2	9	999	23	85	100	9	41	1	1	11.2	8.3	20.1	41.2	130.2	58	4.64	451	12.84	78.2	.363	24.2	9
28	1	55	1	3	25	2	1	4	1	4	1	1	1	1	1	9	2	2	2	3	5	5	4	6	3	4	2	8	15	3	2	4	7	999	99	999	999	10	45	9	9	10.7	6.7	12.0	56.0	143.2	43	3.88	282	5.26	83.8	.325	27.5	3
29	1	52	1	3	7	2	1	9	4	4	1	1	1	1	1	9	2	2	2	3	2	3	4	5	2	2	9	8	77	3	1	4	2	999	79	999	999	10	777	2	5	14.2	99.9	99.9	99.9	999.9	25	5.49	371	8.06	82.4	.453	25.8	9
30	1	44	1	3	2	2	2	1	1	1	1	1	1	1	1	9	2	2	2	3	2	9	2	8	2	3	2	9	77	3	1	4	9	360	15	100	51	4	777	2	2	13.9	7.5	14.1	53.3	68.0	69	4.66	314	18.01	90.4	.422	29.9	9
31	1	50	1	2	3	2	1	6	2	1	1	1	1	1	1	9	4	2	2	3	6	3	3	7	3	3	2	2	99	9	9	4	2	60	10	95	87	4	999	1	1	14.0	9.1	13.3	68.4	34.9	59	5.16	472	10.47	84.9	.438	27.1	0
32	1	44	1	3	4	2	1	2	2	1	1	3	3	1	1	4	2	3	4	3	5	5	2	2	3	3	2	8	1	2	2	4	3	999	99	999	999	10	999	2	5	14.3	14.0	18.1	77.3	77.3	20	4.50	509	10.22	91.6	.412	31.7	9
33	1	57	1	3	27	2	2	4	1	1	1	1	1	5	20	9	4	2	3	3	3	3	2	8	3	5	2	2	2	3	1	3	9	30	3	95	87	0	777	2	6	11.4	10.2	14.8	68.9	28.1	40	4.34	602	9.62	82.3	.357	26.2	1
34	1	34	1	2	2	2	3	1	5	6	1	1	1	1	1	9	5	2	3	3	6	3	2	8	3	6	3	9	99	9	1	3	2	60	25	100	97	4	777	1	1	12.8	13.9	22.1	63.0	10.9	28	4.77	328	8.23	87.5	.418	26.7	9
35	1	57	1	2	7	2	1	4	3	5	2	1	1	1	1	9	4	2	2	3	4	9	2	8	9	3	2	2	99	3	1	4	9	240	26	105	63	8	41	2	1	9.5	4.7	6.6	71.2	176.0	35	4.17	591	13.09	73.7	.305	22.4	1
36	1	64	1	2	3	2	1	4	6	2	2	1	1	1	1	9	2	3	3	3	3	4	4	2	4	3	2	9	55	9	1	4	3	30	19	125	99	3	777	2	1	11.0	12.8	19.5	65.7	15.6	20	3.70	305	5.99	89.0	.330	29.6	0
37	1	38	1	3	9	2	1	4	2	4	1	1	1	1	1	9	2	2	5	3	5	2	8	5	2	3	2	8	1	4	9	3	2	999	99	999	999	10	27	2	5	11.6	7.3	13.7	53.3	87.5	60	4.60	327	7.74	73.9	.340	25.2	9
38	1	67	1	99	25	2	1	4	5	1	1	1	1	12	1	1	2	3	3	2	5	2	4	8	3	4	2	2	1	3	1	3	2	999	99	999	999	10	999	9	9	99.9	99.9	99.9	99.9	999.9	999	9.99	999	99.99	999.9	9.999	99.9	9
39	1	53	1	99	25	2	1	1	1	4	1	2	1	5	22	5	4	8	3	3	6	2	3	8	2	3	2	2	66	3	1	3	2	999	99	999	999	10	999	9	9	99.9	99.9	99.9	99.9	999.9	999	9.99	999	99.99	999.9	9.999	99.9	9
40	1	34	1	2	4	2	1	9	9	2	9	9	9	99	99	9	3	2	2	2	2	2	2	8	2	7	2	8	66	3	2	3	2	999	99	999	999	10	777	2	1	11.0	16.7	20.3	82.4	5.6	4	4.02	280	4.73	84.2	.339	27.4	9
41	1	66	1	2	17	2	3	4	2	1	1	1	1	1	1	9	2	8	5	3	2	2	8	5	2	5	2	9	99	3	1	3	9	30	11	100	86	3	41	1	1	11.4	12.3	17.0	72.4	9.6	30	4.38	307	6.74	77.9	.341	26.0	9
42	1	55	1	2	3	2	1	1	7	3	1	1	1	1	1	9	9	9	9	9	9	9	9	9	9	9	9	9	99	9	9	9	9	90	12	105	66	4	82	2	1	13.6	7.1	12.3	57.5	53.5	60	5.43	498	5.28	76.2	.414	24.1	1
43	1	38	1	2	5	2	1	1	8	4	1	1	1	1	1	9	4	2	2	3	5	5	4	5	8	2	4	9	99	2	3	4	2	999	99	999	999	10	777	1	1	10.7	5.3	7.2	73.3	8.7	33	4.18	432	9.31	80.5	.336	25.6	9
44	1	66	1	3	25	2	1	4	8	1	1	1	3	2	1	9	2	2	3	3	3	4	2	8	3	6	3	2	44	3	3	9	9	999	99	999	999	10	29	2	6	9.7	6.2	8.7	71.0	11.9	55	3.87	326	7.39	77.8	.301	25.0	1
45	1	46	2	3	13	2	1	4	6	2	1	2	1	1	1	9	4	2	3	3	5	3	2	6	9	3	2	8	1	2	1	4	2	90	5	85	99	0	999	2	2	11.0	10.7	16.3	65.6	73.3	73	3.89	654	12.18	81.9	.319	28.2	1
46	1	68	1	3	2	2	1	9	5	2	1	1	1	1	1	2	2	2	3	3	5	4	3	8	4	3	2	8	44	9	1	4	2	999	99	999	999	10	999	2	6	8.3	4.2	8.6	49.1	22.4	60	3.98	816	8.95	69.2	.275	21.0	1
47	1	58	1	3	15	2	2	1	6	1	1	2	1	1	1	9	4	2	2	3	5	3	8	8	3	4	2	8	44	9	1	4	9	180	2	80	112	0	999	2	2	8.0	4.1	7.0	58.9	8.8	47	4.16	680	9.58	60.8	.253	19.2	1
48	1	57	1	2	3	2	1	3	1	4	1	1	1	4	1	9	3	2	2	3	5	9	3	8	9	7	9	9	55	9	2	9	9	999	99	999	999	10	999	1	1	14.2	22.2	37.3	59.5	59.6	16	4.81	391	10.89	89.6	.431	29.4	9
49	1	61	1	2	4	2	1	1	5	2	1	1	1	1	1	9	3	8	3	3	5	3	2	8	2	5	2	2	77	3	1	9	9	10	6	75	142	0	999	2	1	11.7	9.3	16.3	57.2	17.8	30	4.12	245	5.30	85.1	.351	28.5	9
50	1	63	1	2	3	2	1	2	8	4	1	1	1	1	1	1	2	2	2	3	5	9	4	8	4	2	2	9	55	9	1	4	9	0	17	60	89	1	129	2	1	13.7	5.1	8.3	61.7	191.0	53	4.80	413	14.80	86.3	.420	28.5	9
51	1	62	1	3	6	2	2	1	3	4	1	1	1	6	1	9	9	9	9	9	9	9	9	9	9	9	9	9	99	9	9	9	9	30	0	70	72	3	29	2	4	10.6	5.2	8.4	61.6	25.4	45	4.90	359	6.50	70.6	.350	21.7	1
52	1	61	2	3	2	2	1	1	8	4	2	1	1	1	1	9	2	2	3	2	5	2	3	8	2	3	2	8	2	3	1	2	9	999	99	999	999	10	777	2	3	15.3	99.9	99.9	99.9	999.9	30	4.98	540	15.01	91.2	.454	30.7	9
53	1	77	1	2	3	2	1	1	1	2	1	1	1	5	22	9	2	2	2	3	5	3	3	5	2	3	2	2	77	3	1	3	7	999	99	999	999	10	777	1	1	12.4	17.8	24.6	72.4	15.1	20	4.51	330	6.66	88.6	.399	27.5	9
54	1	62	1	3	2	2	2	1	5	1	1	1	1	6	1	9	2	3	2	3	3	5	4	8	9	3	2	9	99	9	1	4	9	999	99	999	999	10	32	2	3	9.0	6.6	8.3	79.1	31.1	53	4.05	450	6.06	70.2	.285	22.2	1

Number of cases read: 54 Number of cases listed: 54

Number of cases read: 54 Number of cases listed: 54

Iron deficient subjects without RLS

4-Mar-89 SPSS-X RELEASE 3.1 FOR VAX/VMS
1:59:14 ASTON UNIVERSITY on KIRK:: VMS V4.7 Page

14-Mar-89 SPSS-X RELEASE 3.1 FOR VAX/VMS
11:59:16 ASTON UNIVERSITY on KIRK::

