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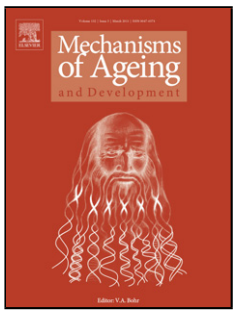

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Authors: Anna A. Wawer, Amy Jennings, Susan J. Fairweather-Tait

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Iron status in the elderly: a review of recent evidence

Anna A Wawer[1], Amy Jennings[2] and Susan J Fairweather-Tait[2]

[1] Discipline of Medicine, University of Adelaide, The Queen Elizabeth Hospital and the Basil Hetzel Institute for Translational Health Research, Woodville, South Australia 5011

[2] Norwich Medical School, University of East Anglia, Norwich NR4 7TJ, UK

Corresponding author: Susan Fairweather-Tait

E-mail address: s.fairweather-tait@uea.ac.uk

Postal address: Norwich Medical School, University of East Anglia, Norwich NR4 7TJ, UK

Highlights

- Perturbations of iron metabolism resulting in changes in iron status are observed in a variety of age-related medical conditions, including kidney disease, cancer, cardiovascular disease, and neurodegenerative diseases.
- Biomarkers of iron status outside the 'normal' range may be indicative of other underlying health conditions and should be investigated, but a consensus for cut-off levels for optimal iron status in the elderly is required in order to establish normal, safe ranges.
- Hormonal treatments, erythropoiesis stimulating agents, hepcidin inhibitors and ferroportin modulators have potential as novel therapies for treating challenging conditions, such as inflammation-related anaemia. The use of conventional treatments with high dose iron supplements needs to be reviewed.
- Lifestyle changes, for example exercise and diet, may help improve iron status in healthy older people.

Abstract

A comprehensive literature review of iron status in the elderly was undertaken in order to update a previous review (Fairweather-Tait et al, 2014); 138 papers were retrieved that described research on the magnitude of the problem, aetiology and age-related physiological changes that may affect iron status, novel strategies for assessing iron status with concurrent health conditions, hepcidin, lifestyle factors, iron supplements, iron status and health outcomes (bone mineral density, frailty, inflammatory bowel disease, kidney failure, cancer, cardiovascular, and neurodegenerative diseases). Each section concludes with key points from the relevant papers. The overall findings were that disturbed iron metabolism plays a major role in a large number of conditions associated with old age. Correction of iron deficiency/overload may improve disease prognosis, but diagnosis of iron deficiency requires appropriate cut-offs for biomarkers of iron status in elderly men and women to be agreed. Iron deficiency (with or without anemia), anemia of inflammation, and anemia of chronic disease are all widespread in the elderly and, once identified, should be investigated further as they are often indicative of underlying disease. Management options should be reviewed and updated, and

novel therapies, which show potential for treating anemia of inflammation or chronic disease, should be considered.

[1]***Abbreviations:*** ACD- Anemia of chronic diseases , AD- Alzheimer's disease, AI- Anemia of inflammation, AS- arterial stiffness, AUC- area under the curve, AUC^{ROC}- The area under the receiver operating characteristic curve, CAD- coronary artery disease, CD- Crhon's disease, CE- capsule endoscopy, CKD- chronic kidney disease, CRP- C-reactive protein, DEXA- dual-energy X-ray absorptiometry, eGFR- estimated glomerular filtration rate, EPO- erythropoietin , FGF-23- fibroblast growth factor-23, Hb- haemoglobin, HCT- haematocrit, IBD- inflammatory bowel disease, IDA- iron deficiency anemia, MRI- magnetic resonance imaging, NAL- neocortical amyloid-ß load , NGAL- neutrophil gelatinase associated lipocalin, OR- odds ratio, PBRD- probable rapid eye movement sleep behaviour disorder, PLC- primary lung carcinoma, RDW- red cell distribution, RR- relative risk, sTfR- soluble transferrin receptor, TBARS- thiobarbituric acid reactive substance , TRAP- tartrate-resistant acid phosphatase, UA-unexplained anemia, UC- ulcerative colitis, UPDRS- unified Parkinson's disease rating scale, WHO- World Health Organisation

Keywords

Iron status, elderly, iron deficiency, anemia

1. Background

The aim of this review was to summarise the latest information on iron status in the elderly in order to update our previous article published in 2013 (Fairweather-tait *et al.*, 2014). A PubMed search was carried out on 14th August 2017 and covered the following date range: 1st June 2013 until 14th August 2017 (detailed information in Figure 1). The following key words were used (number of papers identified in brackets): ferritin and elderly (6); ferritin and inflammation (24); hepcidin and inflammation (31); iron and inflammation (144); iron and status (346); soluble transferrin receptor (28); anemia and elderly (68); anemia elderly (13); ferritin and inflammatory (9); iron and elderly (27). Due to the large number of papers identified genetic components of iron status were not included in this review.

Physiological requirements for iron are mainly provided by the recycling of iron from the breakdown of senescent red blood cells (Hurrell and Egli, 2010). Obligatory daily losses of approximately 1-2mg iron (de Benoist *et al.*, 2008) have to be replaced from absorbed dietary iron. Losses that occur take place through exfoliated intestinal epithelial cells, bile, urine, skin, hair, nails, sweat, semen and blood loss (menstruation or other) (Hentze *et al.*, 2010). There is no specific mechanism for iron removal from the body (Mackenzie and Garrick, 2005).

According to the World Health Organisation (WHO) around 2 billion people worldwide are affected by anemia (de Benoist *et al.*, 2008), which results in a reduced capacity of red blood cells to carry oxygen. In contrast, the iron overload disorder, haemochromatosis, affects approximately 1 in 150

people in populations of Northern European origin (Hurrell and Egli, 2010). Iron is vital for many biochemical processes within the body, such as synthesis of DNA, mitochondrial respiration, thyroid function and other crucial metabolic reactions (Geissler and Hilary, 2005).

The two main causes of iron deficiency anemia (IDA) are diets low in bioavailable iron and/or high iron requirements. Other causes of anemia include acute and chronic infections (Baysoy *et al.*, 2004), blood loss, other micronutrient deficiencies (riboflavin, folate, copper, vitamin A, B12) and haemoglobinopathies affecting African, Southeast Asia and Mediterranean populations. Additional causes of anemia in the elderly include:

- poor quality and/or monotonous diet,
- loss of appetite associated with lower physical activity,
- decreased physical functional capacity (Bosco *et al.*, 2013),
- chronic diseases and inflammation (Lee, 1983; Fleming *et al.*, 2001),
- impaired efficiency of iron absorption (Lopez-Contreras *et al.*, 2010),
- myelodysplastic syndrome (disorders affecting production of blood cells), decreased functionality of erythrocytes (Gershon and Gershon, 1988),
- regular intake of medications (such as aspirin) (Fleming *et al.*, 2001),
- occult blood loss (Lopez-Contreras *et al.*, 2010),
- institutionalisation (Lopez-Contreras *et al.*, 2010) (mainly associated with low BMI, malnutrition, increased degree of dependency and chronic diseases) (Da Silva *et al.*, 2016; Sahin *et al.*, 2016).

Anemia in the elderly is related to several adverse health outcomes, including a decline in physical performance and strength, cognitive impairment, increased susceptibility to falling, frailty, longer hospital stay, post-operative risk, higher number of comorbidities and mortality (Penninx *et al.*, 2003, 2004; Hamer and Molloy, 2009; Migone De Amicis *et al.*, 2015; Chrobak *et al.*, 2017; Munoz *et al.*, 2017).

Conversely a higher occurrence of elevated iron stores in the elderly compared to other age groups has been reported by a number of authors (Fleming *et al.*, 2001; L. Wang *et al.*, 2016). This shift is more pronounced in older women rather than men and, at least in part, is attributed to their ceased reproductive function (no iron losses related to menstruation or pregnancy). Furthermore, elevated iron stores have been linked to exercise in the aged population (Jehn, 2004; Li *et al.*, 2013, 2014; Shoji *et al.*, 2017).

Finally, there is evidence in the literature that genotype is a major determinant of body iron content. The latest research in twins demonstrate that the genetic contribution to iron status is around 50% (Whitfield *et al.*, 2000; Constantine *et al.*, 2009).

In the 2013 review by Bianchi *et al.* (2016) it was hypothesized that anemia in the elderly, when present in a situation of iron sufficiency, stems from a mix of comorbidities and inflammatory state. Those conditions are either impeding haematopoiesis by limiting iron through IL-6 and thus hepcidin increase or through TNF-α pathways resulting in erythropoietin (EPO) resistance (Bianchi, 2013).

One of the challenges of estimating accurate figures for iron deficiency (ID), IDA and anemia around the world is that various cut-off points and different markers of iron status are being used, making the results of different surveys difficult to compare. Further, the description of iron status in a given population is often limited to anemic, iron deficient and subjects with iron status parameters in the normal range. Individuals with elevated iron stores are rarely reported.

The cut-off hemoglobin (Hb) values for anemia in the elderly are based on the WHO definition from 1968 and apply to the general adult population (Hb<12g/dL in women and Hb<13g/dL in men) but not specifically to the elderly. Population specific Hb cut-off levels for the elderly are yet to be established but some authors advocate this approach (Gabriele, 2016). There are, however, different opinions about the consequences of the observed decline in Hb concentration with age, and since lower Hb concentrations are associated with increased risk of hospitalisation, morbidity and mortality, the cut-off values have not been changed (Stauder and Thein, 2014). Finally, correct interpretation of iron status biomarkers (such as serum ferritin, transferrin saturation, serum iron) with concurrent inflammation may be difficult.

1.1. What is the size of the problem?

WHO projects that the percentage of elderly in the population will triple by 2050, when it will reach 1,500 million people (Global health and aging, 2011). It is estimated that by 2020 there will be more people over 65 years of age than children under 5 (Global health and aging, 2011; Agarwalla, 2014). Thus it is very likely that the number of elderly people with ID or IDA will also greatly increase.

A Brazilian group (Bosco *et al.*, 2013) reported that anemia was present in 30% of their hospitalised patients (n=709, aged 60 years and over). In an Austrian hospital cohort of 19,758 patients aged 64-104 years, 24% of men and 19% of women were anemic (Bach *et al*., 2014). A significant increase in the incidence of anemia was observed with age, reaching 31% and 38% for over 80 and over 90 years respectively. The main reasons for anemia in this study were identified as: mild, moderate and severe kidney failure (56% of anemic patients), absolute and functional iron deficiency in 14% and 28% respectively, folate and B_{12} deficiency in 7% and 2% respectively. Macrocytic anemia was diagnosed in 16% of participants and could not be explained by vitamin B12 or folate insufficiency (Bach *et al.*, 2014). Further, C-reactive protein (CRP) was negatively correlated with Hb. The authors noted that in the population investigated, there was often more than one reason for the anemia.

In an elderly Brazilian population (>60 years, n=218, 50-59 years, n=155, 60% women), approximately 11% of the population had anemia (Lacerda *et al.*, 2016), according to the WHO cut-off of Hb<12g/dL in women and Hb<13g/dL in men. As expected, Hb levels were significantly lower in the group aged >60 years compared with those aged 50-59 yeas ($p=0.001$, Mann-Whitey test), and overall Hb levels were inversely correlated with age ($p<0.001$).

In 1,920 elderly Mexicans aged 60 years and over anemia was diagnosed in 13% of women and 15% of men. ID was responsible for approximately 4% of anemias for women as well as for men (Contreras-Manzano, Cruz and Villalpando, 2015). Similar to the Austrian study (Bach *et al.*, 2014), the incidence of ID increased with age and reached 7% for men and women above 80 years. The

authors concluded that the main reason for anemia in this group of elderly was not ID but kidney failure.

Dramatically higher figures for anemia were reported in a German study (Röhrig *et al.*, 2014); 54% of male patients aged 70 and over who were admitted to the emergency department had anemia. Out of a total of 1,045 patients 18% were out-patients and the remaining 858 participants were admitted to the hospital. Anemia (WHO cut-offs of Hb) was present in 36% of out-patients (44% and 28% in men and in women respectively) with a significantly higher prevalence in male patients ($p=0.023$). Within the in-patient group anemia was present in 54% participants (60% of men and 48% women) with significantly higher occurrence in men than in women ($p=0.001$). Interestingly, the authors noted that patients referred to nephrology and hemato-oncology departments had significantly lower levels of Hb ($p<0.001$ and $p=0.044$ respectively) when compared to other anemic in-patients. Patients referred to cardiology departments had significantly higher Hb levels ($p=0.001$) when compared to other anemic in-patients. Finally, the authors reported that only 15% of anemic in-patients had their anemia addressed while 85% were left untreated (Röhrig *et al.*, 2014).

In a cross-sectional prospective study of anemia in elderly in-patients aged 70 and over (n=100, 55% females), Zilinski *et al.* (2014) explored the association of multidimensional loss of function with anemia (WHO cut-offs). All participants had a comprehensive geriatric assessment investigating cognition, transfer skills, mobility, competence in performing the basal activities of daily living and swallowing ability. Multidimensional loss of function was confirmed if patients had atypical results for a minimum of three out of six components of the assessment. The authors reported that 60% of the participants were anemic and 61% had multidimensional loss of function. Further, participants with anemia were more likely to present with renal failure, hyperlipidemia and osteoporosis ($p<0.001$, $p=0.018$ and $p=0.017$ respectively). Interestingly, patients who presented with multidimensional loss of function had significantly lower Hb concentrations. Thus the authors suggest that Hb could be used as a screening tool to identify frailty in older people.

