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**Title: Systematic review of the clinical outcomes of iron reduction in Hereditary Hemochromatosis**

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## ABSTRACT

Hereditary Hemochromatosis is a condition caused by defects in iron-sensing genes leading to parenchymal iron loading. If diagnosed early and treated appropriately, many of the complications, including liver fibrosis, cirrhosis and liver cancer, arthritis, cardiomyopathy and diabetes, were thought to be avoided. As iron reduction by venesection became the mainstay of HH treatment before the introduction of evidence-based medicine, its efficacy has never been the subject of high-level clinical research. Here we provide a systematic review of iron reduction in HH, including 24 studies and 6000 patients. While strong deductions are prohibited by an absence

of robust clinical trial data, the purported benefits of venesection are reviewed and we report an improvement in fatigue, liver function tests and fibrosis, and overall survival. In conclusion, these findings, coupled with the absence of an alternative, low-cost, effective and tolerable therapy, suggests venesection will remain the mainstay of treatment in HH for decades to come.

## INTRODUCTION

Hereditary hemochromatosis (HH) is a relatively common autosomal recessive disorder of iron regulation that results in iron overload and its deposition in multiple organs. Complications include liver cirrhosis and hepatocellular carcinoma (HCC), as well as wide-ranging extrahepatic manifestations including diabetes, cardiovascular disease, arthritis, hypogonadism and osteoporosis ((Figure 1, (1)).

Despite significant advances in our understanding of iron regulation, the treatment of iron-overload conditions has remained relatively static and is largely founded on historical convention. Phlebotomy remains the mainstay of treatment for HH, even in the absence of robust data from randomised trials, and though other options such as iron chelation and erythrocytapheresis are used in a minority, the ideal treatment modality and regimen remains unclear.

A lack of high-quality trial design has restricted our understanding of the clinical outcomes of iron reduction therapy, and the present data is conflicting and discordant. This absence of robust data was highlighted by a recent Cochrane meta-analysis, which attempted to collate evidence on the benefits and harms of iron reduction therapy in HH but found only 2 papers with usable data, precluding any consequential conclusions from being drawn (2). Our narrative review aims to address this deficiency in the literature, with the recognition that a systematic review or meta-analysis is not currently feasible to perform given the dearth of high-quality evidence. The numerous multi-system effects of iron reduction therapy in HH are outlined, citing the best available evidence where possible.

## MATERIALS AND METHODS

The study protocol was registered on the PROSPERO international prospective register of systematic reviews (3) and carried out in accordance with PRISMA guidelines. MEDLINE and EMBASE were searched, studies screened, and data extracted and summarised in the PRISMA diagram (Table 1 and Figure 2).

Data were extracted by two authors independently (AP and TC) into a standardised proforma. We recorded information on; the nature of the study (study design, region where study was performed), participant demographics (age, % male, hemochromatosis diagnosis), iron reduction therapy regimens and clinical outcomes; mortality, cirrhosis, liver fibrosis, portal hypertension, HCC, liver transplant, arthritis, joint replacement, diabetes, cardiovascular disease, heart failure, erectile dysfunction, hypogonadism, quality of life, fatigue, biochemical iron indices, liver function tests (Table 2).

**Quality assessment of included studies**

Two authors independently assessed risk of bias in the included studies (AP and TC). The ROBINS-I tool (4) or the Cochrane risk of bias tool (5) were used to evaluate non-randomised and randomised studies respectively. Disagreements were resolved by discussion and a consensus decision made.

**RESULTS**

**Cohort characteristics**

Of the 64 studies identified by the search, 24 studies from between 1972-2018 were included in the final cohort (5994 patients in total, Supplementary Table 1). The remaining studies were excluded due to reporting on fewer than 20 participants (n=15), duplication (n=5), lack of outcome data (n=15), inclusion of non-hemochromatosis patients (n=4) and having not yet been performed (n=1). One included abstract was subsequently published as a full article in 2019 and so the comprehensive version was included in our analysis.

The majority of studies (n=20, 83%) were retrospective cohort studies, while 3 (13%) were randomised controlled trials and 1 (4 %) was a non-randomised trial. The published data were skewed towards reports from Western countries, with 16 papers from Western Europe (67%), 5 from North America (21%), 2 from Eastern Europe (10%) and 1 from Australasia (4%).

The mean age of individuals, recorded in 14 studies, was 51 years (95% confidence interval (CI) 48-53 years old) and the mean percentage of male participants, recorded in 18 studies, was 70% (CI 64-76 %). 15 studies (63%, 5197 participants) included patients with hereditary hemochromatosis (either confirmed by genotyping or unspecified), 4 studies (17%, 383 participants) included those with hereditary hemochromatosis with fibrosis or cirrhosis and 5 studies (21%, 420 participants) included those with hereditary hemochromatosis and deranged iron indices.