CASE	CC1	AGE	SEX	DISEAS	DURATN	EKBOMS	IRON	ANALGE	NSAIDS	SECOND	STEROD	H2ANTA	BENZOD	OTHER1	OTHER2	RELFAM	FEEL	DEPTH	SITE	BILAT	WHEN	TIME	RELEF1	RELEF2	SLEEP	FREQ	LAST	EXACER	STARTD	CORREL	LEGS	INCONV	OP	EMS	RITCHI	MCKON	AVGRIP	PAIN	CRP	LATEX	RWAAL	HB	FE	SATN	TIBC	FERRIT	ESR	RBC	PLTS	WBC	MCV	PCV	MCH	BM
01	1	59	1	6	77	1	1	1	1	1	1	1	1	27	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	11.6	99.9	99.9	99.9	7.3	999	9.99	999	99.99	80.1	9.999	26.4	9
02	1	75	1	6	77	1	1	2	5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	2	1	1	888	88	888	888	9	888	8	8	7.8	3.0	3.6	84.0	3.9	999	9.99	999	99.99	64.6	9.999	21.1	9
03	1	83	2	6	77	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	2	1	1	888	88	888	888	9	888	8	8	11.2	6.5	10.2	63.5	22.6	999	9.99	999	99.99	68.4	9.999	22.1	9
04	1	77	1	6	77	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	2	1	1	888	88	888	888	9	888	8	8	10.8	6.3	7.7	81.7	15.1	5	4.57	349	7.71	74.0	.338	23.6	9
05	1	73	2	6	77	1	1	1	1	1	1	1	1	22	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	2	1	1	888	88	888	888	9	888	8	8	9.5	3.7	5.3	69.7	2.2	8	3.88	374	7.81	74.6	.290	24.4	9
06	1	58	1	6	3	1	1	4	1	1	1	1	2	6	10	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	7.9	3.9	4.9	80.1	2.5	26	4.04	367	10.26	60.4	.244	19.5	9
07	1	80	1	6	77	1	1	3	8	1	1	1	1	4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	9	1	1	888	88	888	888	9	888	8	8	8.8	43.2	56.6	76.3	38.5	44	4.39	450	5.76	60.8	.267	19.9	9
08	1	52	1	6	77	1	3	1	1	1	1	1	1	1	1	3	1	1	1	1	1	1	1	1	1	1	1	1	88	1	3	1	1	888	88	888	888	9	888	8	8	11.6	17.5	24.1	72.5	13.0	12	4.15	283	5.40	82.9	.344	27.9	9
09	1	80	1	6	77	1	9	9	9	9	9	9	9	99	99	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	3	1	1	888	88	888	888	9	888	8	8	7.0	2.7	3.0	90.0	5.8	999	9.99	999	99.99	58.7	9.999	18.6	9
10	1	77	2	6	77	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	2	1	1	888	88	888	888	9	888	8	8	10.2	5.5	10.4	52.7	24.3	999	9.99	999	99.99	68.9	9.999	22.1	9
11	1	42	1	6	77	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	5	1	1	888	88	888	888	9	888	8	8	8.4	4.8	5.5	87.6	10.9	18	9.99	210	99.99	57.1	9.999	18.1	9
12	1	59	2	6	77	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	2	1	1	888	88	888	888	9	888	8	8	9.0	4.6	4.3	98.9	1.9	40	4.52	395	7.41	61.6	.279	20.0	9
13	1	71	1	6	77	1	1	9	9	1	9	9	9	21	99	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	9	1	1	888	88	888	888	9	888	8	8	7.4	4.0	5.2	77.3	999.9	999	4.24	535	10.92	70.7	.300	21.8	9
14	1	72	2	6	77	1	1	1	1	1	1	1	1	28	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	10.3	13.9	16.7	83.1	12.3	4	4.10	129	13.20	81.5	.334	25.2	9
15	1	68	1	6	77	1	2	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	9.4	99.9	99.9	99.9	999.9	50	4.29	651	10.75	66.4	.285	21.9	9
16	1	50	1	6	25	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	3	1	1	888	88	888	888	9	888	8	8	8.7	99.9	99.9	99.9	5.7	999	4.14	593	11.24	63.1	.261	21.0	9
17	1	73	1	6	3	1	2	1	2	1	1	1	1	29	1	4	1	1	1	1	1	1	1	1	1	1	1	1	88	1	2	1	1	888	88	888	888	9	888	8	8	9.5	23.0	27.2	84.6	3.4	25	3.49	292	6.78	84.2	.293	27.2	9
18	1	38	1	6	2	1	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	9.4	99.9	99.9	99.9	3.3	999	4.21	264	7.01	69.9	.294	22.2	9
19	1	76	2	6	77	1	2	1	1	1	1	1	1	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	9.7	5.7	8.4	68.2	999.9	17	5.42	363	10.11	59.9	.324	17.9	9
20	1	71	2	6	77	1	2	1	1	1	1	1	5	6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	12.5	7.8	9.9	78.5	5.1	9	5.42	374	9.74	74.9	.406	23.0	9
21	1	80	2	6	77	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	6.2	1.9	2.0	94.5	4.6	999	3.78	360	4.03	49.7	.188	16.5	9
22	1	51	1	6	77	1	1	1	1	1	1	1	1	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	2	1	1	888	88	888	888	9	888	8	8	2.9	5.6	6.0	93.5	1.0	999	1.61	490	10.86	52.8	.085	17.9	0
23	1	60	1	6	77	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	8.9	3.9	4.6	85.0	999.9	18	5.56	580	9.85	53.8	.299	16.0	9
24	1	51	1	6	77	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	2	1	1	888	88	888	888	9	888	8	8	9.3	1.0	1.2	85.3	2.9	110	4.21	387	8.05	68.0	.286	22.2	9
25	1	58	2	6	77	1	1	1	1	1	1	1	1	7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	2	1	1	888	88	888	888	9	888	8	8	10.5	38.0	44.4	85.5	5.7	22	5.18	186	9.17	61.6	.319	20.2	9
27	1	44	1	6	77	1	1	1	1	1	1	1	1	5	29	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	5.7	4.5	4.7	96.6	999.9	29	3.35	440	8.20	52.5	.176	17.1	9
28	1	40	1	6	4	1	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	11.9	99.9	99.9	99.9	13.7	20	4.58	294	4.16	77.7	.356	26.1	9

mber of cases read: 27 Number of cases listed: 27

Number of cases read: 27 Number of cases listed: 27

Iron deficient subjects with RLS

14-Mar-89 SPSS-X RELEASE 3.1 FOR VAX/VMS Page
11:59:17 ASTON UNIVERSITY on KIRK:: VMS V4.7

14-Mar-89 SPSS-X RELEASE 3.1 FOR VAX/VMS
11:59:18 ASTON UNIVERSITY on KIRK:: V

CASE	CC1	AGE	SEX	DISEAS	DURATN	EKBOMS	IRON	ANALGE	NSAIDS	SECOND	STEROD	H2ANTA	BENZOD	OTHER1	OTHER2	RELFAM	FEEL	DEPTH	SITE	BILAT	WHEN	TIME	RELEF1	RELEF2	SLEEP	FREQ	LAST	EXACER	STARTD	CORREL	LEGS	INCONV	GP	EMS	RITCHI	MCKON	AVGRIP	PAIN	CRP	LATEX	RWAAL	HB	FE	SATN	TIBC	FERRIT	ESR	RBC	PLTS	WBC	MCV	PCV	MCH	BM
1	1	67	1	6	77	2	1	9	9	9	9	9	9	99	99	1	2	3	2	3	3	5	8	6	3	5	9	4	77	5	2	3	7	888	88	888	888	9	868	8	8	7.7	9.6	11.0	86.9	6.6	999	9.99	999	99.99	57.0	9.999	19.4	9
2	1	45	1	6	77	2	1	9	9	9	9	9	9	99	99	9	5	9	7	3	5	5	8	8	9	4	9	9	99	5	9	3	9	888	88	888	888	9	888	8	8	8.8	2.3	2.3	98.9	6.6	57	9.99	383	99.99	64.8	9.999	21.8	9
3	1	69	1	6	77	2	2	9	9	9	9	9	9	19	1	9	3	2	3	2	5	5	4	8	9	2	2	8	66	5	9	9	9	888	88	888	888	9	888	8	8	6.7	13.3	14.8	90.1	6.3	16	3.71	240	9.30	57.6	.214	18.0	9
4	1	42	1	6	77	2	1	1	2	1	1	1	1	1	1	2	3	9	3	3	2	5	2	8	2	2	2	8	77	5	1	3	2	888	88	888	888	9	888	8	8	8.9	8.8	13.6	64.8	70.8	92	9.99	682	99.99	68.9	9.999	21.5	9
5	1	72	2	6	77	2	2	9	9	9	9	9	9	99	99	2	5	2	5	2	4	2	4	8	3	5	2	8	99	5	1	9	2	888	88	888	888	9	888	8	8	6.2	3.6	4.8	74.5	5.0	999	5.80	350	8.05	50.0	.190	16.4	9
6	1	71	2	6	77	2	1	1	1	1	1	1	2	6	29	9	4	2	3	2	4	3	3	5	3	3	2	9	1	5	5	4	2	888	88	888	888	9	888	8	8	11.2	6.2	7.1	87.5	6.7	999	9.99	999	99.99	74.8	9.999	24.1	9
7	1	45	1	6	77	2	1	1	1	1	1	1	1	30	1	1	5	2	2	3	3	5	3	8	4	2	2	7	2	5	4	4	7	888	88	888	888	9	888	8	8	9.4	99.9	99.9	99.9	999.9	999	4.26	437	8.43	67.9	.289	22.1	9
8	1	65	1	6	77	2	1	1	1	1	1	2	1	1	1	1	2	2	3	3	6	9	3	8	9	4	2	9	2	5	1	4	8	888	88	888	888	9	888	8	8	8.6	99.9	99.9	99.9	999.9	25	4.43	999	10.20	67.0	.290	19.4	9
9	1	39	1	6	77	2	1	1	1	1	1	1	1	1	1	2	2	2	3	3	6	5	4	8	2	2	2	3	4	5	5	2	2	888	88	888	888	9	888	8	8	9.4	5.3	6.2	84.9	10.1	999	9.99	999	99.99	67.0	9.999	20.9	9