In low and middle income countries, the underlying causes of anemia in the elderly are most likely different to developed countries. In a Chinese study of 5,690 women (Song *et al.*, 2014) aged 50-75 who were screened from three rural areas, 23% were diagnosed as anemic according to Hb (90-120g/dL). In total 1,025 and 1,004 of non-anemic and anemic women respectively were enrolled onto the study. Anemic participants had more frequent food shortages and lower BMI's when compared to controls ($p<0.001$ in both instances). The authors concluded that inadequate nutrition was the main reason for anemia but lack of information about chronic diseases and inflammation was a key limitation of the study.

As noted by Stauder *et al.* (Stauder and Thein, 2014) the actual diagnosis of iron deficiency in the elderly should be the starting point for a meticulous process in which the actual cause of ID or IDA is sought. Also, attention should be given to potential malignant causes and/or blood loss. Anemia in the elderly was divided into the following categories:

- Nutritional deficiency (insufficient intake of iron (IDA), folate or B_{12}).
- Anemia of chronic diseases (ACD); involving a number of pathophysiologies, with hepcidin playing a major role, and resulting in functional iron deficiency.

- Unexplained anemia (UA); a diagnosis that is made based on ruling out other possible causes with inconclusive diagnostic methods. Additionally, the common presence of an inflammatory state in the elderly plus other co-morbidities makes the diagnosis more difficult.

Anemias can be further divided into:

- Absolute anemia, presenting with empty iron stores (IDA, UA).
- Functional anemia where the process of iron release from iron stores is dysfunctional (ACD, UA) (Gabriele, 2016).

In a Turkish study of community dwelling elderly (n=827, mean age 70.9±6.2, 50.1% men) 7.3% (5.3% women and 0.2% men) of the population was anemic (WHO criteria) (Yildirim *et al.*, 2015). The prevalence of anemia significantly increased with age (p=0.03) with 50% anemias being hypochromic microcytic anemia. ID and IDA were present in 7.1% (6.3% men and 8% women) and 2.8% of the population, respectively. Vitamin B_{12} deficiency (<200pg/ml) was diagnosed in 64.2% of the population with similar distribution between the genders. Folic acid deficiency (<2.6 ng/ml) was found in 10.9% of the population with more women being deficient (12.1% vs 9.7%). Interestingly, in this population 4.4% had Vitamin B_{12} deficiency anemia and 1% had folic acid deficiency anemia, suggesting that B vitamins are major contributors to anemia in the population studied. These findings demonstrate that the nutritional reasons for anemia will vary depending on the elderly population (developed vs low or middle income countries, geographical region etc.).

A Canadian group retrospectively investigated 570 cases of anemic patients over 60 years old who had EPO levels measured at the time of the referral (Gowanlock *et al.*, 2016). After thorough investigation using pre-defined criteria, three independent reviewers investigated the reasons for anemia in each patient and assigned it to one of 10 categories: iron deficiency, chronic kidney disease, chronic disease, suspected myelodysplastic syndrome, confirmed myelodysplastic syndrome, vitamin B12 deficiency, folate deficiency, anemia of unknown aetiology, multifactorial aetiology or other aetiology. Regression analysis (adjusted for Hb, estimated glomerular filtration rate (eGFR) and comorbidities) revealed that EPO levels in UA, ACD and in chronic kidney disease were lower by 27%, 46% and 45% respectively when compared to the levels in IDA. The authors concluded that perturbed EPO synthesis/response may at least be in part an underlying reason of UA.

Phatlhane *et al.* (Phatlhane *et al.*, 2016) noted that in a study of 214 males and 410 females aged between 18 and 76 years from South Africa, that the percentage of women with anemia was higher than men. Moreover, the author also observed ethnic differences. Out of Caucasian, mixed ancestry and black African participants the latter had the highest incidence of IDA, followed by mixed ancestry and Caucasians (with IDA prevalence of 26.1%, 14% and 4.2% respectively). Similarly, Miller (Miller, 2016) noted that Hb was significantly higher in Caucasian women (aged 50 years and over) when compared to Hispanic and non-Hispanic black women within the same age group. However, ferritin was significantly lower in white women when compared non-Hispanic black women. Similarly, Li *et al.* (Li *et al.*, 2014) reported in their study of 2,600 participants aged 60 and

over (1,100 women and 1,500 men) that ferritin levels were significantly higher in men when compared to women presenting with the same metabolic disorders.

As summarised by Stauder and Thein (2014) (data from eight studies) the prevalence of anemia in the elderly (>65 years of age) in free living individuals, hospitalised and living in residential homes was 12%, 40% and 47% respectively. In 2015, De Amicis *et al.* (2015) reported similar figures in elderly hospitalized patients. In a study of 193 patients admitted to hospital, 48% presented with anemia. Another paper published in 2016 by a Turkish group reported that that in care homes anemia was present in approximately 55% of elderly (n=257, aged 78.5 ± 7.8) (Sahin *et al*., 2016). Summarised data are presented in Figures 2, 3 and 4. Interestingly, the main reason for anemia in patients classified as malnourished (8% of all participants) or at risk of malnutrition (36% of all participants) was ACD. The risk was 5 and 2 fold higher in malnourished and patients at risk of malnutrition respectively.

1.2. Key points:

- The prevalence of anemia increases with age within the elderly population.
- Moderate or severe kidney failure is one of the causes of anemia.
- Malnutrition, iron, vitamin B12 and folate deficiencies often contribute to anemia.
- The presence of complex anemia (more than one reason for anemia) is frequent.
- Unexplained anemia is present in about one third of the cases.
- Nephrology and hemato-oncology patients present with lower levels of Hb whereas cardiology patients present with higher Hb levels.
- A large proportion of elderly anemic patients are left untreated (study in Germany).
- Patients with multidimensional loss of function have a significantly lower Hb.

2. Aetiology, including physiological age-related changes that may affect iron status.

In an American study of 788 men (Roy *et al.*, 2017) aged 65 and over with low testosterone levels 16% were anemic but half had no known cause of anemia. In a double-blind placebo-controlled study, participants were given testosterone (to restore testosterone levels of men of young age) or placebo gel for 12 months. Testosterone treatment resulted in an increase in Hb of 1g/dL in 54% of men with unexplained anemia, shifting 58% (p=0.002) of men to the non-anemic category at the end of the 12 months trial. An increase in Hb in the placebo group was observed in 15% of men but it was not significant. Similarly, in the group with known cause of anemia, an increase in Hb values after testosterone treatment was observed in 52% of men, whereas in the placebo group an increase was observed in 19% of men. Overall, in this study of elderly men with low testosterone it has been shown that red blood cell production is stimulated by testosterone treatment or, as noted by Eisenga *et al.* (Eisenga, Stam and Bakker, 2017), testosterone treatment may be down-regulating serum hepcidin thus resulting in increased iron absorption. Eisenga *et al.* (2017) further commented that the authors could have underestimated the incidence of iron deficiency anemia by using a cut-off for ferritin of 40 ng/ml instead of 75 ng/ml and for vitamin B_{12} 200 pg/mL instead of 350 pg/mL, as suggested by other authors (Holyoake *et al.*, 1993; Lindenbaum J *et al*, 1994). This reinforces the

need for establishing a consensus regarding cut-offs for iron and vitamin B_{12} biomarkers of status for diagnosing anemia in the elderly.

Miller (2016) investigated if hormone replacement therapy in 1,066 post-menopausal women aged 50 and over had any effect on their iron status (Hb and ferritin). Results from animal models and cell studies showed that oestrogen plays a part in the regulation of iron homeostasis through hepcidin expression (Hou *et al.*, 2012; Ikeda *et al.*, 2012) and ferroportin signalling (Qian *et al.*, 2015). In an animal model low oestrogen levels increased hepcidin leading to a reduction in serum iron and increase in tissue iron (Hou *et al.*, 2012). In the study conducted by Miller, the women who were on hormone replacement therapy had significantly lower ferritin levels while their Hb levels were unchanged. The author also noted that ferritin levels were significantly higher in women who had undergone hysterectomy when compared to women that had not (Miller, 2016).

The reasons for anemia in the elderly are unexplained in approximately 25-30% (Migone De Amicis *et al.*, 2015) of the population but it is often accompanied by low plasma EPO (Artz and Thirman, 2011). Artz *et al.* (Artz *et al.*, 2014) investigated if unexplained anemia (UA) in the elderly has comparable traits to the anemia of inflammation (AI). In a small cohort study of 37 subjects (18 controls with median age 75.4, range 65-87, and 19 patients with unexplained anemia with median age 75.3, range 65-89) cut-offs for Hb were as follows: 115g/l, 122g/l, 127g/l and 132g/l for back women, white women, black men and white men respectively. Ferritin, serum iron, EPO and inflammation markers, namely IL6, IL8, IFNγ and neopterin were compared between participants from the control and UA groups. Ferritin levels were significantly higher and serum iron levels significantly lower in participants from UA group. Further, all inflammatory biomarkers were significantly increased in the UA group. However, only neopterin (synthesized by macrophages and currently used as an indicator of specific cancers) significantly correlated with reduced Hb levels in patients with UA. Based on their results the authors concluded that UA has similar traits to AI. Artz *et al.* (2014) also suggest that neopterin could be a useful indicator of UA. The authors suggest that immunomodulatory or anti-inflammatory drugs should be tested in large scale human studies to examine if they would alleviate the incidence of UA.

Lupescu *et al.* (Lupescu *et al.*, 2015) conducted a pilot study (16 elderly, aged 83-96 years and 11 younger controls aged 26-43 years) comparing the percentage of eryptosis between younger and older participants. The authors reported that eryptosis was significantly higher in the elderly when compared to younger participants. Further, reticulocytes in the elderly individuals were inversely correlated with Hb concentration ($p<0.001$, $R^2=0.6$) suggesting increased erythrocyte turnover in the elderly. Larger studies are needed to investigate this hypothesis.

Montero *et al.* (Montero *et al.*, 2016) suggested that increased arterial stiffness (AS) may be the main cause of low EPO through the mechanisms that regulate kidney perfusion. After reviewing the evidence in the literature they suggest the following mechanisms: AS affects blood volume-regulating hormones and EPO synthesis through its (AS) negative effect on baroreceptor; reduced hormone delivery to EPO producing cells due to reduction of renal perfusion is caused by AS. Appropriately designed human studies are needed to test the hypothesis that AS negatively affects iron status through reductions in EPO.

Halawi *et al.* (Halawi, Moukhadder and Taher, 2017) concluded in their review that anemia in the elderly is not a pure consequence of ageing. The authors emphasised the importance of investigating the underlying cause of anemia in order to implement effective treatment. They also noted the difficulty in managing anemia of chronic disease, especially the kidney, and suggested exploring potential mechanisms which might help elucidate cases of unknown anemia, such as decrease of androgen levels, role of inflammation, EPO synthesis and under-production of stem cells.

2.1. Key Points:

-Restoring testosterone levels in elderly men to those found in young men seems to be effective in alleviating anemia.
- Hormone replacement therapy, as well as hysterectomy, may change the iron status of elderly women.
- Elevated ferritin levels, decreased serum iron levels and neopterin may be useful indicators of UA.
- Increased arterial stiffness may be the main cause of low EPO levels.
- There is a need to establish a consensus for cut-offs of iron and vitamin B_{12} biomarkers of status for diagnosing anemia in the elderly.
-Immunomodulatory or anti-inflammatory drugs should be tested in large-scale human studies to determine if they would alleviate the incidence of UA.

3. Novel strategies for assessing iron status with concurrent chronic health conditions.

Interesting findings were recently published by a British group investigating the relationship between inflammatory markers, namely CRP and albumin and iron status indicators (total iron, transferrin, transferrin saturation and ferritin) (Mcsorley *et al.*, 2016). The strength of this retrospective study is in quantitative data from 23,778 individuals with mean age 68 years (56% women). The data were split into 3 groups according to inflammatory biomarkers:

1. CRP <10 mg/L and albumin >35 g/L,
2. CRP 11–80 mg/L and albumin 25-35 mg/L,
3. CRP >80 mg/L and albumin <25 g/L

The median serum ferritin was 77, 173, and 445 ng/dL, respectively ($p<0.001$). The median serum total iron was 15.0, 7.0, and 3.0mmol/L, respectively. The median serum transferrin concentration was 2.6, 2.0, and 1.3mmol/L, respectively. The median transferrin saturation was 23%, 13%, and 10% respectively ($p<0.001$ for all iron indicators). All of the iron parameters that were investigated were independently and significantly associated with inflammatory markers analysed in the study. This study shows the importance of quantifying inflammatory biomarkers when investigating iron status, as well as the unreliability of the aforementioned iron status indicators in inflammatory states. Corrections for inflammation have been suggested by Thurnham *et al.* (Thurnham *et al.*, 2010)

A Polish group measured the two forms of fibroblast growth factor 23 (FGF-23 responsible for phosphate and vitamin D metabolism), namely cFGF-23 and iFGF-23 in a large, cross-sectional, multicentre study of the elderly (>65 years, n=3780, 48% women) (Bozentowicz-Wikarek *et al.*, 2015). They found that participants with the lowest serum iron had the highest levels of cFGF-23 and

iFGF-23 ($p<0.001$) and this association was independent of low-grade inflammation. Interestingly, iFGF-23 and cFGF-23 levels remained constant until certain serum iron levels were reached, (59.3µg/dL and 57.3µg/dL respectively), then they observed a near-linear increase with 0.285 pg/mL of iFGF-23 and 3.742 RU/mL of cFGF-23 for each unit of serum iron increase (R^2 of normalized slopes -0.989 and -0.988 for iFGF-23 and cFGF-23 respectively). The authors conclude that low iron status stimulates the synthesis of FGF-23 and the actual mechanism responsible for this observation should be investigated in the future. They also point out that there is a contradictory study reported in the literature where the actual relationship is not observed (Lukaszyk *et al.*, 2015) but note that the latter study was in a different population group and not sufficiently powered to see a relationship.