The study intervention was venesection/phlebotomy in 18 studies (75%, 5737 participants), erythrocytapheresis in 4 studies (17%, 185 participants), iron chelation in 1 study (4%, 49 participants) and phlebotomy and erythrocytapheresis in 1 study (4%, 49 participants).

**Quality of Evidence**

The quality of evidence assessments for included studies is shown in Table 3. Of the 3 randomised controlled trials, 1 was judged to be of good quality, 1 of fair quality and 1 of poor quality. Of the 21 non-randomised studies of intervention, 19 (90%) had a serious risk of bias and 2 studies had a moderate risk. None of the included studies had a low risk of bias.

**Mortality**

Our knowledge of the natural history of HH has been derived from retrospective follow up of longitudinal cohorts and survival has been found to be the same as the general population, provided treatment is initiated in time (6). In our search, 7 reported on mortality in HH patients (n=1708, mean age 50.2 years, mean follow up 7.7 years (range 0-31) (7–13). All included patients were diagnosed as having primary HH on clinical grounds, with 5 of the 7 studies predating HH genotyping, but were heterogeneous with respect to exact venesection procedure as well as to the presence of HH complications.

Crude mean overall survival was 63.7%, SD 30.1 (4 studies, n=436, mean follow up 7.5 years, range 0-31 years) (7,10,12,13) and cumulative survival after follow-up was reported to be between 61%-92% at 5 years, 61%-81% at 10 years and 49-71% at 20 years (3 studies, n=315) (7,9,13).

Even in the absence of controlled trials, there is some evidence that survival of patients with HH has improved over time, coinciding with iron depletion therapy becoming the cornerstone of HH treatment. One study of HH patients diagnosed between 1948 to 1985 found the standardised mortality ratio (SMR) was significantly raised at 3.68 (3.07-4.39). They also found 10 year cumulative survival had increased within this time period (38% if diagnosed between 1948-1968 vs. 48% between 1969 and 1979 and 58% between 1980-1985) (12). A more recent study of patients diagnosed between 1996 and 2010 calculated the SMR as 0.94 (0.71-1.22), indicating that overall survival of HH might not differ from the general population in contemporary cohorts (8).

Regarding the effects of treatment, a single study reported higher cumulative survival in patients that had been treated compared to those who did not receive treatment (96% vs. 45% at 1 year, 96 vs. 5% at 5 years) (9). Similarly, patients that achieved iron depletion or were treated 'adequately' had significantly higher cumulative survival compared to those that did not at 10 years (76% vs. 36%) and at 20 years (40% vs 4% and 70% vs. 30%) with median survivals of 24 vs 13 years and 16 vs 5 years (Figure 3) (12,13).

Analysis of survival based on the intensity of treatment received revealed patients that had received a low (1-8) or high (over 64) number of venesections had lower overall survival (60% and 70% respectively) than those in whom between 9 and 63 venesections has been performed (92.5% overall survival) (7). In patients in whom a mild amount of iron had been removed (2-10g), SMR was low 0.23 (0.08-0.5) but in patients with over 10g of iron depleted mortality was no different to the general population 0.94 (0.71-1.22) (8).

Finally, several studies have focused on the mortality of patients with HH related cirrhosis. Five studies investigated the relationship between cirrhosis and mortality in cohorts of patients with HH (n=1611 patients). Four of these studies reported cumulative survival was significantly lower in patients with HH that had cirrhosis compared to those without cirrhosis (7,8,12,13). The largest of

these cohorts reported SMR of all-cause mortality in 1085 HH patients with liver fibrosis 1-2, liver fibrosis 3-4 and cirrhosis (METAVIR scoring system) and found it to be significantly higher in patients with cirrhosis 4.43 (2.53-7.19). The SMR for liver-causes of death (37.04 (18.5-66.3)) and deaths from liver cancer (86.1(37.0-169.5)) were also significantly raised (8). These data support cirrhosis as being associated with higher mortality in cohorts of HH, however only one study has looked at whether iron depletion altered survival differences and found no effect (12).

Together these data suggest that iron depletion therapy may have a positive effect on survival in HH, however it is impossible to draw firm conclusions as patient and treatment related confounding factors are not consistently accounted for.