Number of cases read: 9 Number of cases listed: 9

Number of cases read: 9 Number of cases listed: 9

Normal healthy subjects without RLS

14-Mar-89 SPSS-X RELEASE 3.1 FOR VAX/VMS Page
11:59:08 ASTON UNIVERSITY on KIRK:: VMS V4.7

14-Mar-89 SPSS-X RELEASE 3.1 FOR VAX/VMS
11:59:10 ASTON UNIVERSITY on KIRK:: V

CASE	CC1	AGE	SEX	DISEAS	DURATN	EKBOMS	IRON	ANALGE	NSAIDS	SECOND	STEROD	H2ANTA	BENZOD	OTHER1	OTHER2	RELFAM	FEEL	DEPTH	SITE	BILAT	WHEN	TIME	RELEF1	RELEF2	SLEEP	FREQ	LAST	EXACER	STARTD	CORREL	LEGS	INCONV	GP	EMS	RITCHI	MCKON	AVGRIP	PAIN	CRP	LATEX	RWAAL	HB	FE	SATN	TIBC	FERRIT	ESR	RBC	PLTS	WBC	MCV	PCV	MCH	BM
001	1	75	2	1	77	1	1	1	1	1	1	1	1	5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	13.7	10.3	13.9	73.9	999.9	57	4.81	309	6.85	84.6	.406	28.6	9
002	1	63	2	1	77	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	14.8	22.5	31.2	72.0	999.9	999	4.40	235	5.91	102.5	.451	33.6	9
003	1	66	2	1	77	1	1	1	1	1	1	1	1	23	1	3	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	14.7	19.6	26.4	74.2	999.9	999	4.76	233	6.39	92.7	.441	30.8	9
004	1	37	2	1	77	1	1	1	1	1	1	1	1	1	1	3	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	14.3	14.7	23.2	63.2	999.9	999	4.22	239	8.75	99.4	.419	34.0	9
005	1	28	2	1	77	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	14.1	10.4	14.7	70.3	999.9	999	5.12	237	5.77	82.1	.420	27.5	9
006	1	29	2	1	77	1	1	1	1	1	1	1	1	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	9	1	1	888	88	888	888	9	888	8	8	13.9	14.3	21.7	65.9	71.7	999	4.59	205	4.99	87.6	.402	30.4	9
007	1	35	2	1	77	1	1	1	1	1	1	1	1	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	9	1	1	888	88	888	888	9	888	8	8	16.2	20.7	46.5	45.8	110.2	999	4.71	336	10.98	100.2	.472	34.5	9
008	1	24	2	1	77	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	15.3	19.2	36.6	52.5	202.8	999	5.15	318	5.20	87.9	.453	29.7	9
009	1	29	2	1	77	1	1	1	1	1	1	1	1	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	9	1	1	888	88	888	888	9	888	8	8	14.5	41.8	59.6	70.1	10.6	999	4.92	286	8.06	85.8	.422	29.5	9
010	1	54	2	1	77	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	14.6	21.9	26.6	82.2	999.9	12	4.60	280	6.15	92.6	.426	31.9	9
011	1	64	2	1	77	1	1	1	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	15.8	16.7	24.8	67.4	999.9	999	5.25	330	8.86	91.8	.482	30.1	9
012	1	31	2	1	77	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	15.6	17.0	26.0	65.2	999.9	999	5.10	313	9.61	90.8	.463	30.5	9
013	1	30	2	1	77	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	14.6	22.7	25.8	87.9	999.9	999	5.24	209	4.87	86.3	.452	27.9	9
014	1	57	2	1	77	1	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	13.6	21.8	28.3	76.9	999.9	999	4.19	242	5.29	96.2	.403	32.5	9
015	1	56	2	1	77	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	14.4	16.8	27.9	60.3	999.9	999	4.98	232	8.11	88.3	.440	28.9	9
016	1	42	2	1	77	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	15.7	15.3	21.0	72.8	999.9	12	5.14	313	7.23	90.4	.465	30.5	9
017	1	42	2	1	77	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	2	1	1	888	88	888	888	9	888	8	8	14.1	13.0	20.2	64.1	999.9	1	4.29	270	10.71	96.7	.415	33.0	9
018	1	33	2	1	77	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	99.9	99.9	99.9	99.9	999.9	999	9.99	999	99.99	999.9	9.999	99.9	9
019	1	42	2	1	77	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	99.9	99.9	99.9	99.9	999.9	999	9.99	999	99.99	999.9	9.999	99.9	9
020	1	28	1	1	77	1	1	1	1	1	1	1	1	24	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	2	1	1	888	88	888	888	9	888	8	8	14.6	23.8	30.9	77.0	997.9	999	4.79	336	5.47	90.6	.434	30.5	9
021	1	26	1	1	77	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	2	1	1	888	88	888	888	9	888	8	8	12.8	11.4	15.3	74.3	999.9	9	4.31	384	8.61	87.5	.378	29.8	9
022	1	28	1	1	77	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	14.1	7.0	10.0	69.9	7.8	999	4.38	209	5.38	88.9	.407	30.8	9
023	1	34	1	1	77	1	9	9	9	9	9	9	9	99	99	9	9	9	9	9	9	9	9	9	9	9	9	9	99	9	9	9	9	888	88	888	888	9	888	8	8	13.6	16.2	29.6	54.7	26.7	999	6.07	310	6.07	89.4	.382	31.8	9
024	1	43	1	1	77	1	1	1	1	1	1	1	1	12	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	9	1	1	888	88	888	888	9	888	8	8	14.2	27.9	37.8	73.8	55.7	999	4.46	216	6.27	91.6	.409	31.8	9
025	1	30	1	1	77	1	1	1	1	1	1	1	1	24	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	13.0	13.6	18.5	73.1	999.9	999	4.44	361	15.11	86.2	.383	29.3	9
026	1	19	1	1	77	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	88	1	2	1	1	888	88	888	888	9	888	8	8	13.6	33.2	50.9	65.1	999.9	6	4.53	322	8.19	89.7	.406	30.1	9
027	1	26	1	1	77	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	15.1	21.1	33.8	62.4	65.2	999	5.08	226	6.01	89.7	.456	29.6	9
028	1	25	1	1	77	1	1	1	1	1	1	1	1	1	1	9	9	9	9	9	9	9	9	9	9	9	9	9	88	1	9	1	1	888	88	888	888	9	888	8	8	15.3	29.6	52.5	56.4	42.4	999	4.64	266	5.34	93.4	.433	33.0	9
029	1	24	1	1	77	1	1	1	1	1	1	1	1	25	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	11.7	17.1	20.6	82.8	999.9	2	4.50	330	7.80	84.4	.380	26.1	9
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031	1	44	1	1	77	1	3	1	1	1	1	1	1	26	1	4	1	1	1	1	1	1	1	1	1	1	1	1	88	1	2	1	1	888	88	888	888	9	888	8	8	9.5	5.2	5.6	92.3	999.9	999	4.18	354	9.29	70.9	.296	22.8	9
032	1	32	1	1	77	1	1	1	1	1	1	1	1	22	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	14.0	22.3	29.4	75.7	999.9	999	4.57	186	6.73	92.9	.424	30.6	9
033	1	38	1	1	77	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	12.3	11.1	20.9	52.9	999.9	999	4.62	232	10.37	83.2	.384	26.7	9
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7	1	40	2	1	77	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	16.0	25.2	27.8	90.6	999.9	999	5.43	288	6.63	91.2	.495	29.5	9
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9	1	37	1	1	77	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	2	1	1	888	88	888	888	9	888	8	8	13.4	37.7	49.2	76.6	999.9	999	4.31	299	7.31	95.3	.411	31.2	9
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2	1	38	1	1	77	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	88	1	4	1	1	888	88	888	888	9	888	8	8	13.9	23.6	27.4	85.1	999.9	999	5.17	239	6.27	83.2	.431	26.8	9
3	1	38	1	1	77	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	14.0	22.4	29.8	75.2	999.9	999	4.66	235	6.08	91.0	.424	29.9	9
4	1	20	1	1	77	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	15.3	15.2	19.3	78.7	999.9	999	5.06	309	7.76	92.3	.467	30.2	9
5	1	25	1	1	77	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	13.9	23.7	30.3	78.3	999.9	999	4.68	259	3.48	90.9	.425	29.7	9
6	1	26	1	1	77	1	1	1	1	1	1	1	1	1	1	4	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	13.9	23.1	28.2	81.8	999.9	999	4.62	331	9.11	91.5	.423	30.2	9
7	1	39	2	1	77	1	1	1	1	1	1	1	1	1	1	3	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	14.5	19.0	24.3	77.9	999.9	999	5.10	237	4.25	89.8	.459	28.5	9
8	1	37	2	1	77	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	4	1	1	888	88	888	888	9	888	8	8	99.9	99.9	99.9	99.9	999.9	999	9.99	999	99.99	999.9	9.999	99.9	9
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2	1	22	2	1	77	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	99.9	99.9	99.9	99.9	999.9	999	9.99	999	99.99	999.9	9.999	99.9	9
3	1	29	2	1	77	1	1	1	1	1	1	1	1	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	99.9	99.9	99.9	99.9	999.9	999	9.99	999	99.99	999.9	9.999	99.9	9
	1	55	2	1	77	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	3	1	1	888	88	888	888	9	888	8	8	99.9	99.9	99.9	99.9	999.9	999	9.99	999	99.99	999.9	9.999	99.9	9
	1	39	2	1	77	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	99.9	99.9	99.9	99.9	999.9	999	9.99	999	99.99	999.9	9.999	99.9	9
	1	32	2	2	77	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	99.9	99.9	99.9	99.9	999.9	999	9.99	999	99.99	999.9	9.999	99.9	9
	1	35	1	1	77	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	3	1	1	888	88	888	888	9	888	8	8	99.9	99.9	99.9	99.9	999.9	999	9.99	999	99.99	999.9	9.999	99.9	9
	1	28	2	1	77	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	99.9	99.9	99.9	99.9	999.9	999	9.99	999	99.99	999.9	9.999	99.9	9
	1	73	1	1	77	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	2	1	1	888	88	888	888	9	888	8	8	99.9	99.9	99.9	99.9	999.9	999	9.99	999	99.99	999.9	9.999	99.9	9
	1	27	1	1	77	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	99.9	99.9	99.9	99.9	999.9	999	9.99	999	99.99	999.9	9.999	99.9	9
	1	21	1	1	77	1	1	1	1	1	1	1	1	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	99.9	99.9	99.9	99.9	999.9	999	9.99	999	99.99	999.9	9.999	99.9	9
	1	48	1	1	77	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	99.9	99.9	99.9	99.9	999.9	999	9.99	999	99.99	999.9	9.999	99.9	9
	1	23	1	1	77	1	1	1	1	1	1	1	1	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	2	1	1	888	88	888	888	9	888	8	8	99.9	99.9	99.9	99.9	999.9	999	9.99	999	99.99	999.9	9.999	99.9	9
	1	19	1	1	77	1	2	1	1	1	1	1	1	24	1	9	1	1	1	1	1	1	1	1	1	1	1	1	88	1	2	1	1	888	88	888	888	9	888	8	8	99.9	99.9	99.9	99.9	999.9	999	9.99	999	99.99	999.9	9.999	99.9	9
	1	64	1	1	77	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	99.9	99.9	99.9	99.9	999.9	999	9.99	999	99.99	999.9	9.999	99.9	9
	1	27	1	1	77	1	1	1	1	1	1	1	1	11	1	0	1	3	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	99.9	99.9	99.9	99.9	999.9	999	9.99	999	99.99	999.9	9.999	99.9	9
	1	28	1	1	77	1	1	1	1	1	1	1	1	11	1	0	1	2	1	1	1	1	1	1	1	1	1	1	88	1	4	1	1	888	88	888	888	9	888	8	8	99.9	99.9	99.9	99.9	999.9	999	9.99	999	99.99	999.9	9.999	99.9	9
	1	44	1	1	77	1	1	1	1	1	1	1	1	11	1	0	1	1	1	1	1	1	1	1	1	1	1	1	88	1	3	1	1	888	88	888	888	9	888	8	8	99.9	99.9	99.9	99.9	999.9	999	9.99	999	99.99	999.9	9.999	99.9	9
	1	50	1	1	77	1	1	1	1	1	1	1	1	11	1	0	1	1	1	1	1	1	1	1	1	1	1	1	88	1	3	1	1	888	88	888	888	9	888	8	8	99.9	99.9	99.9	99.9	999.9	999	9.99	999	99.99	999.9	9.999	99.9	9
	1	39	1	1	77	1	1	1	1	1	1	1	1	11	1	0	1	9	1	1	1	1	1	1	1	1	1	1	88	1	1	1	1	888	88	888	888	9	888	8	8	99.9	99.9	99.9	99.9	999.9	999	9.99	999	99.99	999.9	9.999	99.9	9
	1	40	1	1	77	1	1	1	1	1	1	1	1	11	1	0	1	3	1	1	1	1	1	1	1	1	1	1	88	1	3	1	1	888	88	888	888	9	888	8	8	99.9	99.9	99.9	99.9	999.9	999	9.99	999	99.99	999.9	9.999	99.9	9