Similar findings were recently reported by a Swedish group in an observational study of community-based elderly men (n=977, mean age 75.3, range 70-81) (Lewerin *et al.*, 2017). Mean Hb of participants was close to 147g/L and iron deficiency (transferrin saturation <15%, ferritin < 30 ng/ml)) was found in only 3.5% and 3.9% of participants respectively. Multiple step-wise regression analysis revealed that transferrin saturation and serum iron were independent predictors of iFGF-23 (ß values -0.10 and $p<0.001$ in both instances). The authors commented that FGF-23 might be regulated via an iron related pathway since the negative correlation between low iron and high levels of iFGF-23 were independent of biomarkers of renal function and inflammation. In support of this is the review by Kanbay *et.al* (Kanbay *et al.*, 2017) in which he concludes that FGF-23 appears to be involved in iron metabolism, erythropoiesis and other conditions such as inflammation, acute kidney injury, insulin resistance and others.

Potential new pathways of treating disorders of iron metabolism, especially in conditions of inflammation, may become possible by identifying the roles that Lipocalin 2 (an acute phase protein), plays in mammalian iron homeostasis within the circulation, at the mucosal surface and intracellularly, see review by Xiao *et al.* (Xiao, Yeoh and Vijay-kumar, 2017)

An interesting paper was recently published by Enculescu *et al.* (Enculescu *et al.*, 2017) in which the authors have developed a mathematical model of systemic iron regulation. The model was calibrated and tested using physiological responses in mice which were in a state of inflammation and/or exposed to iron overload. The authors predicted that iron uptake from the diet is regulated via the hepcidin pathway by downregulating or upregulating ferroportin to maintain homeostasis. However, under conditions of inflammation circulating iron in the blood is reduced by the decrease of intracellular ferroportin transcription. In order to fully account for observed changes of serum iron levels in the inflammatory state, exposure to high doses of dietary iron or preferential accumulation of iron in the liver under iron overload conditions, the model was expanded by adding other regulatory mechanisms, such as an iron labile pool, or loss of iron via the enterocyte desquamation.

Shin *et al.* (Shin *et al.*, 2015) evaluated the first automated immunoassay for soluble transferrin receptor (sTfR) quantification and tested it in a hospital setting. A number of authors have discussed the usefulness of sTfR as an indicator of iron status, but it is not being used routinely in the hospitals. Therefore they compared Access sTfR immunoassay with ferritin, hepcidin, total iron binding capacity, CRP and other markers. The blood samples were obtained from 367 patients with IDA or ACD and non-anemic controls (n=157; 210 and 80, respectively, age range 37-80 years). The area under the receiver operating characteristic curve (AUC) revealed that the most precise single test in

distinguishing between IDA and ACD was ferritin, AUC 0.989; sensitivity 96.8%; specificity 93.3%. The STfR's AUC was 0.944 with 85.4% sensitivity and 91.9% specificity, emphasizing its limited use in discriminating between IDA and ACD. In patients with ferritin levels between 10-100ng/ml, the grey zone due to difficult interpretation of the results (possible inflammation affecting the results), the sTfR assay proved to be the most accurate as a single test (AUC 0.931). The best performance was noted for sTfR/log ferritin index (AUC 0.994 sensitivity 95.5%; specificity 98.6% and AUC 0.962 for patients in the grey zone with sensitivity 87.0% and specificity 96.7%). The authors concluded that the sTfR assay and the sTfR/log ferritin would be of particular benefit for differentiating patients with ACD and IDA. However, it was also noted that if the patients were split into IDA, IDA with ACD, and ACD groups, the findings could be different. Similarly, Harms and Kaiser (Harms and Kaiser, 2015) concluded that sTfR/Ferritin ratio can be a valuable measure in complicated anemia, especially in combination with reticulocyte Hb. They also advocate the use of the plot published by Thomas *et al.* (Thomas *et al.*, 2006) as it allows for differential diagnosis between ACD, latent ID, classic IDA and ACD with functional iron deficiency and iron restricted erythropoiesis.

Harms *et al.* (Harms and Kaiser, 2015)also support the use of sTfR measurements for the general population in clinical settings. STfR levels are elevated with increased erythropoiesis (in conditions such as: haemolytic anemia, hereditary spherocytosis, sickle cell anemia, thalassaemia, iron deficiency anemia, and secondary polycythaemia). Reduced sTfR levels are observed in erythroid dysplasia conditions (such as chronic renal failure, hyper-transfusion, intensive chemotherapy). Interestingly, in patients with malignancies, the sTfR levels are not elevated (Beguin, 2003; Harms and Kaiser, 2015). The exceptions are non-Hodgkin's lymphoma, chronic lymphocytic leukaemia and hepatocellular carcinoma.

Braga *et al.* (Braga *et al.*, 2014) undertook a critical review in order to assess the usefulness of sTfR as a biomarker of IDA in patients presenting with concurrent inflammatory disease. Unfortunately, it was not possible to conduct a meta-analysis as the studies were too heterogeneous. However, from 6 studies that met the criteria the authors concluded that sTfR was more sensitive than ferritin, but ferritin sensitivity was affected by the selected cut-offs. This highlights the need to conduct more studies in order to identify the best markers of iron status when concurrent inflammatory diseases occur, which is a very common situation in the elderly.

Babaei *et al.* (Babaei *et al.*, 2017) investigated the use of ferritin to differentiate between participants with and without iron deficiency anemia (WHO cut-offs). IDA was diagnosed when transferrin saturation <15%. Final figures included 80 participants with IDA and 160 anemic participants without ID (mean age 72.9±8 and 71.6±7.6 respectively). There were significantly different iron parameter profiles between the two groups for ferritin, Hb, serum iron, transferrin saturation and transferrin ($p=0.036$, $p=0.018$ and $p=0.001$ for all remaining parameters) with only transferrin saturation values being higher in IDA group. The cut-off level of ferritin of 100µg/L (as established by AUC^{ROC} method) was able to predict 50% of participants with absolute IDA. Therefore the authors concluded that serum ferritin levels in their study were not a suitable tool to identify IDA in the elderly population. It seems that depending on the population studied (especially age and the prevalence of most common chronic conditions) the sensitivity and specificity of ferritin for diagnosing IDA varies greatly. More importantly, ferritin cut-off levels vary greatly.

In a large cohort of elderly (n=36,226, aged 65 and over; 62% women) Lam *et al.* (Lam *et al.*, 2013) investigated the association between an elevated red cell distribution width (RDW) and adverse outcomes. Patients were followed for up to 10 years. Out of the whole cohort 29% of participants were anemic at baseline, and out of those 53% had elevated RDW. In non-anemic patients elevated RDW was noted only in 2.5% of patients. Interestingly, elevated RDW was associated with increased mortality in both anemic and non-anemic patients with higher hazard ratios (Cox regression analysis adjusted for age, gender and Hb) in non-anemic patients (HR=1.87 and HR=3.66 respectively, $p<0.05$ in both instances). When the authors used Kaplan–Meier survival curves to investigate the predictive value of both RDW and MCV for risk stratification by anemia status, they found that when MCV and RDW was elevated, the outcome was worse and the survival was reduced from >10 and 8.6 years to 2.7 and 2.2 in non-anemic and anemic patients respectively. Therefore, the authors concluded that MCV advances the predictive value of RDW.

Diagnostic superiority of capsule endoscopy (CE) over other endoscopic equipment in the examination of the small bowel for potential pathologies has made it possible to pinpoint the reasons for IDA or ID in the elderly (Muhammad, Vidyarthi and Brady, 2014). Assessment with the use of CE provides a complete picture of small bowel submucosa. CE detects arterio-venous malformation, ulceration related to nonsteroidal anti-inflammatory drugs (which is not possible with the use of other techniques), Crohn's disease and other abnormalities of the mucosa. At the same time CE is less invasive than deep enteroscopy or colonoscopy and well tolerated by the patients. Utilisation of CE in patients with unexplained IDA may be particularly useful.

3.1. Key points:

- It is important to quantify inflammatory biomarkers when investigating iron status.
- Low iron status stimulates the synthesis of FGF-23 independently from low grade inflammation; potential for new diagnostic tool.
- Further exploration of Lipocalin 2 may lead to new pathways of treating iron metabolism disorders.
- The usefulness of mathematical models of systemic iron regulation warrant further investigation.
- STfR/log ferritin seems to be particularly useful in differentiating patients with ACD and IDA, especially in combination with reticulocyte Hb.
- There are contradictory findings about the usefulness of sTfR in concurrent inflammatory disease. STfR dynamics might vary in different inflammatory conditions.
- Serum ferritin has limited diagnostic capacity for estimating IDA in the elderly.
- Further investigations are warranted on the usefulness of Thomas *et al.* (Thomas *et al.*, 2006) plot in clinical settings.
- An elevated RDW is associated with increased mortality in anemic and non-anemic patients.
- Capsule endoscopy has potential for evaluating the reasons for IDA or ID in the elderly.

4. Hepcidin

Anemia of inflammation (AI) occurs when despite normal or increased iron stores in bone marrow, iron incorporation during erythropoiesis is decreased (Bianchi, 2013). Patients usually present with normal or increased levels of ferritin, normal or reduced iron binding capacity and reduced serum

iron levels (Weiss and Goodnough, 2005). It is also worth noting that anemia is not caused by iron deficiency if ferritin levels are above 200mg/dL (North *et al.*, 1997). In many cases of AI, cancer, inflammatory conditions and acute and chronic infections are the actual cause of AI (Nairz *et al.*, 2016). Nairz *et al.* (Nairz *et al.*, 2016) have reviewed the mechanisms initiating and maintaining AI, and the usefulness of diagnostic tools to identify AI.

Hepcidin is a key systemic iron metabolism regulator. "Three major pathophysiological mechanisms influence hepcidin production: elevated iron status and inflammation are stimulatory, and EPO-stimulated expansion of erythroid precursors is inhibitory" (Ganz and Nemeth, 2015). Hepcidin reduces iron absorption by degrading ferroportin, and iron release from macrophages (Bianchi, 2013) and hepatocytes (Ganz and Nemeth, 2015) thus preventing the release of iron into the plasma. Increase in hepcidin levels is mediated by IL-6 (pro-inflammatory cytokine) linking inflammatory state with AI through reduced iron accessibility for erythropoiesis (Ganz and Nemeth, 2015; Schmidt, 2015). Hepcidin response to an infection may be tissue, pathogen and inflammation specific (Schmidt, 2015). New hepcidin-ferroportin axis therapies have been proposed which have the potential to increase iron availability for erythropoiesis (Wang and Babitt, 2016; Langer and Ginzburg, 2017). However, the increase of hepcidin is not always present in the elderly with an inflammatory state, suggesting a more complex interaction (Ferrucci *et al.*, 2010). In healthy individuals low levels of hepcidin are found with ID or IDA, permitting maximal iron uptake from the gut and release from macrophages. Further as mentioned by Harms and Kaiser "hepcidin-25 is not a marker of iron-restricted erythropoiesis, but rather of iron supply and retention" (Harms and Kaiser, 2015).

Karlsson (Karlsson, 2015) investigated the use of mass spectrometry to evaluate the hepcidin-25 assay in the differential diagnosis of iron deficiency anemia with concurrent inflammation and AI. A study was undertaken in Uppsala Hospital, Sweden, where patients (n=31, aged 65 and over) were admitted to the Haematology Department because of anemia (WHO criteria). All patients had elevated CRP levels. According to bone marrow smears patients were divided into two groups: AI-patients with stainable bone marrow (n=20) and ID-IA -patients with no stainable bone iron and with concurrent inflammation (n=11). All patients from the ID-IA group suffered from gastrointestinal haemorrhage. In the AI group the most common diagnoses were cancer, congestive heart failure and chronic kidney disease. The hepcidin-25 assay was compared with the gold standard method (bone marrow staining) and with ferritin (amongst other blood parameters) in order to differentiate between the two groups. Hepcidin-25 was significantly higher in the AI population when compared to ID-IA population ($p<0.001$; 112.8ng/mL and 24.4ng/mL respectively). There was also a significant positive correlation between hepcidin-25 and ferritin as well as CRP ($r=0.870$ and $r=0.463$ respectively). Also, a negative correlation was found between hepcidin-25 and transferrin ($r= -0.775$). The best sensitivity and specificity (82% and 95% respectively) for hepcidin-25 was a cut-off of 31.5ng/mL to estimate iron deficiency (method: area under the curve for Receiver operating characteristic (AUC^{ROC})). The best sensitivity and specificity (70% and 100% respectively) for ferritin was a cut-off of 41.5µg/L. Since the AUC^{ROC} did not differ statistically between hepcidin-25 and ferritin the author concluded that the former method is no more advantageous than ferritin in the population studied. The mean ferritin concentrations were 65µg/L and 535µg/L in the ID-IA and AI groups respectively.

In a study by Petrova *et al.* (Petrova *et al.*, 2016) (n=148, average age 54.1±4.9y) significant increases in serum hepcidin, iron levels and IL-6 levels were observed in patients with ischemic stroke when compared to healthy control group ($p<0.001$ in all instances). The authors point towards elevated hypoxia inducible factor-1 as well as inflammatory cytokines as the triggers for elevated hepcidin in patients with ischemic stroke, which, in turn, causes iron build-up in ischemic tissue.