**Liver Cirrhosis, Fibrosis and Portal Hypertension**

Liver cirrhosis and its sequalae are well-documented complications of HH and where present, survival is reduced (7,8,12,13). Several reports have investigated the relationship between iron overload indices, iron depletion therapy and the presence of fibrosis and cirrhosis. A paper which evaluated liver biopsies in a HH cohort including both paediatric and adult patients found that liver fibrosis improved following phlebotomy in all 19 patients who did not have coincident heavy alcohol intake, and that fibrosis was completely reversed in 15 of these 19 subjects (14). Current evidence also suggests that high serum ferritin levels (>1000 μg/L) are associated with an increased risk of cirrhosis (15,16) and that cirrhosis prevalence is significantly higher amongst patients with inadequate phlebotomy treatment (93% vs 68%, p=0.0002 (12)). However, the direction of these associations is unclear, and the evidence is insufficient to infer causality.

Several reports have assessed the effect of iron depletion on the histological appearance of liver cirrhosis or fibrosis by comparing pre and post treatment liver biopsy (13,17–19). They all find evidence of histological regression of fibrosis following iron depletion in a proportion of patients. The largest and most recent study of treated HH patients (median follow up of 9.5 years) reported fibrosis stage improvement in 44 of 106 patients with HH related F3/4 fibrosis. Fibrosis stage also improved in 23 percent of the 66 patients in this cohort with cirrhosis at diagnosis. Older age at diagnosis, the presence of diabetes and higher GGT were negatively associated with regression of fibrosis to stage <2 (17).

Further evidence supporting the benefits of iron depletion in cirrhotic cohorts includes a study that found overall survival was higher in patients with treated HH cirrhosis than patients with non-HH cirrhosis (75.5 vs. 66.6%) and that only 1 HH cirrhotic patient had worsening varices during follow up (11). Another study assessed non-invasive markers of fibrosis in HH patients with moderate iron overload (ferritin 300-1000 ug/L) randomised to erythrocytapheresis or sham plasmapheresis (20). The mean change in transient elastography (an imaging-based non-invasive test of liver fibrosis) was similar between both groups following treatment, but the hepascore (a blood-based non-invasive test of liver fibrosis) decreased in the treatment group and increased in the control group ($p = 0.049$).

These data suggest that iron depletion does contribute to regression of fibrosis and cirrhosis in a subset of patients with HH, but further work needs to be done to be able to identify a priori which individuals might benefit from treatment and whether reversal of fibrosis stage is associated with clinical benefits including mortality.

**Hepatocellular Carcinoma (HCC)**

HCC is a well-recognised complication of HH, including in the absence of cirrhosis, though those with cirrhosis have an up to 200-fold increased risk (1).

4 papers (n=538) from Europe and the USA commented on the relationship between HCC and treatment, with differing results. One study reported 11 of 13 (84.6%) HCCs were in livers that were completely depleted of iron (13) whereas another found that 80% were from an untreated pool of patients (9). Another study found that fibrosis regression was associated with reduced HCC risk (Figure 4) (17). However, a recent abstract found no association between successful phlebotomy treatment and risk of HCC (OR 0.91, 95% CI), (21).

Overall, though the aim of reducing the risk of HCC often forms part of the rationale for offering treatment, the evidence that phlebotomy has any influence on the development of HCC in hemochromatosis patients is weak.

**Diabetes**

The prevalence of diabetes in C282Y homozygotes has been decreasing over the past century, largely due to timely diagnosis, with one study reporting a prevalence of 7% in 2008 (22). In the 2019 UK Biobank study (23), including 2890 C282Y homozygotes, only male C282Y homozygotes had a significantly increased rate of type 1 or type 2 diabetes (OR 1.52, 1.18-1.98).

5 included studies (n=554), all cohort, commented on diabetes in hemochromatosis with 4 published pre-1986. One study (n=49) analysed changes in insulin requirements or oral hypoglycaemic agent doses pre- and post-venesection in those with idiopathic hemochromatosis and cirrhosis and found there was no significant difference in 21 patients (43%), a significant reduction in 17 patients (35%) and an increase in the insulin requirement of at least 8 units per day in 8 patients (16%) (24). Similarly, another noted that the frequency of type 1 diabetes did not significantly differ between adequately and inadequately treated groups (12). Conversely, one study found that the clinical features of diabetes improved in 45% of insulin-dependent and 50% of non-insulin-dependent diabetes patients after biopsy confirmed iron depletion, though clinical features worsened in 5% and 4%, respectively (13). They also commented that the daily dose of insulin reduced in 45% of those undergoing iron depletion.

In general, while it is possible that phlebotomy can reverse diabetes to some extent, the available evidence is scarce and far from conclusive.