Number of cases read: 71 Number of cases listed: 71

Number of cases read: 71 Number of cases listed: 71

Normal healthy subjects with RLS

14-Mar-89 SPSS-X RELEASE 3.1 FOR VAX/VMS Page
11:59:12 ASTON UNIVERSITY on KIRK:: VMS V4.7

14-Mar-89 SPSS-X RELEASE 3.1 FOR VAX/VMS
11:59:13 ASTON UNIVERSITY on KIRK:: V

CASE	CC1	AGE	SEX	DISEAS	DURATN	EKBOMS	IRON	ANALGE	NSAIDS	SECOND	STEROD	H2ANTA	BENZOD	OTHER1	OTHER2	RELFAM	FEEL	DEPTH	SITE	BILAT	WHEN	TIME	RELEF1	RELEF2	SLEEP	FREQ	LAST	EXACER	STARTD	CORREL	LEGS	INCONV	GP	EMS	RITCHI	MCKON	AVGRIP	PAIN	CRP	LATEX	RWAAL	HB	FE	SATN	TIBC	FERRIT	ESR	RBC	PLTS	WBC	MCV	PCV	MCH	BM
1	1	26	1	1	77	2	1	1	1	1	1	1	1	1	1	2	3	2	3	3	5	3	4	8	2	5	2	8	77	5	1	9	9	888	88	888	888	9	888	8	8	13.5	18.9	23.0	82.5	999.9	999	4.95	.413	7.30	81.5	.403	27.2	9
2	1	37	1	1	77	2	1	1	1	1	1	1	1	1	1	5	4	2	3	3	6	3	4	8	3	3	2	2	13	5	1	4	2	888	88	888	888	9	888	8	8	13.4	7.7	11.8	65.5	7.6	999	4.61	241	6.09	86.4	.399	29.0	9
3	1	35	1	1	77	2	1	1	1	1	1	1	1	1	1	4	2	2	2	3	5	5	4	8	3	5	2	2	4	5	1	4	2	888	88	888	888	9	888	8	8	13.0	15.9	23.7	67.0	999.9	8	4.26	188	6.71	88.6	.377	30.5	9
4	1	47	1	1	77	2	1	1	1	1	1	1	2	1	1	6	4	2	2	2	6	4	2	8	4	3	2	7	2	5	1	4	7	888	88	888	888	9	888	8	8	12.0	8.3	13.8	60.0	999.9	999	4.17	368	7.93	88.2	.368	28.7	9
5	1	24	1	1	77	2	1	1	1	1	1	1	1	1	1	9	3	9	5	3	3	3	3	8	3	5	2	2	77	5	1	3	2	888	88	888	888	9	888	8	8	12.9	23.6	35.6	66.2	999.9	999	4.27	219	7.11	91.9	.392	30.3	9
6	1	55	1	1	77	2	1	1	1	1	1	1	1	1	1	9	2	9	2	2	3	2	8	5	2	4	2	8	77	5	9	2	2	888	88	888	888	9	888	8	8	13.0	20.7	33.0	62.8	999.9	3	3.97	278	6.17	93.6	.371	32.7	9
7	1	41	1	1	77	2	1	1	1	1	1	1	1	1	1	9	4	9	5	3	6	5	3	8	2	5	2	8	20	5	2	3	8	888	88	888	888	9	888	8	8	14.2	21.3	23.6	90.3	999.9	999	4.80	265	4.58	90.2	.433	29.6	9
8	1	27	2	1	77	2	1	1	1	1	1	1	1	1	1	9	2	2	3	2	5	4	2	3	3	6	4	8	13	5	3	4	2	888	88	888	888	9	888	8	8	99.9	99.9	99.9	99.9	999.9	999	9.99	999	99.99	999.9	9.999	99.9	9

Number of cases read: 8 Number of cases listed: 8

Number of cases read: 8 Number of cases listed: 8

EXAMPLES OF STATISTICAL ANALYSIS PERFORMED USING SPSS[x]

```
14-Mar-89   SPSS-X RELEASE 3.1 FOR VAX/VMS
13:31:37    ASTON UNIVERSITY                 on KIRK::          VMS V4.7

EKBOMS  by  SEX

                    SEX            Page 1 of 1
            Count  I
          Row Pct  IFEMALE   MALE
          Col Pct  I                    Row
                   I     1  I     2  I Total
EKBOMS    ---------+--------+--------+
                1  I    59  I    38  I    97
  NEGATIVE         I  60.8  I  39.2  I  64.2
                   I  54.1  I  90.5  I
                   +--------+--------+
                2  I    50  I     4  I    54
  POSITIVE         I  92.6  I   7.4  I  35.8
                   I  45.9  I   9.5  I
                   +--------+--------+
           Column     109       42      151
            Total    72.2     27.8    100.0

      Chi-Square                 Value          DF              Significance
--------------------          -----------      ----            ------------

Pearson                        17.43582          1                 .00003
Continuity Correction          15.88950          1                 .00007
Likelihood Ratio               20.13484          1                 .00001
Mantel-Haenszel                17.32035          1                 .00003
Fisher's Exact Test:
   One-Tail                                                        .00001
   Two-Tail                                                        .00002

Minimum Expected Frequency -   15.020


Number of Missing Observations:  0
```

```
14-Mar-89   SPSS-X RELEASE 3.1 FOR VAX/VMS
13:31:37    ASTON UNIVERSITY                 on KIRK::          VMS V4.7

Preceding task required 1.33 seconds CPU time;  4.88 seconds elapsed.

 111  0  TEMPORARY
 112  0  SELECT IF        SUBOP EQ 1
 113  0  NPAR TESTS       M-W=AGE BY EKBOMS(1,2)

There are 611,392 bytes of memory available.

***** Workspace allows for 16383 cases for NPAR tests *****
```

```
14-Mar-89   SPSS-X RELEASE 3.1 FOR VAX/VMS
13:31:39    ASTON UNIVERSITY                 on KIRK::          VMS V4.7

- - - - - Mann-Whitney U - Wilcoxon Rank Sum W Test

     AGE
  by EKBOMS

      Mean Rank    Cases

        79.14         97  EKBOMS = 1  NEGATIVE
        70.35         54  EKBOMS = 2  POSITIVE
                     ---
                     151  Total

                                    Corrected for ties
          U            W                 Z      2-Tailed P
      2314.0        3799.0          -1.1848        .2361
```

```
14-Mar-89   SPSS-X RELEASE 3.1 FOR VAX/VMS
13:31:39    ASTON UNIVERSITY                 on KIRK::          VMS V4.7

Preceding task required .27 seconds CPU time;  1.96 seconds elapsed.

 114  0  TEMPORARY
 115  0  SELECT IF        SUBOP EQ 1 AND EKBOMS EQ 1
 116  0  RECODE           AGE(LO THRU 29=1)(30 THRU 39=2)(40 THRU 49=3)(50 THRU 59=4)
 117  0                   (60 THRU 69=5)(70 THRU 79=6)(80 THRU 89=7)
 118  0  FREQUENCIES      VARIABLES=AGE

There are 610,752 bytes of memory available.

Memory allows a total of 20,828 values accumulated across all variables.
There may be up to 5,207 value labels for each variable.
```

```
14-Mar-89   SPSS-X RELEASE 3.1 FOR VAX/VMS
13:31:41    ASTON UNIVERSITY                 on KIRK::          VMS V4.7

AGE

                                                        Valid      Cum
  Value Label                 Value  Frequency  Percent  Percent  Percent

                                  1          2      2.1      2.1      2.1
                                  2          5      5.2      5.2      7.2
                                  3         16     16.5     16.5     23.7
                                  4         26     26.8     26.8     50.5
                                  5         28     28.9     28.9     79.4
                                  6         20     20.6     20.6    100.0
                                        -------  -------  -------
                              Total         97    100.0    100.0

Valid cases      97      Missing cases      0
```

```
14-Mar-89   SPSS-X RELEASE 3.1 FOR VAX/VMS
13:31:41    ASTON UNIVERSITY                 on KIRK::          VMS V4.7

Preceding task required .27 seconds CPU time;  1.58 seconds elapsed.

 119  0  TEMPORARY
 120  0  SELECT IF        SUBOP EQ 1
 121  0  DESCRIPTIVES     VARIABLES=AGE HB/

There are 611,136 bytes of memory available.

148 bytes of memory required for the DESCRIPTIVES procedure.
4 bytes have already been acquired.
144 bytes remain to be acquired.
```

APPENDIX A9

DEMOGRAPHIC DETAILS OF SUBJECTS RECRUITED TO CLINICAL TRIAL

Rheumatoid subjects

Subject A

Age (years)	44	
Sex	Female	
Duration of rheumatoid disease (years)		3
Rheumatoid factor	Latex	+ve
	Rose Waaler	1/32
Drug therapy		
Analgesics	Nil	
NSAID	Indomethacin SR	
2nd line agent	Hydroxychloroquine	
Other	Nil	
Duration of RLS	-	

Subject B

Age (years)	55	
Sex	Female	
Duration of rheumatoid disease (years)		25
Rheumatoid factor	Latex	-ve
	Rose Waaler	-
Drug therapy		
Analgesics	Co-proxamol	
NSAID	Piroxicam	
2nd line agent	Sulphasalazine	
Other	Temazepam	
Duration of RLS (years)		15
Onset with respect to RA		After