Drakou *et al.* (Drakou *et al.*, 2016) conducted a prospective study to investigate whether sTfR, hepcidin-25 and their ratio could be used as predictors of effectiveness of intravenous iron supplementation in iron deficient, pre-dialysis patients with CKD (n=78, 77% of men). Patients were split into two groups depending on their response to intravenous iron supplementation, with sufficient (n=40) and insufficient (n=38) erythropoiesis one month after giving iron. It was hypothesised that with iron deficiency, levels of sTfR increase while levels of hepcidin decrease, thus the sTfR/hepcidin index will decrease, whereas with inflammation or functional iron deficiency sTfR is not likely to change while hepcidin will increase, thus the sTfR/hepcidin index will increase. The authors found that sTfR and sTfR/hepcidin ratio were higher and hepcidin levels lower in patients who responded to intravenous iron supplementation ($p=0.01$, $p=0.002$ and $p=0.025$ respectively) when compared to non-responders (patients with functional iron deficiency). Interestingly, AUC ROC analysis showed that sTfR/hepcidin-25 index had better sensitivity than sTfR/log ferritin index in predicting a positive response after intravenous iron supplementation. It is also worth noting that in participants with functional iron deficiency, log sTfR/hepcidin-25 index was negatively correlated with IL-6 and high sensitivity CRP ($p<0.04$ and $p=0.005$). The authors concluded that sTfR/hepcidin-25 index at the cut-off point of 1.21 (with 52% specificity and sensitivity of 82%) could be used as a predictor of the effectiveness of intravenous iron therapy in patients with pre-dialysis CKD.

Interestingly, Jaspers *et al.* (Jaspers *et al.*, 2017) reported that in patients (n=45, mean age 55±10 years) with autologous haematopoietic cell transplantation, serum hepcidin levels before and after the transplant were driven by iron stores and EPO rather than inflammation.

4.1. Key points:

- Hepcidin-25 is significantly higher in AI population when compared to ID-IA population.
- Hepcidin-25 and ferritin values performed similarly in the differential diagnosis of iron deficiency anemia with concurrent inflammation and AI.
- Elevated hypoxia inducible factor 1 and inflammatory cytokines are most likely the triggers for elevated hepcidin in patients with ischemic stroke and iron build-up in ischemic tissue.
- There is potential to increase iron availability for erythropoiesis due to new hepcidin-ferroportin axis therapies.
- sTfR/hepcidin-25 index has potential as a predictor of the effectiveness of intravenous iron therapy in patients with pre-dialysis CKD.

5. Effect of lifestyle factors on iron status.

5.1. BMI and iron status.

Obesity negatively affects iron metabolism in the body (Nead *et al.*, 2004; Yanoff *et al.*, 2007; Zimmermann *et al.*, 2008). Changes in the circulating concentrations of hepcidin have been identified as one of the links between obesity and ID/IDA (del Giudice *et al.*, 2009; Dao *et al.*, 2013). In obese individuals, the enlarged adipocytes release cytokines which elicit chronic inflammation (Gustafson, 2010) and increased levels of leptin (del Giudice *et al.*, 2009). Both the increased inflammation and leptin promote hepcidin production in the liver (Weiss and Goodnough, 2005; Chung *et al.*, 2007). Elevated hepcidin affects iron metabolism by direct influence on ferroportin (present in enterocytes or in the plasma membrane of macrophages). Hepcidin binds to iron loaded ferroportin as it exits the enterocyte or macrophages, causing its internalisation and degradation. Consequently iron efflux into the circulation is prevented (Nemeth *et al.*, 2004). A study in obese and lean Indian women (aged 18-35 years) revealed that fractional iron absorption from a test meal with stable iron isotope ^{57}Fe was significantly lower in the obese group (Herter-Aeberli *et al.*, 2016). Also, overweight and obese individuals (between 18-50 years old) are less responsive to iron fortification (Zimmermann *et al.*, 2008), thus this strategy to improve iron status may not be as successful in a group with a high prevalence of obesity.

In a cross-sectional study of South Africans (88 women and 16 men above 60 years old) (Oldewage-Theron, Egal and Grobler, 2014) a negative relationship between low-grade inflammation in obesity and iron status was observed in the elderly.

Simon *et al* (2015) investigated the independent effect of BMI and waist circumference on iron status in 225 men (146 lean and 73 obese/overweight) and 905 women (349 lean and 544 overweight/obese). The mean age for all participants was 55 years. Hb, serum ferritin, serum iron, TIBC concentrations and CRP were measured. There was a gender specific trend: in women with a BMI above the normal category (above BMI 22±2.2) their transferrin saturation and serum iron were significantly lower when compared to lean women. Perhaps due to the smaller sample size and relatively good iron status of male participants this relationship was not noted in men. Regression analysis revealed a significant negative association in women with high BMI accompanied by normal waist circumference and iron status parameters when compared to participants in the lean category for BMI and waist circumference. These results suggest that the site of fat deposition, namely across the whole body or around the waist may have an effect on iron deficiency or iron overload, respectively.

A nested case control study conducted by a Spanish group (Fernández-Cao *et al.*, 2017) investigated the relationship between sTfR and the risk of type 2 diabetes and cardiovascular events in obese and non-obese individuals (participants with diabetes n=73 obese and 80 non-obese, and non-diabetic controls n=166 non-obese and 138 obese, mean age 66). Median follow-up was 6 years. The authors reported that the association between sTfR and waist circumference was found in both non-obese and obese individuals (ß=0.455, p=0.003 and ß=0.802, p<0.001), being stronger in the latter group. Major sTfR determinants were waist circumference, ferritin and use of alcoholic and caffeine drinks. When waist circumference was replaced with BMI in the regression model, BMI and ferritin were

the only predictors of sTfR and only in obese individuals (ß=0.030, p=0.017). Comparison of the lowest and highest tertile revealed that the odds ratio (OR) of the association of sTfR with the risk of type 2 diabetes was 0.40 (95% CI: 0.20–0.79, p=0.015) in non-obese subjects and 2.79 (95%CI: 1.35–5.77, p=0.005) in obese subject. The analysis was adjusted for a number of factors such as fasting glucose, lifestyle, sociodemographic status, anthropometric data, dietary and biochemistry variables of the risk of type 2 diabetes. The authors concluded that the nature of the relationship between the risk of type 2 diabetes and sTfR is different in the presence or absence of obesity and that sTfR may not be an accurate tool to measure iron status in obese populations.

Sun *et al.* (Sun *et al.*, 2013) investigated the association between the onset of type 2 diabetes and elevated levels of ferritin. Community dwelling Asians (n= 2198) aged between 50-70 years took part in this prospective study and were followed-up for 6 years. When the authors compared the highest quintile of ferritin with the lowest, the adjusted regression analysis revealed that relative risk (RR) of type 2 diabetes was 1.90 (95% CI: 1.37, 2.65). The authors also conducted meta-analysis of 9 studies which resulted in similar findings of RR 1.60 (95% CI: 1.25, 2.04, heterogeneity I^2 =49.0%; p=0.03). Thus it was concluded that increased ferritin (or body iron stores) are an independent risk factor for developing type 2 diabetes.

Similarly, Andrews *et al.* (Andrews, Soto and Arredondo-Olguin, 2015) investigated the association between iron status and inflammation in obese and non-non obese subjects with and without diabetes (n=444; 146 controls, 106 obese with type 2 diabetes, 132 obese patients without diabetes and 60 non obese individuals with type 2 diabetes). The authors reported that obesity alone was associated with elevated ferritin levels (p=0.0001). Type 2 diabetes was independently associated with elevated ferritin, high sensitivity CRP and thiobarbituric acid reactive substance (TBARS), a surrogate measure of the reactive oxygen species (p=0.001, p=0.008 and p<0.001 respectively). In obese patients with type 2 diabetes the association increased further for ferritin and TBARS. In the adjusted model (age, BMI, high sensitivity CRP) for the development of type 2 diabetes high odds risk was reported in the upper ferritin quartile and for increased TBARS levels (p<0.01 and p<0.05 respectively). The authors concluded that since the inflammation in individuals with type 2 diabetes was present irrespective of adiposity, other mechanisms such as oxidative stress or elevated insulin levels may be responsible for the inflammatory state, and diabetes is associated with modified body iron stores irrespective of obesity.

5.1.1. Key points:

- In obesity, ferritin acts as a marker of inflammation rather than reflecting iron status.
- In obesity, a negative relationship is observed between low-grade inflammation and iron status.
- Fat deposition across the whole body or around the waist may result in iron deficiency or iron overload, respectively.
- The nature of the relationship between the risk of type 2 diabetes and sTfR is different in the presence or absence of obesity.
- sTfR may not be an accurate tool to investigate iron status in an obese population.
- Increased body iron stores are an independent risk factor for developing type 2 diabetes.
- Type 2 diabetes is independently associated with elevated ferritin, high sensitivity CRP and

thiobarbituric acid reactive substance.

5.2. Iron status and diet.

A systematic review and meta-analysis conducted by Haider *et al.* (Haider *et al.*, 2016) investigated the effect of vegetarian diets on iron status. Out of 24 studies included in the analysis 6 included an elderly age group. The authors reported that vegetarians have significantly lower ferritin levels including the elderly age group. It was also reported that adult vegetarians have significantly lower ferritin levels than meat eaters (-29.71ng/mL, 95%CI (-39.69, -19.73), $p<0.01$). The addition of participants who ate meat occasionally (<1 a week) only slightly adjusted the results (-23.27ng/mL, 95%CI (-29.77, -16.76), $p<0.01$). It is also worth noting that the effect of the diet was more evident in men than in women (-61.88ng/mL vs -13.50ng/mL respectively, $p<0.001$ in both instances). It was concluded that diet could potentially be used as means to change iron status.
Jackson *et al.* (Jackson *et al.*, 2016) undertook a meta-analysis on the effect of increased consumption of meat on iron status in adults from industrialized countries. A total of 49 studies met the criteria, out of which 10 included people over 65 years. The authors reported highly conflicting results. Out of 7 studies which were high quality, 5 reported a positive association between meat consumption (85-300g/day) and iron status, but these studies were primarily conducted in women of childbearing age.

It has long been known that vitamin C enhances iron uptake in the duodenum. However Lane *et al.* (2016) point out that vitamin C is not only increasing dietary iron absorption but also is crucial at cellular level for efficient uptake of iron from transferrin (intracellular reductive mechanism), the only source of iron for erythropoiesis (Lane and Richardson, 2014; Suárez-Ortegón *et al.*, 2016). Whilst vitamin C deficiency is considered to be uncommon, a 2005 publication from the UK found that around 40% of elderly individuals over 65 years of age were vitamin C deficient (Elia and Stratton, 2005). Taking into account that critical care patients and dialysis patients (Singer *et al.*, 2008; Berger and Oudemans-Van Straaten, 2015) are often vitamin C deficient, there is potentially a chance of improving iron status by correcting vitamin C deficiency.

Bianchi (2016), in his review from 2016, concludes that adequate nutrition could and should be used in the elderly in order to prevent IDA and if coupled with exercise it should reduce the rates of mortality and improve overall wellbeing. Bianchi points out that inadequate intake of protein followed by malnutrition and potentially anorexia triggers systemic inflammation, immunodeficiency disorders and anemia. On the other hand obesity also activates inflammatory pathway, increases hepcidin and downregulates uptake or release from iron storage in the body.

5.2.1. Key points:

- Vegetarians are at higher risk of ID and IDA than regular meat eaters.
- Assessment of vitamin C status in the elderly and addressing deficiency may have a beneficial impact on iron status.

5.3. The role of exercise in iron homeostasis and iron status in the elderly.

Reports in the literature suggest that body iron stores increase with ageing and with the pathogenesis of a number of diseases (Sullivan, 2004). The link between declining health in the presence of excess body iron stores may, at least in part, be caused by conditions of stress (through activated protein kinase) in which ferritin may release some of the stored iron starting the cascade of free radical mediated damage (Borkowska *et al.*, 2011).

Kortas *et al.* (2017) investigated whether regular Nordic walking would reduce body iron stores and therefore reduce oxidative stress in apparently healthy 35 women aged 60 and over. The women participated in the exercise program for 12 weeks, meeting 3 times per week for approximately 50min of Nordic walking training at 60-70% intensity of maximal heart rate. Blood samples were collected at baseline and at the end of 12 weeks of the exercise program. Markers of iron levels, lipid profile and oxidative stress indicators were measured in the blood. The authors reported significant reduction in ferritin and serum iron ($p=0.00$ in both instances) and in oxidative stress parameters, namely in malondialdehyde and advanced oxidation protein products ($p=0.01$ in both instances) after 12 weeks of intervention. In another paper published by the same group similar trends in serum ferritin were noted after 32 weeks of Nordic walking training (Kortas *et al.*, 2015). Thirty-seven women aged 67.7±5.3 completed the intervention which comprised of exercising 3 times per week for an hour. The authors noted a number of changes in hematological parameters. Significant reductions in Hb, hematocrit and ferritin were observed ($p=0.00$ in all instances), and they remained in the normal range, but no changes in hepcidin levels. The authors speculated that "effects of training on hepcidin may be dependent on the subjects' body iron levels". The use of physical exercise to control body iron levels is an interesting concept and worth further investigation.

An Egyptian (Mohamady, Elsisi and Aneis, 2017) group conducted a study in women with breast cancer (n=30, age 60-70) undergoing chemotherapy. The 12 week randomized intervention was an aerobic exercise (walking on a treadmill) 3 times per week for 25-40 min. within 50-70% maximum heartrate or no exercise beyond the usual day-to-day activities. Patients were not on EPO therapy and their BMI was between 30 and 35. Since patients undergoing cancer treatment often suffer from chemotherapy-induced anemia the authors wanted to investigate if regular exercise could improve iron status parameters. Hb and red blood cell count were analysed prior and after the 12 week period. In comparison to the baseline, Hb levels significantly increased (5.0% change) in the group that exercised whereas in the group that did not train Hb levels significantly decreased (-11.8% change) after 12 weeks ($p<0.001$ in both instances). Further, the group that completed the 12 week course of training had significantly higher Hb levels than the group that did not participate in training ($p=0.001$). Exactly the same trend was observed for RBC with significant increase (5.9% change) in the group that exercised and significant decrease (-13.0% change) in the group that did not train. Also RBC values were significantly higher at the end of the 12 week period in the group that exercised when compared to the group that did not undertake the training ($p=0.001$). The authors hypothesize that the observed effect is due to the challenge of weight bearing exercise and the effect it has on bone marrow and thus blood formation. The study could benefit from including ferritin analysis and dietary data as then it would help to clarify whether the observed effect was due to the

release of iron from body iron stores or an increased uptake of iron from foods. Nonetheless, exercise could be used in clinical settings to alleviate chemotherapy-induced anemia.