**Cardiovascular disease**

The prevalence of cardiac failure symptoms in HH has been reported as high as 35% (15), though in the UK Biobank Study (23) C282Y homozygosity was associated with significantly reduced prevalence of coronary artery disease in men but not women.

4 studies commented on cardiovascular disease in hemochromatosis (n=4033) but only 2 reported on outcomes following treatment. In a questionnaire study (n=2,851), of 679 patients who complained of symptoms of heart fluttering, 42 (6.2%) reported an improvement in symptoms following treatment while 69 (10.1%) reported a worsening of symptoms (25). The utility of this finding in the context of evaluating the effects of treatment on the heart is questionable, given that the symptom of heart fluttering certainly cannot be assumed to be a manifestation of arrhythmia and therefore cannot be used as a proxy for underlying cardiovascular disease. A retrospective

analysis of death certificates (n=1085) found that treated patients with serum ferritin between normal and 1000 ug/L had a lower cardiovascular mortality than the general population (SMR: 0.27 CI: 0.1–0.5) (8) and, interestingly, these patients did not have a compensatory higher mortality from liver disease.

Clearly, there is very little available evidence on the effects of treatment on cardiovascular disease, particularly that relating to HH-related cardiomyopathy and arrhythmias. While there are suggestions that treatment can limit cardiovascular mortality, there is too little data to draw any decisive conclusions.

**Adverse Events with treatment**

There were 8 studies (n=544) that commented on adverse events or side effects associated with treatment of HH. The studies relate to all treatment modalities, with most reported adverse events being mild in nature.

Studies involving patients undergoing phlebotomy treatment frequently reported side effects including tiredness, fainting, loss of appetite and needle intolerance in 19-52% of patients, with tiredness being most frequently reported (26–28).

Numerous studies reporting on therapeutic erythrocytapheresis found that up to 25% of collections had complications - mostly light citrate toxicity but also hypotension-related reactions and vein ruptures (20,27–31).

A randomised controlled trial of therapeutic erythrocytapheresis versus phlebotomy found no significant difference in numbers of adverse events per number of procedures (27) (4.7% vs 1.9%) while a further trial also found little difference between the 2 groups, finding procedural discomfort in 19% of their phlebotomy group versus 23% in their erythrocytapheresis group (28).

A single study, a phase 1/2 trial of desferasirox, commented on side effects with iron chelation therapy including diarrhoea (49%), headache (27%) and nausea (22%) (32).

Though common, it appears that the majority of adverse events with phlebotomy and erythrocytapheresis are mild. They relate mainly to the effects of hypovolaemia and prehydration is now widely recommended in clinical practice (33).

**Quality of Life and Mental Health**

The 2017 HH patient survey, in which nearly 2,000 hemochromatosis patients from around the world commented on their symptomology, found that symptomatic disease has long been associated with poor quality of life and psychological wellbeing (34).

3 studies (n=2993), of which 2 were randomised controlled studies (n=142), provided mixed outcomes. In a survey study, of 592 patients with self-reported depression, 242 (40.8%) reported improved symptoms with phlebotomy while 61 (10.3%) worsened (25). The 2 randomised controlled trials, 1 evaluating perceived health status with phlebotomy versus erythrocytapheresis and another assessing depression in erythrocytapheresis versus sham, found no significant differences (20,27).

There are discrepancies within the scant data on the ability of treatment to improve quality of life and mental health, and little to convincingly suggest that treatment has a positive effect.

**Fatigue**

4 of the included studies in this paper (n=3297) commented on the effects of treatment on fatigue. One controlled trial found the mean decrease in the Modified Fatigue Impact Scale score was greater in the erythrocytapheresis group compared to sham (mean difference -6.3, 95% CI -11.1 to -1.4, p=0.013) (20). From the cohort studies, one found that weakness/lethargy improved in 51% after iron depletion (13) though another found no significant difference in fatigue symptoms when comparing adequately and inadequately phlebotomy-treated groups (12). A further study described mixed results, with 54.4% of patients reporting improved symptoms, while 17.2% worsened (25).

This data does suggest the possibility that treatment with either phlebotomy or erythrocytapheresis could have positive impact on symptoms of fatigue.

**Arthralgia**

Joint pain is the most frequently reported symptom and both rheumatoid arthritis and osteoarthritis are independently associated with HH (23,34).

7 included studies (n=3585) described arthralgia in hemochromatosis with a reported improvement in up to 62% of patients following phlebotomy, though one study reported a worsening of arthralgia in 34%. (13,25,35,36). A randomised controlled trial found no significant changes to subjective scores of arthralgia following treatment erythrocytapheresis or sham plasmapheresis (20).