Subject C

Age (years)	60	
Sex	Male	
Duration of rheumatoid disease (years)		4
Rheumatoid factor	Latex	-ve
	Rose Waaler	-
Drug therapy		
Analgesics	Nil	
NSAID	Indomethacin	
2nd line agent	Penicillamine	
Other	Lorazepam	
Duration of RLS (years)		<4
Onset with respect to RA		After

Subject D

Age (years) 80
Sex Female

Duration of rheumatoid disease (years) 19
Rheumatoid factor Latex -ve
Rose Waaler -

Drug therapy
Analgesics Paracetamol
NSAID Fenbufen
2nd line agent Nil
Other Frumil

Duration of RLS (years) 2
Onset with respect to RA After

Subject E

Age (years) 50
Sex Female

Duration of rheumatoid disease (years) 3
Rheumatoid factor Latex -ve
Rose Waaler -

Drug therapy
Analgesics Nil
NSAID Indomethacin
2nd line agent Nil
Other Nil

Duration of RLS (years) -

Subject F

Age (years) 47
Sex Male

Duration of rheumatoid disease (years) 20
Rheumatoid factor Latex +ve
Rose Waaler -ve

Drug therapy
Analgesiscs Co-proxamol
NSAID Piroxicam
2nd line agent Sulphasalazine
Other Ranitidine

Duration of RLS (years) 1
Onset with respect to RA After

Subject G

Age (years) 65
Sex Female

Duration of rheumatoid disease (years) 2
Rheumatoid factor Latex +ve
Rose Waaler -ve

Drug therapy
Analgesiscs Co-proxomol

	NSAID	Tenoxicam
	3rd line agent	Prednisolone
	Other	Nil
	Duration of RLS (years)	2.5
	Onset with respect to RA	Before

Subject H

	Age (years)	68
	Sex	Female
	Duration of rheumatoid disease (years)	3
	Rheumatoid factor Latex	-ve
	Rose Waaler	-
	Drug therapy	
	Analgesics	Nil
	NSAID	Diclofenac
	2nd line agent	Gold, Sulphasalazine
	Other	Nil
	Duration of RLS (years)	>3
	Onset with respect to RA	Before

Subject I

	Age (years)	35
	Sex	Female
	Duration of rheumatoid disease (years)	3
	Rheumatoid factor Latex	-ve
	Rose Waaler	-
	Drug therapy	
	Analgesics	Paracetamol
	NSAID	Diclofenac
	2nd line agent	Sulphasalazine
	Other	Nil
	Duration of RLS (years)	-

Subject J

	Age (years)	71
	Sex	Female
	Duration of rheumatoid disease (years)	18
	Rheumatoid factor Latex	+ve
	Rose Waaler	-ve
	Drug therapy	
	Analgesics	Nil
	NSAID	Sulindac
	2nd line agent	Hydroxychloroquine
	Other	Dipyridamole
	Duration of RLS (years)	years
	Onset with respect to RA	After

Subject K

Age (years) 63
Sex Female

Duration of rheumatoid disease (years) 30
Rheumatoid factor Latex +ve
Rose Waaler -ve
Drug therapy
Analgesics Co-proxomol
NSAID Nil
2nd line agent Sulphasalazine
Other Salbutamol, Beclomethasone inhalers

Duration of RLS (years) -

Subject L

Age (years) 53
Sex Female

Duration of rheumatoid disease (years) 15
Rheumatoid factor Latex -ve
Rose Waaler -

Drug therapy
Analgesics Dihydrocodeine
NSAID Indomethacin
2nd line agent Nil
Other Bendrofluazide, Verapamil

Subject M

Age (years) 62
Sex Female

Duration of rheumatoid disease (years) 6
Rheumatoid factor Latex +ve
Rose Waaler -ve

Drug therapy
Analgesics Nil
NSAID Sulindac
2nd line agent Sulphasalazine
Other Ranitidine, Gaviscon

Duration of RLS (years) 0.75
Onset with respect to RA After
Other Bendrofluazide, Verapamil

<u>Non-rheumatoid subjects</u>

Subject X

Age (years) 58
Sex Male

Concurrent disease Nil known

Drug therapy
Garlic pills occasionally

Subject Y

Age (years) 49
Sex Female

Concurrent disease Osteoarthritis

Drug therapy
Naproxen

VALUES FOR VARIABLES MONITORED DURING TRIAL FOR INDIVIDUAL SUBJECTS

Haemotological variables

HAEMOGLOBIN (g/dl)

Patient	Control	Values at beginning of phase: Placebo run-in	Iron	Placebo	Follow-up
Rheumatoid subjects					
A	11.0	10.2	10.1	N/A	11.9
C	14.1	13.4	12.4	N/A	13.9
F	11.0	11.0	10.6	N/A	11.2
G	12.7	12.3	11.6	N/A	11.5
H	9.1	8.8	9.2	10.4	10.7
I	10.9	10.6	10.3	12.4	12.7
K	13.1	13.0	12.7	13.1	13.1

	Control	Placebo run-in	Placebo	Iron	Follow-up
B	11.1	11.4	10.7	N/A	10.3
D	11.2	10.3	10.4	N/A	11.6
E	14.0	13.6	13.6	12.2	13.1
J	11.1	-	11.5	11.1	11.1
L	12.1	11.4	12.0	12.1	12.5
Non-rheumatoid subjects					
X	-	-	15.6		-
Y	14.2	13.8	13.7	12.6	-

SERUM IRON (umol/l)

Patient	Control	Values at beginning of phase: Placebo run-in	Iron	Placebo	Follow-up
Rheumatoid subjects					
A	6.0	3.2	3.5	N/A	5.3
C	21.6	18.7	24.8	N/A	29.1
F	10.7	5.1	5.3	N/A	10.1
G	10.4	5.0	7.5	N/A	7.4
H	-	5.3	4.7	6.0	5.4
I	4.9	7.7	5.4	19.0	11.9
K	18.9	-	12.6	11.7	14.1
	Control	Placebo run-in	Placebo	Iron	Follow-up
B	8.4	-	6.4	N/A	8.3
D	7.9	-	3.8	N/A	5.0
E	20.7	11.0	11.2	7.9	7.4
J	25.1	-	19.5	13.7	16.5
L	4.8	12.0	5.0	19.3	8.8
Non-rheumatoid subjects					
X	-	-	-	16.0	-
Y	21.0	18.0	21.0	9.8	-

TRANSFERRIN SATURATION %

Patient	Values at beginning of phase				
	Control	Placebo run-in	Iron	Placebo	Follow-up
Rheumatoid subjects					
A	8.3	5.3	5.3	N/A	9.5
C	29.7	22.9	31.2	N/A	-
F	16.3	8.2	8.9	N/A	16.8
G	15.8	16.9	11.7	N/A	10.7
H	-	7.7	6.1	8.3	7.0
I	7.7	9.6	13.4	31.7	15.8
K	26.3	-	20.5	17.2	21.1
	Control	Placebo run-in	Placebo	Iron	Follow-up
B	14.0	-	10.9	N/A	15.4
D	9.7	-	4.9	N/A	5.8
E	28.7	16.1	18.1	9.7	9.5
J	36.8	-	27.9	22.4	23.7
L	7.1	14.4	5.7	21.6	10.2
Non-rheumatoid subjects					
X	-	-	-	28.8	-
Y	32.5	23.0	26.2	13.1	

TOTAL IRON BINDING CAPACITY (umol/l)

Patients	Values at beginning of phase				
	Control	Placebo run-in	Iron	Placebo	Follow-up
Rheumatoid subjects					
A	72.4	60.7	66.2	N/A	55.7
C	72.7	81.8	79.4	N/A	54.0
F	65.6	62.3	59.7	N/A	60.1
G	66.0	69.5	63.9	N/A	68.9
H	-	68.4	77.4	72.0	76.8
I	63.7	80.0	40.2	60.0	75.3
K	71.8	-	61.4	68.2	66.7

	Control	Placebo run-in	Placebo	Iron	Follow-up
B	60.0	-	45.4	N/A	53.9
D	81.4	-	77.5	N/A	85.6
E	72.1	68.4	61.9	81.6	77.9
J	68.2	-	70.0	61.2	69.6
L	67.3	83.3	88.0	89.3	86.4
Non-rheumatoid					
X	-	-	-	55.6	-
Y	64.6	78.3	80.0	74.6	-

SERUM FERRITIN CONCENTRATION (ug/l)

Patients	Values at beginning of phase				
	Control	Placebo run-in	Iron	Placebo	Follow-up
Rheumatoid subjects					
A	3.8	5.0	4.3	N/A	14.4
C	19.5	36.6	18.8	N/A	62.6
F	73.3	34.2	37.4	N/A	46.2
G	7.4	11.4	18.5	N/A	23.5
H	-	13.2	8.5	19.4	19.2
I	6.3	4.4	8.1	15.0	8.5
K	46.8	-	30.5	52.0	48.1

	Control	Placebo run-in	Placebo	Iron	Follow-up
B	57.0	-	45.4	N/A	82.8
D	4.7	23.7	6.8	N/A	11.4
E	32.8	22.4	29.3	-	6.7
J	43.7	-	28.5	30.0	38.5
L	16.9	9.9	9.8	16.8	20.2
Non-rheumatoid subjects					
X	-	-	-	87.8	-
Y	30.8	44.8	38.4	40.5	

MEAN CELL VOLUME (fl)

Patients	Control	Values at beginning of phase Placebo run-in	Iron	Placebo	Follow-up
Rheumatoid subjects					
A	71.2	69.9	68.0	N/A	77.6
C	90.1	89.8	88.4	N/A	87.7
F	81.9	84.9	82.1	N/A	77.5
G	87.7	87.1	89.4	N/A	88.4
H	69.3	68.7	67.9	74.0	76.4
I	79.8	79.5	78.2	85.3	88.2
K	86.9	84.9	87.3	87.8	89.4
	Control	Placebo run-in	Placebo	Iron	Follow-up
B	77.7	79.6	80.0	N/A	79.5
D	84.7	74.8	77.0	N/A	74.6
E	85.6	86.0	86.8	82.1	81.5
J	83.5	-	81.6	82.9	83.8
L	75.7	73.5	75.9	76.1	76.3
Non-rheumatoid subjects					
X	-	-	-	87.8	-
Y	94.9	96.7	96.1	93.9	-

Clinical and haematological indicators of disease activity

ERYTHROCYTE SEDIMENTATION RATE (mm/1st hour)