5.3.1. Key points:

- There is potential to use exercise to modify body iron status in apparently healthy elderly people.
- Exercise has potential to alleviate chemotherapy-induced anemia.

5.4. Use of iron supplements in ID and anemia.

The most common strategies to treat ID and anemia are oral iron supplementation, intravenous iron and blood transfusion, with the latter method best evaluated individually (Goodnough and Schrier, 2014) and considered to be the last resort (Stauder and Thein, 2014). The actual strategies to correct anemia should be dependent on the individual assessment in order to provide the most effective and appropriate treatment. For example in patients with ACD oral iron supplementation will be ineffective. Novel therapies, including hepcidin inhibitors (Eijk *et al.*, 2014; M. Wang *et al.*, 2016; Sebastiani *et al*, 2016) may prove to be particularly useful in cases of AI.

The causes of iron deficiency anemia in the elderly are numerous and complex, therefore iron supplementation may only be beneficial for some patients. Usually, high daily doses of iron (150mg and more) are prescribed and these may cause various adverse effects in a large proportion of patients, resulting in low compliance. Also, around 50% of over 80 year olds (Salles, 2007) suffer from low or absent production of hydrochloric acid in the stomach due prescribed proton pump inhibitors (Hamzat *et al.*, 2012) or hypochlorhydria (Mabuchi *et al.*, 2017), thus dramatically limiting iron absorption. Tay and Soiza (2015) examined the feasibility of oral iron supplementation in the elderly in a systematic review. The meta-analysis comprised only 3 studies with a daily dose of 200mg of ferrous sulphate. In total, the data from 440 participants was used, of which 8% reported adverse effects with 3% stopping the supplementation. The authors found that 4–6 weeks treatment of oral iron supplementation augmented Hb levels with a mean difference of 0.35g/dL ($p= 0.003$). One of the limitations of this review is that all participants were hospitalised for orthopaedic reasons while taking part in the study, limiting generalisability. Lindblad *et al.* (2015) point out that in older individuals (mean age 85y) a low vs high dose of elemental iron (15mg vs 150mg per day) supplement is as effective and causes significantly less side effects. Further, Prentice *et al.* (2016) conclude that the use of higher doses of iron provided in a single dose is physiologically inappropriate and unsafe, mainly because iron metabolism is very tightly regulated by hepcidin and ferroportin. Finally, high doses of supplementary iron are unwarranted in patients with inflammation as they are may cause a number of adverse effects and are unlikely to improve iron status (Prentice *et al.*, 2016). Thus the underlying reasons for inflammation should be addressed first. It is also worth mentioning that in a recent paper by Lane and Richardson (2014) optimising the iron:vitamin C ratio in iron supplements helps to minimise potential side effects of iron supplements.

Silay *et al.* (2015) conducted a cross-sectional study (52 woman and 17 men) in which he compared the response to an oral dose of 325mg of iron sulphate (iron content was 65mg) between the elderly

(above 65 years old) and younger (below 65 years old) participants. All participants had IDA. Participants with other causes of anemia were excluded from the study. Fasting serum iron levels were compared with the samples obtained 3 hours post ingestion of iron tablet. According to an independent t-test, the means were significantly higher (p=0.001) in the younger group, 86.1µ/dL in older and 163.µ/dL in younger participants.

Santen *et al.* (2014) investigated if it would be possible to predict a response to iron supplements in 38 anemic patients with chronic rheumatologic disease who used anti-inflammatory drugs shortly after the start of supplementation. The authors measured a number of iron status indicators at baseline and then weekly over a 6 week period while supplementing the participants with 130mg of elemental iron per day. Participants who responded (n=16) well to iron therapy, as defined by an increase in Hb of at least 0.81g/dL at the end of the 6 week period, had significantly lower iron status indicators at the start of intervention. Interestingly, their reticulocyte Hb content increased significantly after just one week of iron supplementation when compared to the group of participants who did not respond to iron supplementation ($p<0.000$). Receiver Operating Characteristics curves revealed that the best predictor for Hb increase after six weeks of iron supplementation was an increase in week one of the intervention in reticulocyte Hb content, transferrin saturation, serum iron levels and reticulocyte count. Conversely, Kurzawa *et al.* (2016) noted that in hemodialyzed patients with chronic renal disease the use of reticulocyte Hb content to identify anemia proved to be ineffective, with accuracy reaching only 45%. In their studies Hb, sTfR, serum iron and CRP proved to be of the most diagnostic value. The above conflicting findings highlight the importance of chronic diseases as a complicating factor for accurate estimation of ID, anemia and its causes. Nonetheless, the assessment of immature red blood cells value as a tool to identify individuals with concurrent chronic inflammation who would benefit from iron supplementation seem particularly relevant in the elderly.

Finally, emerging therapies to combat anemia, such as erythropoiesis stimulating agents require further considerations due to potential side effects. Therapies for AI (currently in pre-clinical stages) may include hepcidin inhibitors (Eijk *et al.*, 2014; M. Wang *et al.*, 2016; Sebastiani, Wilkinson and Pantopoulos, 2016) and other agents modulating ferroportin levels (Fung and Nemeth, 2013). As pointed out by Stauder and Thein (2014) genetic testing will also become a useful tool, especially in the classification of anemia.

5.4.1. Key points:

- As oral iron supplementation in the elderly may not be very effective, strategies predicting the response to oral iron supplementation should be put in place, e.g. iron absorption tests.
- Lower doses of oral iron should be considered.
- There is potential for using erythropoiesis stimulating agents, hepcidin inhibitors and ferroportin modulators.

6. Effect of iron status on health outcomes, including chronic diseases.

In a study of healthy Koreans aged 65 and over a positive association was found between bone mineral density and ferritin levels in men (n=1,374) but not in women (1,569) (Lee *et al.*, 2014). The study measured bone mineral density (g/cm2) using whole-body dual-energy X-ray absorptiometry (DEXA) with lumbar, hip and total femur scans. The analysis was adjusted for age, body mass index, daily intake of protein and calcium, physical activity and socioeconomic status. One of the limitations of the study was that subjects with hormone replacement, osteoporosis treatment or hysterectomy were excluded. Pan *et al.* (2017) reported similar findings on the association between IDA and osteoporosis in the general population but their results were not gender specific. Also, in 2003 Harris *et al.* (2003) reported that the dietary intake of iron is positively associated with mineral bone density in postmenopausal woman aged between 44 and 66 years. However, there are a number of studies which report low bone mineral density or osteoporosis in patients suffering from iron overload/hemoglobin disorders, such as hemochromatosis, thalassemia and sickle cell anemia (Voskaridou and Terpos, 2004; Valenti *et al.*, 2009; Rossi *et al.*, 2014; Valizadeh *et al.*, 2014). Iron accumulation in the bone impairs metabolism through a number of different pathways. Recently, Rossi *et al.* (2014) have shown a direct relationship between bone mineral density and the levels of expression of tartrate-resistant acid phosphatase (TRAP). Metalloprotein enzyme "TRAP is an iron-phosphoesterase and its activation is associated with the redox state of the di-iron metal centre or proteolytic cleavage in an exposed loop domain". The authors have also shown that in patients with thalassemia, their osteoclasts up-regulate TRAP. This up-regulation is correlated with bone mass decline (Rossi *et al.*, 2014). Those findings suggest that osteoporosis due to high levels of iron is limited to patients with iron overload disorders. Since osteoporosis is a major health issue in the elderly, the positive relationship between dietary iron intake, ferritin levels and bone mineral density warrants further investigation.

Based on reports that anemia is independently and significantly associated with old age (Bosco *et al.*, 2013), frailty, morbidity and mortality (although the mechanisms are not known) (Penninx *et al.*, 2006), Cedillo *et al.* (2014) investigated whether there is a relationship between haemoglobin levels and frailty. Volunteers (n=1933 aged 60and over) were randomly selected from the Mexican Study on Ageing and Dementia. In total 8.3% of participants were anemic (Hb below 13g/dL and 12g/dL in men and women respectively) with similar distribution among women and men (4.2% and 4% respectively). The prevalence of anemia in pre-frail and frail group was 2.7% and 2.8% respectively. The authors used Fried and Walston criteria to establish the degree of frailty, including: walking speed, grip strength, weight loss and exhaustion. They found that the risk of frailty increased with the fall in Hb concentration. The likelihood of frailty was as follows:

- For Hb 10.5g/dl the odds ratio was 6.3 (95% CI 5.5 to 7.3)
- For Hb 11.5g/dl the odds ratio was 2.3 (95% CI 2.0 to 2.6)
- For Hb 12.5g/dl the odds ratio was 1.1 (95% CI 1.0 to 2.1)
- For Hb 15g/dl the odds ratio was 0.8 (95% CI 0.7 to 0.8)
- For Hb 16g/dl the odds ratio was 0.9 (95% CI 0.81 to 0.91)

Multivariate analysis revealed that quintiles of Hb were independently associated with frailty. However, as the authors noted, subjects in the lower Hb quintile were older and presented with a higher number of comorbidities. Hypoxia is a potential mechanism for a decline in function of the brain and other organs as well as muscle oxygenation, thus affecting strength, physical and cognitive

performance. Causative mechanisms are still to be elucidated. Nonetheless, in patients with low Hb, risk of frailty should be assessed.

In a more recent review (Gabriele, 2016) the association between frailty, anemia and 'inflammaging' (chronic inflammatory stage due to aging) was explored, together with the latest treatment options (Röhrig *et al.*, 2014). ACD is the most dominant type in the elderly, and elevated levels of hepcidin due to inflammation downregulate ferroportin, keeping iron in the cells and causing functional iron deficiency and impaired erythropoiesis. Another hypothesis considers the negative effect of chronic inflammation on bone marrow and perturbed erythropoiesis (Gomes and Gomes, 2016). The exact mechanisms are yet to be described.

6.1. Key points:

- The reported positive relationship between dietary iron intake, ferritin levels and bone mineral density warrants further investigation.
- Quintiles of Hb are independently associated with frailty, with hypoxia being a potential mechanism affecting strength, physical and cognitive performance.
- Patients with low Hb should be assessed for frailty.

6.2. Iron status in inflammatory bowel disease (IBD) patients.

The changes occurring in the gastrointestinal tract with age and the effect it might have on iron uptake from the gut were reviewed in 2014 by Busti *et al.* (2014). Since then other papers have been published.

IBD can be categorised into ulcerative colitis (UC) in which the gut inflammation is limited to the colon or Crohn's disease (CD) in which the whole gastrointestinal tract is affected. Both UC and CD have a number of similar symptoms (Verma and Cherayil, 2017). The underlying reason of anemia in IBD patients is often multifactorial. Two most common reasons are IDA due to blood loss in the intestinal mucosa and consequently reduced iron absorption and anemia of chronic disease (Stein and Dignass, 2015).

In a prospective study conducted by Abitbol *et al.* (2015), out of 150 (81 women) enrolled patients (median age 38, range 16-78 years) anemia was present in 42 patients (28%) which is similar to the findings reported by others (Filmann *et al.*, 2014). Patients suffered from Crohn disease, ulcerative colitis and unclassified colitis (n=105, 43 and 2 respectively). The active form of IBD was diagnosed in 45.3% participants. As with other chronic conditions assessment of ID or IDA is difficult. The authors investigated if transferrin-ferritin index (sTfR/log) would be a useful measure of iron status in patients with IBD. They also measured CRP, fecal calprotectin, vitamin B_9 and B_{12}, hemoglobin, haptoglobin, lactate dehydrogenase, and performed endoscopy. The ID cut-off for ferritin was <30ng/mL or sTfR/log ferritin > 2 if inflammation was present. ID was present in 49 participants (32.7%). 24% of participants were diagnosed with ID based on ferritin cut-off. A further 8.7% were diagnosed using the transferrin-ferritin index. Twelve percent of patients had vitamin B deficiency. It

is worth mentioning that out of 49 participants with ID, 21 did not have anemia. The authors concluded that to ensure cost effectiveness a serum ferritin cut-off of <30ng/mL should be used first followed by transferrin-ferritin index as a second step analysis in patients with inflammation and ferritin in a normal range.

A Swiss group (Mecklenburg *et al.*, 2014) analysed serum hepcidin concentrations in IBD active and inactive patients presenting with IDA or with anemia of other causes and compared the results with healthy controls (n=130, age range 17-72 years). In this retrospective observational study 130 Out of 247 participants had Crohn's disease and 117 had ulcerative colitis. Based on the IBD status (active or inactive) participants were split into 5 groups. Interestingly, anemic participants with active disease or in remission stage of the disease who had ferritin levels below <30ng/ml had significantly lower hepcidin levels when compared to participants with ferritin >30ng/ml (patients and healthy controls). Backward multi-linear stepwise regression revealed that only ferritin was significantly associated with hepcidin (p=0.005). The authors therefore concluded that ferritin, not inflammatory biomarkers, is responsible for controlling hepcidin levels in their group of IBD patients. These findings are potentially opening the possibility of using hepcidin suppressant (Eijk *et al.*, 2014; M. Wang *et al.*, 2016; Sebastiani, Wilkinson and Pantopoulos, 2016) drugs in order to correct IDA in patients with IBD.