Patients	Control	Placebo run-in	Iron	Placebo	Follow-up
Rheumatoid subjects					
A	12	10	10	N/A	14
C	5	6	8	N/A	10
F	73	58	67	N/A	52
G	7	42	37	N/A	34
H	40	48	30	24	40
I	30	36	26	10	9
K	40	25	40	30	42
	Control	Placebo run-in	Placebo	Iron	Follow-up
B	70	52	67	N/A	83
D	19	32	33	N/A	41
E	22	40	41	58	25
J	35	-	23	20	30
L	36	35	25	24	52
Non-rheumatoid subjects					
X	-	-	-	-	-
Y	-	4	16	23	-

RITCHIE ARTICULAR INDEX

Patients	Values at beginning of phase				
	Control	Placebo run-in	Iron	Placebo	Follow-up
Rheumatoid subjects only					
A	-	7	9	N/A	15
C	-	15	-	N/A	15
F	5	13	-	N/A	-
G	-	-	-	N/A	27
H	-	-	9	8	12
I	-	-	17	17	31
K	-	-	25	26	29
	Control	Placebo run-in	Placebo	Iron	Follow-up
B	-	-	-	N/A	11
D	-	-	28	N/A	31
E	-	10	-	2	16
J	-	-	19	24	14
L	-	-	28	25	-

EARLY MORNING STIFFNESS (mins)

Patient	Value at beginning of phase				
	Control	Placebo run-in	Iron	Placebo	Follow-up
Rheumatoid subjects only					
A	-	0	60	N/A	30
C	-	20	-	N/A	20
F	90	90	-	N/A	-
G	-	-	-	N/A	480
H	-	-	0	120	60
I	-	-	30	30	30
K	-	-	0	30	60

	Control	Placebo run-in	Placebo	Iron	Follow-up
B	-	-	-	N/A	11
D	-	-	20	N/A	30
E	-	60	-	60	120
J	-	-	0	0	0
L	-	-	360	90	-

AVERAGE GRIP STRENGTHS OF BOTH HANDS (mmHg)

Patients		Values at beginning of phase				
		Control	Placebo run-in	Iron	Placebo	Follow-up
Rheumatoid subjects only						
A	R	-	62	86	N/A	46
	L	-	70	108		58
C	R	-	230	-	N/A	300
	L	-	236	-		260
F	R	96	62	-	N/A	-
	L	102	70	-		-
G	R	-	-	-	N/A	68
	L	-	-	-		86
H	R	-	-	83	88	87
	L	-	-	99	89	105
I	R	-	-	185	133	93
	L	-	-	116	107	106
K	R	-	-	127	111	131
	L	-	-	103	99	112

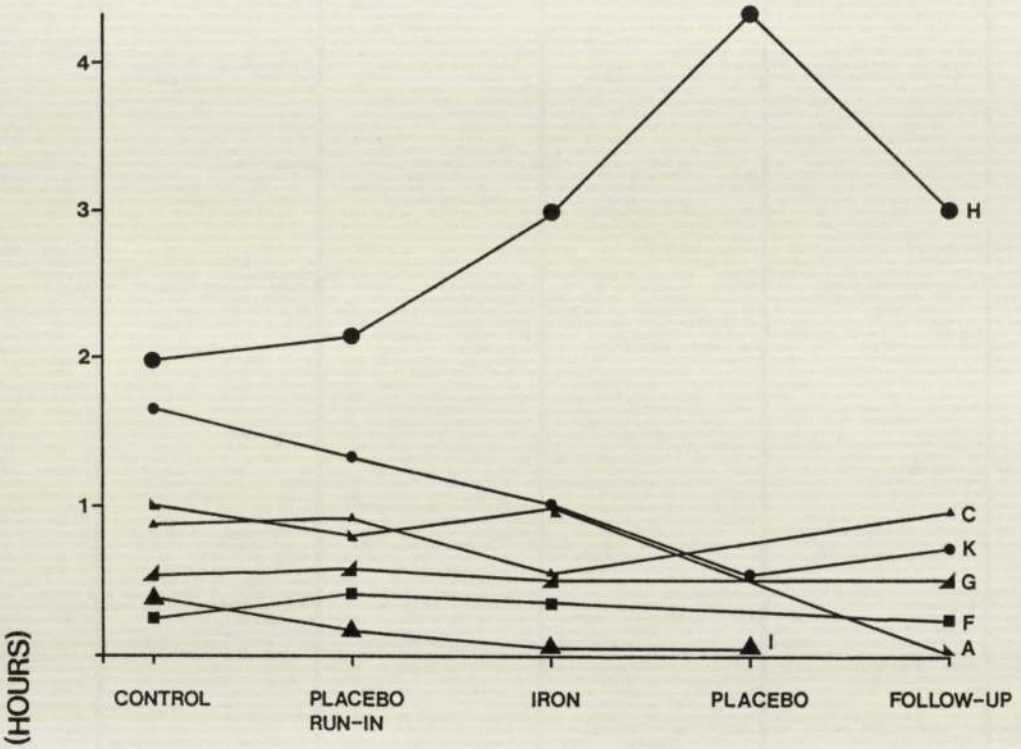
		Control	Placebo run-in	Placebo	Iron	Follow-up
B	R	-	-	-	-	250
	L	-	-	-	-	170
D	R	-	-	47	N/A	57
	L	-	-	48		57
E	R	-	80	-	138	40
	L	-	94	-	53	42
J	R	-	-	73	78	72
	L	-	-	63	58	60
L	R	-	-	85	69	-
	L	-	-	67	79	-

PAIN SCORE (Visual analogue scale 1=no pain 10=worst pain)

Patients	Control	Values at beginning of phase Placebo run-in	Iron	Placebo	Follow-up
Rheumatoid subjects only					
A	-	4.0	5.0	N/A	4.5
C	-	1.5	-	N/A	-
F	0	0	-	N/A	-
G	-	-	-	N/A	9.0
H	-	-	7.0	0	3.0
I	-	-	5.5	0	1.0
K	-	-	1.0	3.0	6.5

	Control	Placebo run-in	Placebo	Iron	Follow-up
B	-	-	-	N/A	2.5
D	-	-	0.5	N/A	3.0
E	-	4.0	-	7.5	-
J	-	-	0	0	0
L	-	-	4.5	6.0	-

Patient	Control	Placebo run-in	Iron	Placebo	Follow-up
A	1.00	0.77	1.00	-	0.00
C	0.84	0.89	0.56	-	0.92
F	0.26	0.39	0.33	-	0.28
G	0.56	0.60	0.50	-	0.50
H	1.97	2.10	2.96	4.31	3.00
I	0.39	0.13	0.00	0.00	-
K	1.64	1.31	1.03	0.55	0.70



Patient	Control	Placebo run-in	Iron	Placebo	Follow-up
B	5.00	5.00	5.00	-	-
D	1.75	1.67	1.58	-	-
E	0.23	0.25	0.26	0.27	-
J	1.56	1.32	1.75	1.50	1.13
L	1.69	1.79	1.94	2.00	2.08

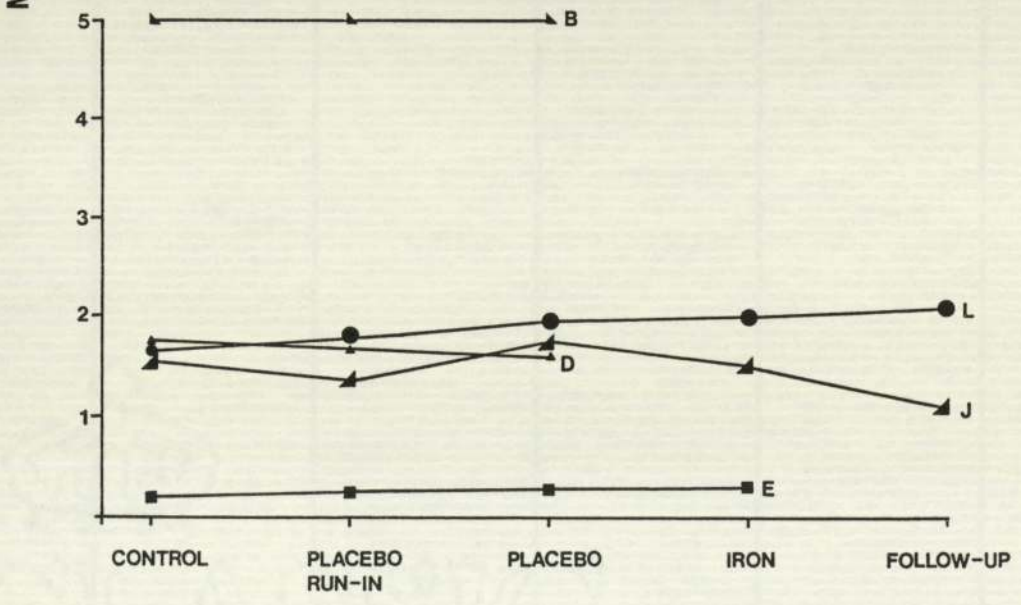
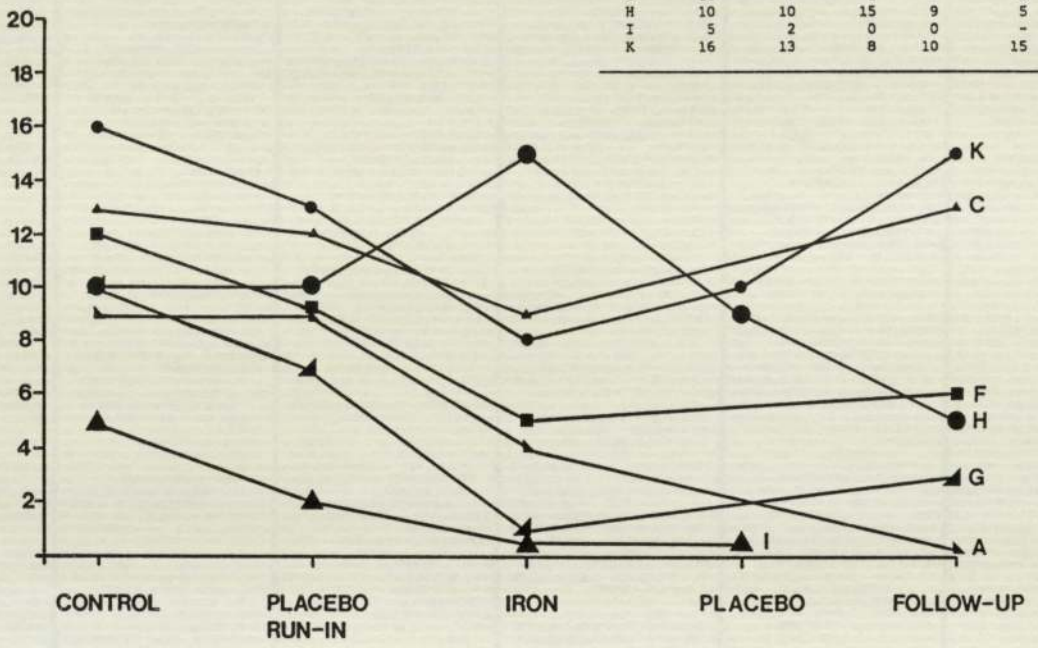
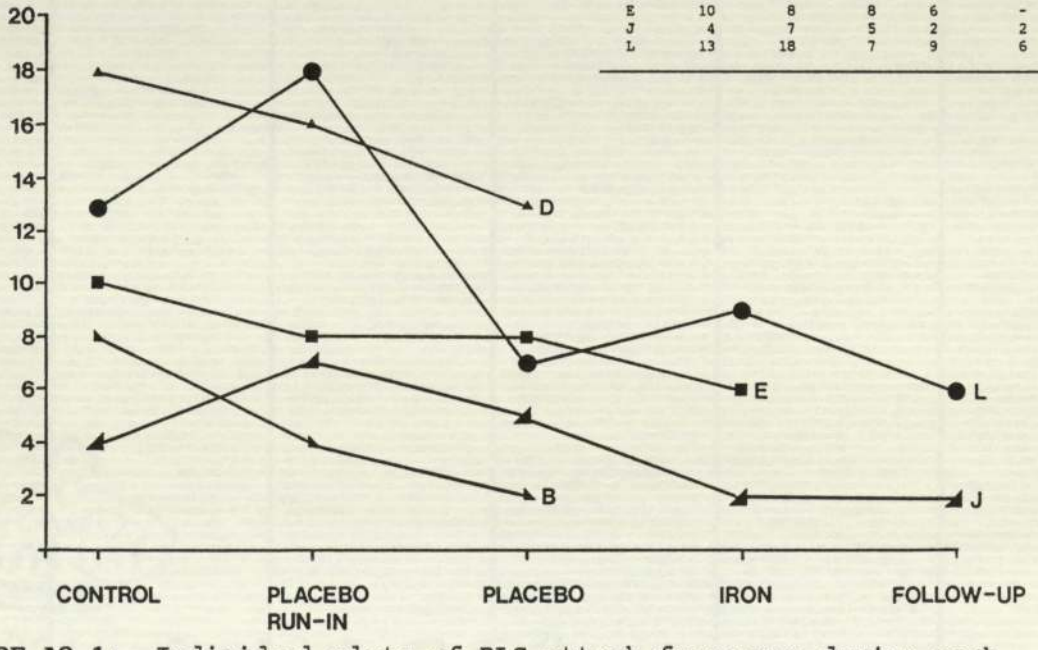