Blumenstein *et al.* (2014) conducted a retrospective analysis from the prospective, observational, multicentre study to investigate the trends in current practice of treating anemia in IBD patients. In patients, age range 18-83 years (mean age 39, 41% men), 193 IBD patients with anemia were analysed. Anemia was primarily assessed by Hb, serum ferritin or transferrin saturation (100%, 97% and 82% respectively). However, only 43.5% of participants were supplemented with iron to alleviate anemia, out of which 56% were prescribed oral supplementation, 15% had intravenous iron, 19% had both oral and intravenous supplementation and 10% had blood transfusions. The authors emphasise the need for a change in German supplementation practices in IBD patients from oral to intravenous, since current methods are clearly not effective.

In order to treat ID and IDA Stein and Dignass (2015) suggest that the use of intravenous iron supplementation is superior to oral iron supplementation, which in patients with IBD may cause a number of adverse side effects (Stein and Dignass, 2015; Verma and Cherayil, 2017). In patients who do not respond to the supplementation, recombinant human EPO could be of potential help (Blumenstein *et al.*, 2014). This method however requires further investigations, especially in IBD patients with AI (Verma and Cherayil, 2017).

6.2.1. Key points:

- Diagnosis of ID in patients with IBD should be a two-step process: first serum ferritin cut-off of <30ng/mL should be applied, followed by transferrin-ferritin index as a second step analysis in patients with inflammation and ferritin in a normal range.
- It is likely that ferritin, not biomarkers of inflammation, is responsible for controlling hepcidin levels in IBD patients.

- The potential to use hepcidin suppressant drugs in order to correct IDA in IBD patients warrants further investigation.
- There is an urgent need to review supplementation practices in IBD patients, with potential for changing to intravenous treatment.

6.3. Assessment of iron status in kidney failure and health outcome.

In a study of 2,606 (mean age 64 years) patients on hemodialysis without inflammation, Shoji *et al.* (2017) reported that both low and high ferritin levels were significantly associated with all-cause mortality (Cox proportional hazard ratios: 1.79 and 1.55 respectively). Similarly, Park *et al.* (2015) in a prospective cohort study of 946 (mean age 60 years, range 49-70, 61% males) patients found that serum ferritin levels at the start of hemodialysis were independently associated with all-cause mortality risk, infection related and cardiovascular mortality risk (p=0.003, HR1.5, 95% CI 1.156-2.069; p=0.032, HR1.9, 95%CI 1.056–3.476 and p=0.033 HR1.6, 95%CI 1.040–2.474 respectively). The authors concluded that serum ferritin could be used as a predictor of mortality irrespective of the presence of inflammation or the nutritional status of patients on haemodialysis.

In an Australian study of 197 haemodialysed patients (60% of women; 178 indigenous and 19 non-indigenous aged 50±8.1 and 58±8.7 respectively)) 57% of participants had CRP above 10mg/L, and 16% had ferritin above 1,500µg/L (Majoni et al., 2014). Indigenous patients had significantly higher ferritin levels and significantly lower responsiveness to erythropoiesis stimulating drugs than the rest of the group (p=0.002 and p<0.001 respectively). All participants with normal CRP levels had ferritin above 500µg/L. As the authors pointed out there are no guidelines for iron supplementation for patients on hemodialysis if their ferritin is above 500µg/L. In this study 99% of participants had a ferritin levels above >500µg/L clearly identifying urgent need to update the guidelines for clinical practice.

Based on fairly recent findings that release of cell free DNA takes place during hemodialysis (Tovbin *et al.*, 2012) due to cell death, Kohlova *et al.* (2013) investigated the levels of cell-free DNA in end-stage renal disease patients (58% men, mean age 65.7±14.4 years, n=153 and 20 controls) and its association with other hematological, inflammatory and iron status indicators. The patients had significantly lower serum iron and transferrin and significantly higher sTfR, transferrin saturation, ferritin, hepcidin CRP, IL-6 and circulating free DNA (p<0.05 in all instances). CRP was independently associated with circulating free DNA (ß=0.372, p<0.0001). Significant correlations were found between circulating free DNA and inflammatory biomarkers (CRP and IL-6 p<0.001 in both cases), iron status indicators (sTfR, transferrin and serum iron; p<0.001, p<0.001 and p=0.002 respectively) and EPO doses (p<0.001). The authors concluded that elevated levels of circulating free DNA in aemodialysis patients are associated with the inflammatory status. Since it has been reported that circulating free DNA can trigger IL-6 synthesis by monocytes (Atamaniuk *et al.*, 2012), it could be an additional pathway to further stimulate the inflammatory cascade, affect iron metabolism and decrease response to EPO therapy that is so frequent in end-stage renal disease patients on hemodialysis.

In a study of 69 patients with early stage chronic kidney disease (age range 30-86) Lukaszyk *et al.* (2015) reported that sTfR remained unaffected by the inflammatory status in patients with functional

iron deficiency. These findings were supported by another group (Yin *et al*, 2017) and suggest that sTfR could be very useful in distinguishing between anemia of chronic inflammation and IDA in patients with early stages chronic kidney disease. The authors also conclude that "any iron deficiency should guide a physician to rule out inflammation/infection as a priority".

Interestingly, a Chinese group reported that switching from low-flux to high flux dialysis reduces anemia in patients with chronic kidney disease (n=40, 55.83 ± 14.54 years, 68% males) (Yin *et al.*, 2017). Bloods were collected at the start of high flux dialysis then at 2, 6 and 12 months. Hb values significantly increased, sTfR and the use of EPO agents significantly decreased after 12 months of high flux haemodialysis ($p=0.004$, $p<0.001$ and $p<0.001$ respectively), suggesting that high flux dialysis might be a better option in preserving/ correcting iron status.

A potentially new iron status indicator (in early chronic kidney disease patients) namely growth differentiation factor 15 (GDF-15), an anti-inflammatory cytokine playing its role in hepcidin metabolism, was reported by Lukaszyk *et al*. (2016) (n=56, age 77.5±6.8,). A negative correlation was observed between Hb and GDF-15 ($r=-0.52$, $p<0.05$), serum iron and IL-6 ($r=-0.38$ and $r=-0.34$, respectively, $p<0.05$ in both instances). Finally, GDF-15 was significantly higher in anemic patients when compared to non-anemic peers. No correlation with hepcidin was observed.

Another potential iron status indicator was reported by a Japanese group (Kim *et al.*, 2017). Neutrophil gelatinase associated lipocalin (NGAL), marker of acute kidney injury was measured in patients with chronic kidney disease (CKD) with anemia in pre-dialysis stage (288 patients, mean age 60±9.5, 59% males and 131 controls, mean age 57±5.0, 65% males). Anemia was diagnosed based on TSAT <30%. Multivariate analysis (including hsCRP and eGFR) revealed that NGAL was independently associated with TSAT. Further, plasma NGAL (cut-off value of ≤394 ng/ml) had better sensitivity and specificity in estimating ID than serum ferritin (cut-off value of ≤500 ng/ ml) in anemic CKD patients (84.2% and 50% vs 50.9% and 66.7% respectively). The authors concluded that NGAL is a more reliable indicator of iron status in the presence of inflammation than ferritin.

In a cross-sectional study Mercadel *et al.* (2014) investigated the metabolism of hepcidin in relation to inflammation and iron parameters in patients with chronic kidney disease (n=199; age 58.±14.8, 57.3% of males). The study revealed that 'the median hepcidin increased from 23.3 ng/ mL (8.8–28.7) to 36.1 ng/mL (14.1–92.3) when glomerular filtration rate (mGFR) decreased from ≥60 to <15 mL/min/1.73 m2 ($p=0.02$)'. However, in patients with absolute iron deficiency, hepcidin values plummeted irrespective of the mGFR decrease. The highest levels of hepcidin were present in patients with normal iron parameters (ferritin >40 ng/mL and TSAT>20%). Multivariate analysis revealed that mGFR, EPO, BMI ≥30 and oral iron supplementation were all significantly associated with hepcidin. Overall hepcidin levels are high in chronic kidney disease patients, except in absolute iron deficiency. Therefore oral iron supplementation will not be effective in patients with chronic kidney disease.

Toblli and Gennaro (2015) conducted a single centre pilot study from which they reported that in patients with non-dialysis chronic kidney disease (n=30, age 70.1±11.4 years) changing oral to intravenous iron therapy was more effective for improving iron and hematological parameters. Within six months Hb, ferritin and transferrin saturation increased by 0.7±0.3 g/dL, 196.0±38.7 μg/L

and 5.3±2.9% respectively ($p<0.01$ in all instances). Also the use of erythropoiesis-stimulating agent decreased significantly ($p<0.01$). Thus intravenous therapy might be more appropriate for patients with non-dialysis chronic kidney disease.

A Japanese group investigated the relationship between Hb and mortality in elderly patients on hemodialysis (n=3341, age 63.6±12.7, 63% men) (Hanafusa *et al.*, 2014). Hb was measured every 4 months, with median follow-up 2.64 years. When the analysis was split into elderly (>75 years) and non-elderly group (<75 years) it revealed that Hb levels below 9g/dL and below 10g/dL respectively were associated with higher mortality ($p=0.023$ and $p=0.044$ respectively), suggesting that the elderly might tolerate lower levels of Hb than the rest of the population. However, significant interaction between age and Hb on mortality was only observed in time dependant analysis. It might be that the Hb trend is more accurate in forecasting diagnosis than a single Hb value.

Thamer *et al.* (2014) investigated if high Hct (at 34.5-<39.0% (n=5390)) or low Hct (at 30.0-<34.5% (n=3637)) levels would be more beneficial in patients were over 65 years old with diabetes as a primary reason for haemodialysis. Maintenance of Hct levels was achieved by administration of erythropoiesis-stimulating agents. The HR for high vs low Hct treatment were 1.01 (0.83; 1.38, 95%CI) for all-cause mortality and 1.00 (0.81, 1.24) for multiple factors mortality and cardiovascular outcome. Similar findings were reported in the elderly patients who were on dialysis, had diabetes and also cardiovascular disease (n= 22,474; the United States Renal Data System) (Zhang *et al.*, 2014). Therefore the authors support the Food and Drug Administration's recommendation of terminating erythropoiesis-stimulating agents treatment once Hct reaches 33% in patients who are on dialysis, have diabetes and cardiovascular disease.

6.3.1. Key points:

- Elevated levels of circulating free DNA in patients on hemodialysis are associated with an inflammatory state, and negatively affect iron metabolism and response to EPO therapy.
- sTfR appears to be an accurate marker of iron status and could be used to differentiate between anemia of chronic inflammation and IDA in patients with CKD.
- Switching from low to high-flux dialysis reduces anemia in patients with CKD.
- Oral iron supplementation will most likely be ineffective in CKD patients due to elevated hepcidin.
- Elderly patients on hemodialysis may have lower levels of Hb than younger patients.
- There is an urgent need to establish guidelines on iron supplementation for patients on hemodialysis with ferritin above 500µg/L.

6.4. Iron status in cancer patients.

Since anemia is a common complication impeding the quality of cancer patients life, Maccio *et al.* (2015) conducted a prospective, observational study to investigate the prevalence of anemia in patients presenting with solid cancers (n=888, mean age 65.1±11, 51% men) prior to the start of anticancer treatment. They found 63% of oncology patients to be anemic at the time of diagnosis according to WHO Hb cut-offs. Ludwig *et al.* (2013) in a study of 1528 cancer patients (mean age 65) reported that 33.3% of patients were anemic and 42.6% were ID, with a higher prevalence of ID

found in patients with solid tumours than hematological malignancies. In both studies (Ludwig *et al.*, 2013; Macciò *et al.*, 2015) iron status declined with advancement of the disease stage, with significant differences reported between patients in stage III and IV when compared with stage I and II ($p<0.05$). Interestingly, they also reported significant differences in Hb values and inflammatory parameters between different cancer sites ($p=0.013$). The highest mean Hb and the lowest inflammatory parameters were noted in breast cancer patients and the lowest Hb and the highest inflammatory parameters in ovarian cancer patients (Macciò *et al.*, 2015). Finally the authors point out that with progression of the diseas,e reduced food intake, malnutrition and cancer anorexia play an important role in anemia. Also, as reported by other groups (Durigova Anna *et al.*, 2013; Kurtz *et al.*, 2016), during chemotherapy a large proportion of cancer patients (86.2%) become anemic. Nutritional support in cancer patients could be beneficial in improving well-being and iron status of patients and the evaluation of anemia in cancer patients should be expanded by appropriate markers.

In a large case control population based study (aged 66-99 years; 100,000 cancer free controls and 1,138,390 individuals with cancer from Surveillance, Epidemiology, and End Results Medicare database, USA) (Murphy *et al.* (2015) vitamin B_{12} anemia caused by autoimmune gastritis damaging parietal cells in the stomach was linked with increased cancer risk. Pernicious anemia (body's inability to absorb vitamin B_{12}) affects between 2 and 5% of the aged population. The authors found that people with pernicious anemia had significantly higher ($p<0.0008$, Bonferroni correction) risk of developing the following cancers: stomach, tonsil, throat, oesophagus, small intestine, liver, leukemia, myeloma and carcinoid tumors when compared with general population. Thus people presenting with pernicious anemia would benefit from cancer screening tests, especially given that the increased risk continues even after correction of anemia.

Castelli *et al.* (2014) conducted a pilot study (n=24, aged 65-84, average age 72 years, 58% men) investigating the usefulness of biosimilar epoetin-α (an erythroid-stimulating agent) to correct anemia in patients with myelodysplastic syndromes who are likely to develop acute myeloid leukemia. Epoetin-α was administered once a week for a period of 12 weeks with responders continuing for another 12 weeks. Hb values increased significantly ($p<0.001$) after 12 weeks of treatment with 67% patients responding well to epoetin-α treatment. Also, 63% of patients no longer needed blood transfusion. The effect continued for at least 3 months (follow-up period). Erythroid stimulating agents could be an effective intervention in correcting anemia in patients with myelodysplastic syndromes and cancer.