FIGURE A9.2: Individual plots of mean attack duration of RLS during each trial phase in rheumatoid subjects

Patient	Control	Placebo run-in	Iron	Placebo	Follow-up
A	9	9	4	-	0
C	13	12	9	-	13
F	12	9	5	-	6
G	10	7	1	-	3
H	10	10	15	9	5
I	5	2	0	0	-
K	16	13	8	10	15



Patient	Control	Placebo run-in	Iron	Placebo	Follow-up
B	8	4	2	-	-
D	18	16	13	-	-
E	10	8	8	6	-
J	4	7	5	2	2
L	13	18	7	9	6



Nº OF ATTACKS

FIGURE A9.1: Individual plots of RLS attack frequency during each trial phase in rheumatoid subjects

APPENDIX A10

DEMOGRAPHIC AND BASELINE HAEMATOLOGICAL PARAMETERS
FOR IRON ABSORPTION TEST SUBJECTS

Subject	Sex	Age	Hb	S-ferritin	S-iron	satn%	TIBC	MCV	ESR
Sub-group (i) Healthy iron replete volunteers									
TB	M	29	15.0	71.7	14.3	20.5	65.9	87.6	-
RC	F	33	13.6	26.7	16.2	29.6	54.7	89.4	-
AF	F	25	15.1	65.2	21.1	33.8	62.4	89.7	-
SH	F	25	15.3	42.4	29.6	52.5	56.4	93.4	-
LI	F	25	13.4	52.3	30.1	40.0	75.2	91.2	-
FR	F	23	13.5	127.0	12.7	30.2	56.3	88.7	-
JU	M	30	14.5	173.9	34.0	55.4	61.4	89.3	-
Mean		27.1	14.3	79.9	22.6	37.4	61.8	89.9	
SD		3.6	0.8	52.2	8.6	12.7	7.1	1.9	

Subject	Sex	Age	Hb	S-ferritin	S-iron	satn%	TIBC	MCV	ESR
Sub-group (ii) Iron deficient subjects									
RB	M	36	12.3	11.0	2.9	4.8	60.8	74.5	7
SB	F	34	13.4	7.6	7.7	11.8	65.5	86.4	-
JD	F	27	14.1	7.8	7.0	10.0	69.9	88.9	-
BT	F	44	5.7	1.1	4.5	4.7	96.6	52.5	29
AW	F	75	7.8	26.3	4.0	4.4	90.0	65.3	8
DP	F	67	9.0	14.1	5.3	7.9	66.9	73.3	30
Mean		47.2	10.4	11.3	5.2	7.3	75.0	73.5	18.5
SD		19.4	3.4	8.5	1.8	3.1	14.7	13.5	

Subject	Sex	Age	Hb	S-ferritin	S-iron	satn%	TIBC	MCV	ESR
Sub-group (iii) Rheumatoid with absent iron stores									
MB	F	46	8.5	11.0	5.9	8.3	71.3	59.6	40
SC	F	39	12.6	4.0	4.5	5.5	81.9	85.4	23
FH	F	69	11.0	7.8	5.0	6.1	82.0	80.8	25
RM	F	50	8.1	13.5	4.0	5.1	78.2	59.7	48
JP	F	55	12.6	8.3	11.5	13.8	83.4	90.1	14
CR	F	80	8.6	5.4	7.6	9.2	82.3	66.8	50
DG	F	77	10.4	11.0	5.5	7.9	70.0	78.0	45
HH	F	65	8.0	15.6	7.6	10.6	48.9	57.2	85
CH	F	53	10.5	18.7	8.2	9.2	89.1	81.5	8
Mean		59.3	10.0	10.6	6.6	7.1	79.9	73.2	37.6
SD		14.2	1.8	4.8	2.3	2.7	4.5	12.5	23.4

Subject	Sex	Age	Hb	S-ferritin	S-iron	satn%	TIBC	MCV	ESR
Sub-group (iv) Rheumatoid subjects with reduced marrow iron stores									
TB	F	22	7.9	9.4	4.7	7.6	62.2	60.4	59
CB	F	66	11.3	140	8.9	16.8	58.0	81.3	60
IE	F	79	9.2	63.2	8.0	16.2	49.4	78.3	38
LE	F	70	10.3	70.2	7.0	12.2	57.5	78.8	44
MR	F	39	11.1	29.1	17.3	11.5	57.4	86.2	11
MS	F	35	12.0	7.7	20.9	28.7	72.8	91.3	5
DS	F	68	8.5	43.6	4.1	8.4	48.8	68.4	85
Mean		54.1	10.0	51.9	10.1	14.5	58.0	77.8	43.1
SD		21.7	1.5	45.8	6.4	7.2	8.1	10.5	28.3

Subject	Sex	Age	Hb	S-ferritin	S-iron	satn%	TIBC	MCV	ESR
Sub-group (v) Rheumatoid subjects with normal marrow iron stores									
RA	M	56	14.9	66.9	19.2	30.0	64.1	-	8
DG	M	55	9.8	189.3	5.6	9.8	56.9	75.8	94
FH	F	68	10.3	953.8	10.5	22.8	46.0	90.1	64
OH	M	55	14.6	122.0	25.7	37.7	71.3	92.6	5
CH	F	79	11.3	40.2	9.2	14.9	61.6	79.7	52
MJ	F	55	11.6	172.3	6.5	11.2	58.1	84.2	49
IS	F	56	11.8	178.0	14.5	20.7	70.0	81.2	105
ES	M	53	14.3	360.8	11.6	24.6	47.1	94.0	26
RW	M	57	13.4	110.8	10.0	17.3	57.8	83.5	34
Mean		59.3	12.4	243.8	12.5	21.0	59.2	86.4	48.6
SD		8.6	1.9	282	6.4	9.0	8.8	7.1	34.9

IRON ABSORPTION TEST RESULTS FOR INDIVIDUAL SUBJECTS

Sub-group (i) Healthy iron replete volunteers

Subject	Pre-test value	Change in serum iron values at sample times (umol/l)					
		30 mins	1 hour	1.5 hours	2 hours	3hours	4 hours
TB	14.3	2.5	3.9	4.7	3.5	5.7	4.0
RC	16.2	5.2	7.5	7.0	8.9	7.5	7.7
AF	21.1	-4.3	-	2.3	4.0	3.9	-
SH	29.6	0.9	-0.3	-0.3	0	-0.1	-1.0
LI	30.1	0.8	-10.3	-2.3	0.8	-0.1	0.3
FR	12.7	0.8	1.7	1.7	1.7	-0.8	-0.1
JU	34.0	-3.9	1.8	3.1	2.9	1.4	2.0

Sub-group (ii) Iron deficient subjects

Subject	Pre-test value	Change in serum iron values at sample times (umol/l)					
		30 mins	1 hour	1.5 hours	2 hours	3hours	4 hours
RB	2.9	18.3	16.4	15.7	14.2	12.6	10.8
SB	7.7	4.6	8.7	10.4	9.2	6.8	
JD	7.0	5.6	6.5	4.7	5.0	5.0	5.2
BT	4.5	4.0	2.4	-	-	-	-0.4
AW	4.0	14.9	19.3	16.2	-	10.3	6.3
DP	5.3	2.4	-	-	6.9	6.1	-

Sub-group (iii) Rheumatoid subjects with absent iron stores

Subject	Pre-test value	Change in serum iron values at sample times (umol/l)					
		30 mins	1 hour	1.5 hours	2 hours	3hours	4 hours
MB	5.9	4.4	5.6	4.6	2.6	0.5	-1.0
SC	4.5	6.4	7.2	5.5	4.6	2.2	1.6
FH	5.0	5.5	11.0	13.5	11.5	11.5	-
RM	4.0	5.1	5.1	2.8	2.0	0.4	-0.2
JP	11.5	7.1	15.0	18.8	19.0	17.4	16.1
CR	7.6	2.0	3.6	2.9	2.7	2.5	1.8
DG	5.5	6.1	4.7	4.3	3.9	3.2	2.9
HH	7.6	2.4	3.1	2.0	0.4	-1.1	-1.4
CH	8.2	6.5	8.2	8.6	-	6.1	5.4