A review of ferritin in cancer (Alkhateeb and Connor, 2013) reported that higher levels of ferritin very often occur in cancer patients and are associated with poor clinical outcome and the aggressiveness of the disease (Ji *et al.*, 2014). Ferritin expression takes place in tumour-associated macrophages which play a key role in cancer advancement as well as in chemotherapy resistance. It has been hypothesized that reducing ferritin expression will impede tumour growth and at the same time increase responsiveness to medications. Thus investigations into ways of down-regulating ferritin in cancer cells could potentially have therapeutic consequences.

6.4.1. Key points:

- 63% of oncology patients are anemic at the time of diagnosis.
- A large proportion of cancer patients becomes anemic during chemotherapy.
- Cancer related anemia is comprised of immune, metabolic and nutritional constituents.
- Nutritional support in cancer patients could be beneficial in improving the iron status of the patients.
- Down-regulation of ferritin in cancer cells could have potential therapeutic consequences.
- Pernicious anemia patients are at higher risk of developing cancer, thus screening tests are advised.
- Erythroid stimulating drugs could be an effective intervention in correcting anemia in patients with cancer.

6.5. Iron status and cardiovascular health.

Associations between iron status and poorer cardiovascular health have been reported by a number of groups, as discussed in our previous review (Fairweather-tait *et al.*, 2014). Briefly, the mechanisms include iron/ferritin accumulation in atherosclerotic lesions and in arterial plaque, creating a potential negative effect on endothelial cells (scavenging of nitric oxide) and formation of free radicals (Day, 2003; Mascitelli *et al.*, 2009; Sullivan, 2009). Conversely, findings reported by Habib and Finn (2014) suggest that areas of intraplaque hemorrhage, which are rich in iron, show decreased levels of reactive oxygen species, reduced inflammation, tissue damage and lipid retention (Habib and Finn, 2014) (Habib and Finn, 2014). Moreover, whereas severe anemia has shown to negatively affect cardiovascular health, mild ID may have a protective effect (Vaquero *et al.*, 2017).

A Spanish group (Comín-Colet *et al.*, 2013) investigated the effect of ID on health related quality of life in patients with chronic heart failure (n=552 average age 72, 43% of women, single-centre cohort study). Post-hoc analysis investigating intellectual capabilities of elderly participants was conducted. The authors reported that patients with ID, or increased levels of sTfR, reported poorer health related quality of life when compared to iron sufficient patients ($p=0.003$ and $p\leq0.001$). However, when the same analysis was conducted for the associations with anemia, it was not significant.

Ariza-Solé *et al.* (2015) conducted a single centre observational study in which they recruited all consecutive patients admitted to the Coronary Care Hospital Unit (n=2,128, mean age 62.3, 80% men). The authors reported that in patients with acute coronary syndromes aged 75 years and over (n=394) there was no significant association between anemia and mortality. The authors suggest that lack of association between anemia and mortality in their sub-group of elderly may be due to other underlying health conditions, which may in turn cause the anemia. Conversely, a Polish (Ponikowska *et al.*, 2013) group reported that in diabetic patients with CAD (n=287, 778% men, aged 65±9 years), iron status was a strong predictor of 5 years all-cause mortality, independent of other variables. The authors concluded that high sTfR and low or high ferritin are predictors of poor outcome in diabetic patients with CAD. Similarly, Zhou *et al.* (2014) investigated the association of iron status parameters with coronary artery disease (CAD) risk (n=540, aged 59.6±9.6) and found that serum iron and TIBC have the strongest association with CAD risk ((AUC 0.92 (0.90-0.95); 85%CI)).

Interestingly, in research conducted in chronic heart failure outpatients (Rangel *et al.*, 2014) with left ventricular ejection fraction below 45% (n=127, median age 62), participants with ID suffered from more frequent nonfatal cardiovascular events and mortality when compared to iron sufficient peers (p=0.001), regardless of anemia occurrence. ID and anemia was present in 36% and 22% of participants respectively. Multivariable analysis with relevant adjustments revealed that ID, not anemia, persisted as an autonomous variable for unfavourable outcomes (OR 5.38; p=0.009). The authors do not speculate about the potential mechanisms behind their observation.

Enjuanes *et al.* (2016) investigated submaximal exercise capacity in patients with chronic heart failure and the impact of iron status on physical performance. The average age of participants was 71 years (n=538) and the actual assessment was a 6 minute walking test. Iron sufficient patients walked on average for 322±113 meters whereas the ID group covered a significantly lower distance, 285±101 meters (p<0.005). Impaired exercise capacity was reported for 59% of ID individuals and 43% participants (p<.001) with normal iron status. When a multivariable generalized additive model was used, ID participants as defined by sTfR and ferritin index, had a significantly lower submaximal exercise performance (p=0.023 and p=0.019 respectively), but there was no association with Hb. The authors hypothesize that anemia is a more advanced form of ID and that, apart from carrying oxygen in the blood, iron also plays an important role in exercise capacity, skeletal and muscle function as well as functioning of the mitochondria (Jankowska *et al.*, 2013).

In a study in hospitalised patients Aelst *et al.* (2017) observed that iron status fluctuates during 30 days of hospital stay. In this study (n=47, mean age 70.4±13.7) 83% of patients had ID at admission which decreased to 68% after 30 days (p<0.001). Systemic inflammation which gradually lessens during the hospital stay is probably the main influencing factor underlyingchanges in iron status.

In a systematic review and meta-analysis of prospective studies (De, Krishna and Jethwa, 2015) the association between coronary heart disease and iron status was investigated. The authors included 17 studies of which 2 were conducted in the elderly, and a further 6 included elderly populations. They reported a significant negative association between transferrin saturation and CHD/myocardial infraction. The association was no longer significant, however, after adjustment for baseline chronic diseases or social status.

A recent review conducted by Vaquero *et al.* (2017) suggests an interaction between insulin resistance, lipoprotein metabolism and iron regulation, with dietary fat playing an important role. The authors conclude that saturated fat may enhance iron uptake and whereas severe anemia negatively affects cardiovascular health mild ID may have some protective effect. Furthermore, insulin resistance can be affected to a greater extent by iron overload than ID.

6.5.1. Key points:

- ID or increased levels of sTfR are associated with a poorer health-related quality of life.
- High sTfR and low or high ferritin are predictors of poor outcome in diabetic patients with CAD.

- Serum iron and TIBC are associated with CAD risk.
- ID is an independent variable for unfavourable outcomes in chronic heart failure outpatients.
- Impaired exercise capacity was observed in ID patients with chronic heart failure.
- Iron status is likely to fluctuate during 30 days of hospital stay. Thus repeated measures could be beneficial.

6.6. Iron status and neurodegenerative disorders (Alzheimer's disease (AD), Parkinson's disease PD).

Urrutia (Urrutia *et al*, 2014) conducted a review in 2014 investigating iron homeostasis in mitochondria and the effect of inflammation on iron overload and functioning of mitochondria in neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, Huntington disease, Fridrich's ataxia and amylotrophic lateral sclerosis. Often, the characteristics of these diseases include iron accumulation, oxidative damage, mitochondrial dysfunction and chronic inflammation. The authors point to numerous reports in the literature showing iron accumulation as a part of neurodegenerative disorders (Urrutia, And and Núñez, 2014) (Urrutia, And and Núñez, 2014). However, it is not known if iron accumulation is a result or a cause of other underlying disorders. Since mitochondria have a redox-active iron pool (Petrat *et al.*, 2001) they play a major role in maintaining iron homeostasis. An excess of redox-active iron will result in overproduction of hydroxyl radicals and increase in oxidative damage. On the other hand insufficient iron will impair a number of processes which use iron as a cofactor. In the brain, microglia (a type of glial cell) plays a major role in active immune defence. In inflammatory states however, reactive microglia are responsible for over-production of pro-inflammatory cytokines which result in increased reactive oxygen and nitrogen species, iron accumulation in microglia and neurons and interference with mitochondrial and iron metabolism (Andersen *et al.*, 2014). Drugs to reduce the transfer of iron-containing monocytes into the brain together with the use of chelators to minimise the effects of free iron could be used in order to limit the damage caused by iron excess in the brain (Andersen *et al.*, 2014). However, as highlighted by Wood (2015) appropriate chelation therapy is challenging due to differences in efficacy of chelators in each individual and the unreliability of iron overload indicators in serum. Urrutia *et al.* (2014) summarised the findings by speculating that "mitochondrial dysfunction, iron accumulation and inflammation are part of a synergistic self-feeding cycle that ends in apoptotic cell death, once the antioxidant cellular defence systems are finally overwhelmed".

Faux *et al.* (2014) and Giambattistelli *et al.* (2012) reported that blood ferritin levels were significantly higher in patients with Alzheimer's disease (AD) in comparison to healthy controls. Faux *et al.* (2014) noted further that in individuals with AD the incidence of serum ferritin levels above the normal range were significantly greater. Goozee *et al.* (2017) investigated whether serum or plasma ferritin levels differed between individuals with high and low risk of developing AD, based on increased levels of neocortical amyloid-β load (NAL). Abnormal accumulation of NAL can be observed 20 years prior to any clinical symptoms of AD (Villemagne *et al.*, 2013). However, performing tomography scans in order to assess the levels of NAL are not feasible on a population level, therefore Goozee *et al.* (2017) investigated if serum/plasma ferritin could be used as a preclinical marker of AD. They reported that both plasma and serum ferritin correlated positively with NAL (r_s=0.353, p=0.001 and r_s=0.358, p=0.001). They also evaluated if ferritin could

differentiate between low and high NAL. The addition of ferritin to a base model of AD risk factors including age, gender and APOE e4 increased specificity from 62% to 71%, although ferritin was not a significant predictor in the model. However, since levels of ferritin were strongly correlated with NAL and alterations in ferritin were noted in individuals prior the decline of any cognitive functions the potential of use of ferritin as a preclinical biomarker of AD should be explored. It is also worth noting that in individuals with elevated NAL lower levels of Hb were observed when compared to the group with low NAL (Goozee *et al.*, 2017).

Brain damage in the hippocampus of AD patients arises in combination with accumulation of ferritin iron levels (in the brain); (Raven *et al.*, 2013). There is emerging evidence that serum ferritin is not only a biomarker of iron stores but an indicator of cellular damage, inflammation and oxidative stress (Kell and Pretorius, 2014; Goozee *et al.*, 2017). When ferritin leaks from injured cells it liberates unbound, highly reactive iron which in turn fuels a Haber-Weiss reaction and causes oxidative damage (Kell and Pretorius, 2014).

Frequently coexisting anemia in individuals with AD would also suggest that elevated ferritin in AD is not indicative of iron stores but more likely tissue damage (Faux et al., 2014). In a cross-sectional study of healthy, mildly cognitively impaired and AD participants (768, 133 and 211 individuals respectively), AD was identified as a strong risk factor for anemia. In the study conducted by Faux *et al.* (2014) the incidence of AI in AD was significantly more common when compared with control group. The underlying reasons for anemia in AD are not understood. The authors suggest that perhaps the synthesis of Hb is perturbed in patients with AD. Finally, anemia may be one of the causes of cognitive deterioration in patients with AD.

Hu *et al.* (2015) conducted a study investigating if Parkinson's disease patients (PD, n=210; 115 males and 95 females) with sleep behaviour disorder (probable rapid eye movement sleep behaviour disorder (PBRD) which affects 25-60% cases) have different patterns of neurodegeneration when compared to patients without sleep behaviour disorder. Levels of iron, ferritin, transferrin, lactoferrin together with a number of inflammatory indicators were analysed in cerebrospinal fluid (PRBD group n=16; no PRBD group n=51) and in blood serum (PRBD group n=40; no PRBD group n=102). The levels of transferrin and iron in cerebrospinal fluid were significantly higher in the PRBD group when compared to the no PRBD group ($p<0.017$ and $p<0.01$ respectively) and to the control group ($p<0.017$ and $p<0.01$ respectively). In patients with PD there was also positive association between iron levels and UPDRS III score ($p=0.038$, $r=0.280$) and between iron levels and inflammatory markers (namely NO and IL-1ß) ($r=0.442$, $p=0.000$ and $r=0.230$, $p=0.035$ respectively).

Hu *et al.* (Hu *et al.*, 2015) research suggests that the course of PD is different for patients presenting with or without PRBD with atypical iron metabolism playing an important role.

Andro *et al.* (2013) conducted a systematic review of the literature from the past 30 years to investigate the relationship between anemia and cognitive performance in the elderly, including dementia in a community dwelling population. They reported a significant association between anemia and cognitive functions and between anemia and global cognitive deterioration with low Hb

most likely contributing towards the decline of cognitive functions and progression of mild cognitive impairment to dementia.

An Italian group (Tombini *et al.*, 2013) investigated the link between inflammation and iron metabolism in epileptic patients (n=37, aged 51.3±18.8, 68% women; period between seizures). Epilepsy affects approximately 50 million people worldwide. An analysis of a number of inflammatory cytokines and iron status indicators revealed significant differences between patients with epilepsy and healthy controls. Namely TNF-α was significantly lower and IL-6 significantly higher in patients with epilepsy ($p=0.002$ and $p=0.026$ respectively). Analysis of iron indicators showed that transferrin was lower and ceruloplasmin/transferrin ratio was significantly higher in epileptic patients ($p=0.031$ and $p=0.011$ respectively) compared to controls, suggesting that adjusted levels of inflammatory cytokines trigger chronic immune stimulation which in turn affects iron metabolism.