Sub-group (iv) Rheumatoid subjects with reduced marrow iron stores

Subject	Pre-test value	Change in serum iron values at sample times (umol/l)					
		30 mins	1 hour	1.5 hours	2 hours	3hours	4 hours
TB	4.7	1.9	2.1	0.9	0.4	-0.7	-0.9
CB	8.9	0.4	0.4	0.2	-0.4	-1.3	-2.2
IE	8.0	4.4	4.0	3.1	4.9	3.8	-
LE	7.0	0.6	-0.5	-1.1	-1.1	-0.8	-1.2
MR	17.3	0.5	1.1	1.9	1.1	0	0.3
MS	20.9	2.2	4.2	4.6	4.0	2.5	1.1
DS	4.1	0.8	0.6	0.4	0.2	0.1	0

Sub-group (v) Rheumatoid subjects with normal marrow iron stores

Subject	Pre-test value	Change in serum iron values at sample times (umol/l)					
		30 mins	1 hour	1.5 hours	2 hours	3hours	4 hours
RA	19.2	1.9	5.7	5.3	6.7	2.7	3.9
DG	5.6	-0.7	-0.8	-0.7	-0.6	-0.9	-0.8
FH	10.5	-3.0	-3.0	-3.0	-3.5	-4.0	-4.5
OH	25.7	1.2	0.5	2.7	2.0	1.7	1.7
CH	9.2	1.1	2.8	-	3.0	2.4	1.5
MJ	6.5	-0.7	-0.7	-1.2	-1.3	-2.0	-1.7
IS	14.5	1.5	2.0	-	2.0	2.0	0.5
ES	11.6	-0.2	-0.4	-	-0.9	-1.5	-1.7
RW	10.0	1.0	1.5	0.9	0.9	0.4	0.4

APPENDIX A11

PUBLICATIONS AND PRESENTATIONS

Publications

1. Iron and akathisia. (letter)
   Blake DR, Williams AC, Pall H, Fonseca A, Beswick T
   Br Med J 1986; 292, 1393.

2. Serum salicylate levels in a breast fed infant.
   Unsworth J, d'Assis-Fonseca AE, Beswick DT, Blake DR.
   Ann Rheum Dis 1987; 46, 638-639.

3. Desensitization to allopurinol - a cautionary tale.
   Unsworth J, d'Assis-Fonseca AE, Beswick DT, Blake DR.
   Ann Rheum Dis 1987; 46, 646.

4. Low-dose iron absorption test and the anaemia of rheumatoid disease.
   Fonseca A, Beswick T, Kelsey S, Hayllar J, Unsworth J, Murray J, Blake DR.
   Br J Rheum 1987; 26 (Suppl 2), 110.
   Paper presented in the plenary session at the British Society of Rheumatology Meeting, London, November 1987.

5. Restless legs syndrome.
   Pall HS, Williams AC, Fonseca AE, Blake DR.
   Neurology 1987; 37, 1436-1437.

Oral presentations

1. A low-dose iron absorption test and the anaemia of rheumatoid disease.
   A. Fonseca, T. Beswick, S. Kelsey, J. Hayllar, J. Unsworth, J. Murray DR. Blake.
   European Iron Club meeting, Frankfurt am Main, 5-8th September 1988.

2. Restless legs syndrome - the influence of iron on its pathogenesis and treatment.
   A. Fonseca, DT Beswick, DR Blake.
   European Iron Club meeting, Frankfurt am Main, 5-8th September 1988.

Poster presentations

1. A low-dose iron tolerance test and the anaemia of rheumatoid disease.
   d'Assis-Fonseca AE, Kelsey S, Hayllar JS, Unsworth J, Beswick DT Blake DR.
   Presented at the West Midlands Rheumatology Society Meeting, Coventry 1986 and the Guild of Hospital Pharmacists' Weekend School, Nottingham 1987.

2. A low-dose iron absorption test and the anaemia of rheumatoid disase.
   A Fonseca, T Beswick, S Kelsey, J Hayllar, J Unsworth, DR Blake, DA Lewis.
   Presented at the British Pharmaceutical Conference, Aberdeen, September 1988.

3. Restless legs syndrome, arthritis and iron - a therapeutic and epidemiological study.
   A Fonseca, T Beswick, DR Blake.
   Presented at the British Society of Rheumatology meeting, London, 1988

RESTLESS LEGS SYNDROME - THE INFLUENCE OF IRON ON ITS PATHOGENESIS AND TREATMENT.

A.Fonseca, DT Beswick, DR Blake.
Departments of Pharmacy and Rheumatology, Selly Oak Hospital, Birmingham, UK.

Restless legs syndrome (RLS) is an unpleasant dysaesthesia of the legs, brought on by inactivity and temporarily relieved by movement. Its cause is unknown and treatment empirical. Literature figures suggest an association between RLS and conditions with pure or functional iron deficiency. Anecdotal reports indicate that iron therapy may be useful.

The prevalence of RLS in 3 groups; patients with rheumatoid arthritis (RA) (n=151), pure iron deficiency (n=39) and healthy volunteers (n=79), was assessed by interview using a standard questionaire. 36%, 23% and 10% respectively had experienced symptoms in the previous 12 months. Standard laboratory indices of iron status were measured. Hb, MCV, MCH, s-iron, saturation (%) and s-ferritin were all significantly lower ($p<0.05$) in RA patients with RLS, than without. There was no significant correlation between RLS and clinical and biochemical measurements of rheumatoid disease activity.

A double-blind cross-over trial was undertaken comparing oral ferrous sulphate (200mg tds) with placebo, taken for 28 days. 14 rheumatoid patients (12F, 2M; mean age 57.2yrs, range 35-80yrs) with 'classical' RLS and experiencing symptoms at least once a week, were recruited. Except for one, all had s-ferritin <55ug/l, the lower limit of normal in RA. Frequency and duration of symptoms were recorded on diary cards and assessed by direct questioning. There was improvement in 11/14 patients whilst on oral Fe. Symptoms were 'much better' or completely relieved (for at least 1-5 months) in 7 patients, compared with only 2 on placebo.

Results confirm an increased incidence of RLS in iron deficiency, and indicate that oral iron may be useful in its treatment. Findings support hypotheses relating the pathogenesis of RLS with central dopaminergic and endogenous opiate pathways, where iron is thought to play an integral role.

Presented at the European Iron Club meeting, Frankfurt am Main, September 5-8th September 1988.

A LOW-DOSE IRON ABSORPTION TEST AND THE ANAEMIA OF RHEUMATOID DISEASE.

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Patients with persistent rheumatoid arthritis (RA) develop the anaemia of chronic disease (ACD). A significant proportion (30-70%) also have iron deficiency anaemia (IDA). It is important to distinguish these types of anaemia to avoid overlooking potentially curable sources of iron defiency. Indescriminant treatment with iron will not cure ACD and may augment joint inflammation.

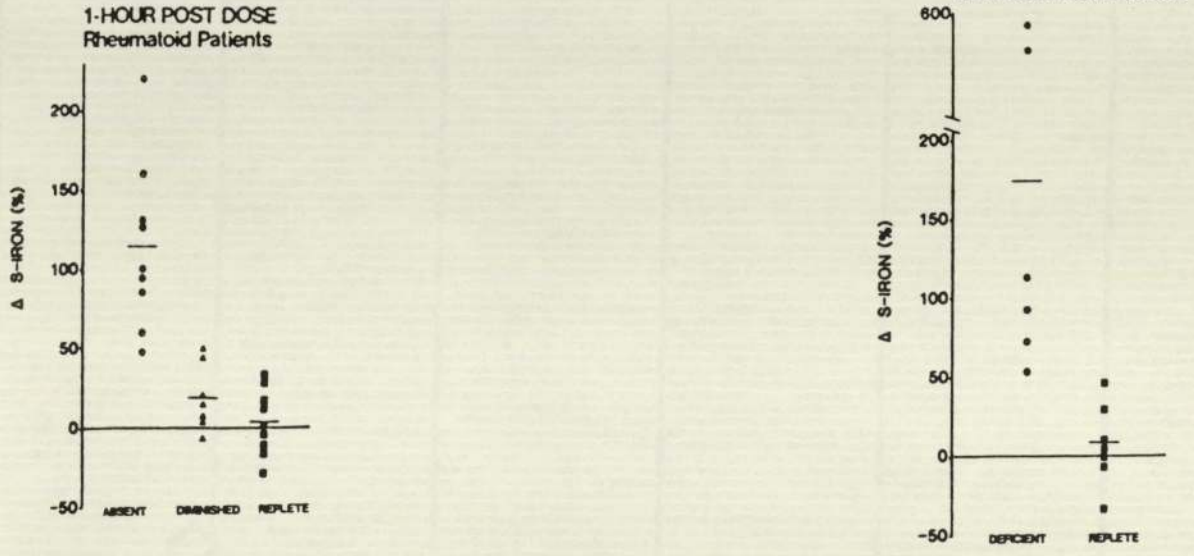
Bone marrow aspiration is considered the definitive method of differentiating genuine IDA from ACD in patients with persistent inflammatory states. However this is a specialist procedure, inconvenient to patients, and results are subjective.

Iron absorption is a function of iron stores. Crosby (1) showed that administration of a physiological dose of iron followed by determination of serum iron at intervals provided a method of differentiating mild iron deficiency from iron repletion in healthy volunteers. We have evaluated this method in differentiating IDA and ACD.

Ten mg elemental iron was administered to fasted subjects as a solution and serum iron measured (deproteinisation-ferrozine assay) hourly over 4h. Five groups were studied; healthy non-iron deficient volunteers (n=7); patients with iron deficiency without concomitant inflammation (n=6); rheumatoid patients who on bone marrow aspiration had normal (n=9), diminished (n=7) or absent (n=9) iron stores.

Maximum discrimination was found at 1h post dose, allowing the test to be simplified.

Fig 1. Graphs showing serum iron (S-iron) 1h post dose.



The study confirmed Crosby's original findings. The test differentiates anaemia due to absent iron stores from that due to chronic disease. It provides a cheap, safe, simple and objective asessment of iron stores; a valuable alternative to bone marrow aspiration.

1. Crosby WH, O'Neill-Cutting MA, JAMA 1984, 251, 1986-1987.

Presented at the European Iron Club meeting, Frankfurt am Main, September 5-8th September 1988.