6.6.1. Key points:

- Both iron accumulation and iron deficiency play a role in neurodegenerative disorders.
- Low Hb levels most likely contribute towards the decline of cognitive functions and progression of mild cognitive impairment in dementia.
- Frequently coexisting anemia in individuals with AD suggests that elevated ferritin is indicative of tissue damage rather than of iron stores.
- There is potential to use NAL and ferritin as early markers of AD.
- Drugs reducing transfer of iron-containing monocytes into the brain together with chelators to minimise the effects of free iron could potentially be used to limit the damage caused by iron excess in the brain.
- There is a different course of PD in patients presenting with or without probable rapid eye movement sleep behaviour disorder, with atypical iron metabolism playing an important role.

7. Conclusions

From this updated literature review it is apparent that iron status plays a major role in a large number of conditions found in older men and women, as summarised in Table1. Elevated levels of ferritin or low levels of Hb seem to be associated with an accelerating trajectory of disease, although changes in iron status are often the result of the actual condition, not causal. Nevertheless, biomarkers of iron status that are outside the normal range are very likely to be indicative of other underlying health conditions and should be investigated.

Efforts should be made to investigate and reach a consensus on optimal cut-off levels of iron status biomarkers to establish normal, safe ranges for the elderly (section 1). These would help in the management of conditions associated with elevated iron stores such as neurodegenerative disorders or disturbed cardiac function, frailty, poor cognitive performance and increased post-operative risk, all of which are associated with depleted iron stores. Within the elderly group, the incidence and severity of anemia seem to progress with age, with kidney failure emerging as one of the main contributors. It is of concern that a many patients diagnosed with anemia or unexplained anemia are

left untreated, exacerbating diminishing quality of life in the elderly. Diagnosis of the underlying reasons of anemia in individuals should be prioritised (section 1.1).

The accuracy of iron status biomarkers in estimating IDA or ID varies depending on other health conditions and on underlying inflammation. For example, sTfR is not a good measure of iron status in obese individuals, but it is a good indicator of iron status in kidney failure patients IBD patients or in differentiating patients with ACD and IDA when used together with ferritin (section 6.2 and 6.3). In obese individuals a negative relationship is observed between low-grade inflammation and iron status, with ferritin reflecting inflammation rather than body iron status (section 5.1). Also, the nature of the relationship between the risk of type 2 diabetes and sTfR is different in the presence or absence of obesity (section 3 and 5.1).

In patients with chronic kidney disease sTfR seem to be effective in differentiating between anemia of chronic inflammation and IDA. However, in patients on hemodiaysis, elevated levels of circulating free DNA are indicative of inflammatory state, which negatively affects iron metabolism and the response to EPO therapy. Also switching from low to high-flux dialysis may reduce anemia. Finally, establishing guidelines on iron supplementation for patients on aemodialysis with ferritin above 500µg/L should be given priority (section 6.3).

Appropriate guidelines should be put in place for practitioners to choose the most reliable and informative markers of iron status for different medical conditions. Information on supplement doses (oral or intravenous) should also be added to the guidelines to avoid situations where patients with ACD, kidney failure or IBD are treated with oral supplementation which is ineffective due to elevated levels of hepcidin or cause a number of adverse side effects. Lower oral iron doses should be considered (section 5.4, 6.2 and 6.3).

Modification of iron status through diet and exercise should be investigated further (section 5.2 and 5.3). From the available reports it seems that simple and cost-effective lifestyle changes could have a powerful effect on iron homeostasis and bone mineral density in the elderly (section 6.1), thereby improving quality of life.

Additional attention should be directed to cancer patients of whom the majority suffers from anemia at some stage of the disease or while undergoing treatment. Nutritional support and erythroid stimulating drugs show promise in improving the iron status of cancer patients (section 6.4).

Future research should also focus on investigating iron deficiency and iron accumulation, and the role both of these conditions play in the development and progression of neurodegenerative disorders. The development of early detection markers of AD, such as NAL, is particularly interesting (section 6.6).

Finally, novel therapies such as hormonal treatments (for example restoring testosterone levels of elderly men or hormone replacement therapies in women (section 2), erythropoiesis stimulating agents, hepcidin inhibitors, ferroportin modulators and identification of new iron and inflammatory status indicators (such as Lipocalin 2 or FGF-23) appear very promising. They may have the potential to treat iron deficiency in patients with AI or ACD, which is currently a very challenging goal.

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Table 1. Conditions associated with iron deficiency or high iron levels in the elderly

Iron status	Condition	Possible mechanisms	Section
Iron deficiency or iron deficiency anemia	Inflammatory bowel disease (IBD)	Blood loss in GI tract	6.2
	Kidney disease/failure	Elevated hepcidin	6.3
	Cancer	High serum ferritin (tumor-derived)	6.4
	Cardiovascular disease	Systemic inflammation	6.5
	Alzheimer's disease	Impaired Hb synthesis	6.6
	Osteoporosis	Unknown	6.1
	Obesity	Low-grade inflammation	5.1
	Frailty	Unknown	6.1
	Vegetarian	Lower iron bioavailability	5.2
High iron levels	Neurodegenerative disorders	Unknown mechanism for iron accumulation	6.6
	Osteoporosis in iron-loading disorders e.g. thalassemia, sickle cell anemia	Up-regulation of TRAP expression	6.1
	Lack of exercise	Unknown	5.3

Figure captions

Figure 1. Literature review selection process.

Figure 2. Summary of anemia prevalence data for in-patients (Bach et al., 2014; Röhrig et al., 2014; Stauder and Thein, 2014; Zilinski et al., 2014; Migone De Amicis et al., 2015; Gabriele, 2016).

Figure 3. Summary of anemia prevalence data for free living/outpatients (Bosco et al., 2013; Röhrig et al., 2014; Song et al., 2014; Stauder and Thein, 2014; Contreras-manzano, Cruz and Villalpando, 2015; Yildirim et al., 2015; Results of the National Diet and Nutrition Survey (NDNS) rolling programme for 2012 to 2013 and 2013 to 2014., 2016; Gabriele, 2016; Lacerda et al., 2016).

Figure 4. Summary of anemia prevalence data for institutionalised elderly (Stauder and Thein, 2014; Sahin et al., 2016).

Figure 1.

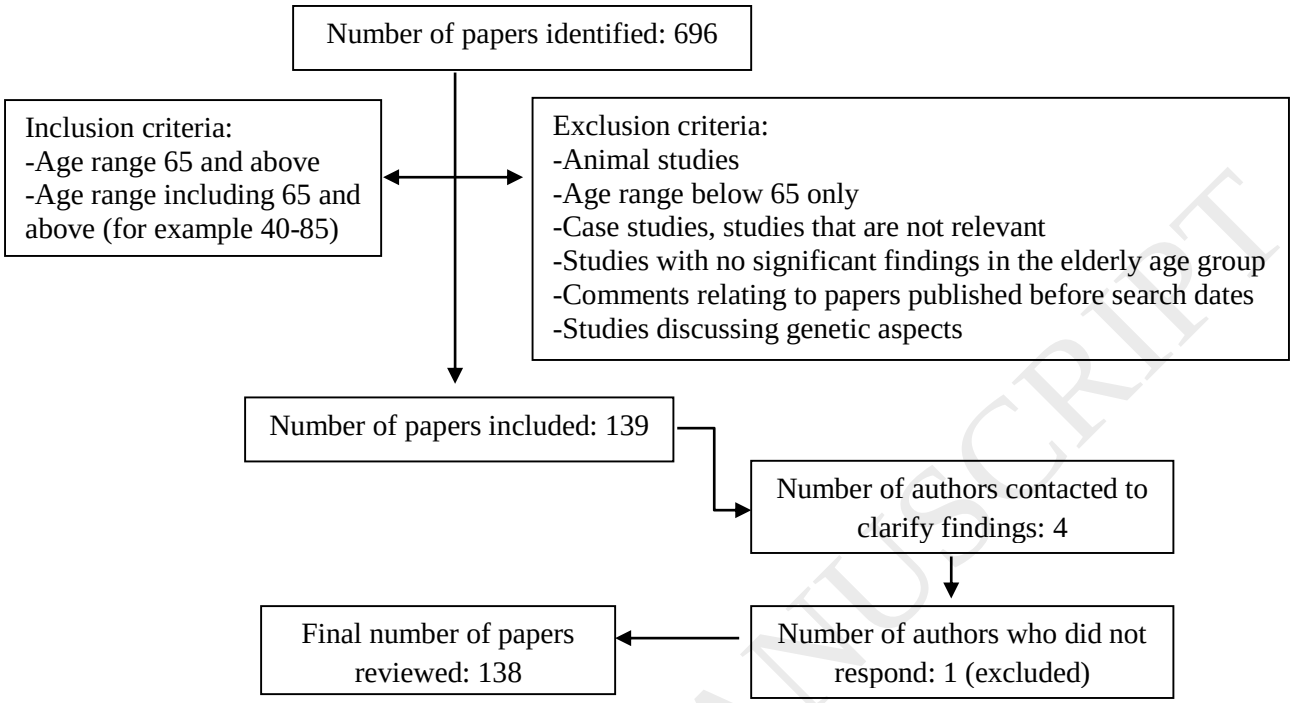
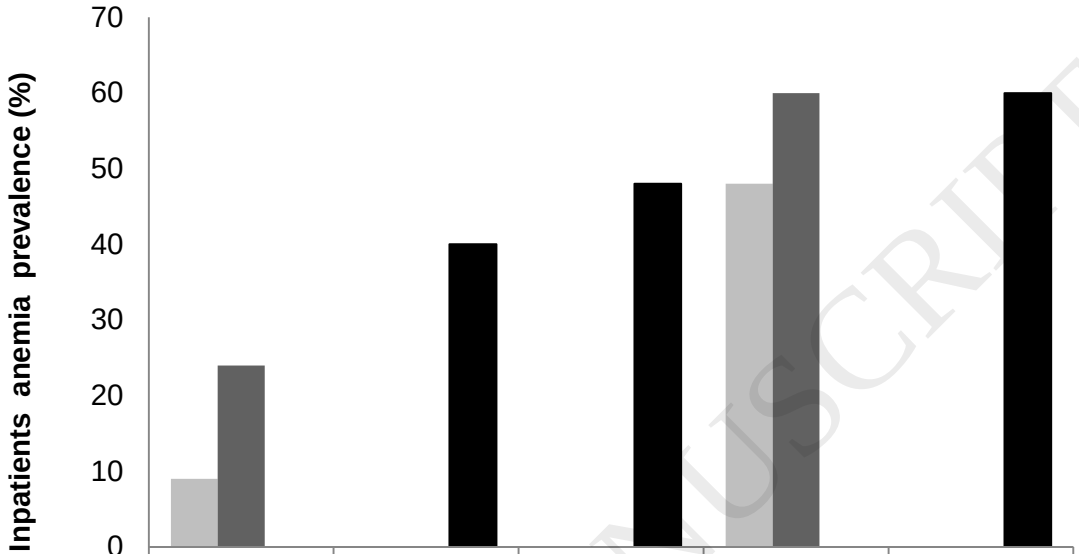
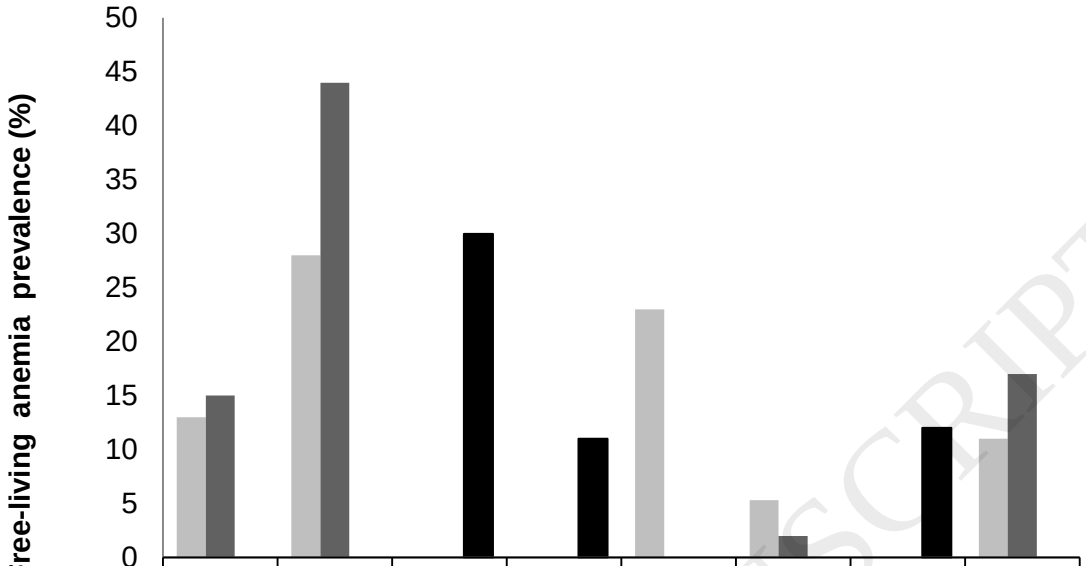


Figure 2.



	>64 yrs Austria	>65 yrs multi-national	>65 yrs Italy	>70 yrs Germany	>70 yrs Poland
women (%)	9			48	
men (%)	24			60	
both genders (%)		40	48		60

Figure 3.



	>60 yrs Mexico	>70 yrs Germa-ny	>60 yrs Brazil	>60 yrs Brazil	>50 yrs China	>60 yrs Turkey	>65 yrs multi-national	>65 yrs UK
women (%)	13	28			23	5.3		11
men (%)	15	44				2		17
both genders (%)			30	11			12	

Figure 4.