Although these results suggest a possible role for phlebotomy in arthralgia, a large proportion also report a worsening of their symptoms following treatment, making it difficult to draw any convincing conclusions.

**Erectile Dysfunction (ED)**

Sexual health issues are commonly experienced, with a high proportion of men experiencing ED (39%) and both men (33.5%) and women (58.4%) experiencing loss of libido (34).

4 studies (n=3111), all cohort designs focusing on phlebotomy, reported on ED and hypogonadism in HH. They found a reversal of ED symptoms in up to 22% following treatment, though one described a worsening in 27.8%. (10,13,25).

While these limited results demonstrate a possible role for phlebotomy in reversing symptoms of erectile dysfunction, there remains the simultaneous possibility that treatment could exacerbate symptoms.

**Biochemical Markers of Disease Severity and Liver Function Tests (LFTs)**

There is mixed evidence on the utility of liver biochemistry as a screening tool for HH. The high prevalence of abnormal liver biochemistry in HH patients had highlighted them as a potentially useful screening tool, with clinical studies having found abnormal serum aminotransferase levels in 65-75% of HH patients (37). Indeed, the incidental finding of abnormal LFTs is often the catalyst for focused investigation and an early diagnosis of HH. However, more recent evidence paradoxically found that the probability of being a C282Y homozygote increased as the ALT and

AST activities decreased, possibly reflecting the lack of inflammation induced by iron deposition in HH in comparison to other liver pathologies (38).

7 papers (n=742), including 2 randomised controlled trials and 1 randomised open-label study, described biochemical markers in hereditary hemochromatosis. One found that both iron and liver indices improved in a greater percentage of adequately treated phlebotomy cohorts and 2 further studies found an improvement in LFTs in up to 93% following phlebotomy (12,13,36).

Regarding iron markers, a randomised controlled trial found that the post-treatment drop in transferrin and transferrin saturation was significant with erythrocytapheresis compared to sham (20). Similarly, an abstract containing 29 patients reported that the median ferritin reduced from 1064 to 421mg/L in a phlebotomy-treated group and from 597 to 50 mg/L in the erythrocytapheresis-treated group (39).

When comparing phlebotomy with erythrocytapheresis, 2 RCTs reported no significant differences in their effect on iron indices or LFTs (27,28).

These results do suggest that both phlebotomy and therapeutic erythrocytapheresis contribute to normalising iron and liver function indices, presumably through the reduction of iron-mediated oxidative stress on the liver, though it is less clear whether one modality is more effective than the other.

**DISCUSSION**

This is the first review to collate and describe results from studies evaluating beneficial and harmful outcomes in patients with HH undergoing iron reduction therapy. Iron reduction therapies, with most studies to date focusing on phlebotomy, have been shown to improve outcomes in a variety of domains though deficiencies in the quality of available evidence makes it difficult to draw meaningful conclusions. Cochrane's recently attempted network meta-analysis supports this opinion on the strength of current evidence (2). They found only two randomised clinical trials with usable data, precluding any consequential deductions to be made.

Though phlebotomy is a well-established treatment in HH, its effects have never been conclusively characterised. Ethical restrictions have limited the design of controlled studies, making the true effects of treatment difficult to clarify. Whilst the use of phlebotomy seems largely based on precedent, untangling the available data and developing an evidence base to justify its continued use remains paramount. In our aim to tackle this through this review we have found that, while there are recurring themes in the available evidence, many discordant results have also been reported.

The Cochrane study found 1 trial that reported on mortality, finding no deaths in either the phlebotomy or erythrocytapheresis groups over eight months, and included no studies that reported on mortality beyond one year (2). Conversely, many of the studies included in our review do suggest a survival benefit with venesection, although there are numerous pitfalls in the evidence base.

For example, the confounding effect of initial disease severity was not controlled for in many of the studies. The studies which demonstrated that those requiring a high number of venesection procedures to achieve iron depletion had a higher mortality rate, possibly represented a population with severe iron loading (13,40). Indeed, the survival curve for non-cirrhotic HH patients was almost identical to that of an unaffected population, while the patients who died from HCC had the highest amount of mobilizable iron. Similarly, the observed high mortality rate in those that underwent very few treatment procedures perhaps represented those with advanced or refractory disease at the time of diagnosis (40). This is supported by the fact that the average follow-up time within the quartile receiving the fewest number of venesections was 1 year (all died), compared with 11 years in the quartile receiving the highest number (5 of 12 died). Conversely, another study found that patients necessitating low amounts of iron removal by phlebotomy had lower overall mortality than the general population, though this subgroup may display a milder disease phenotype (8). One study found that survival was significantly better in those with ‘adequate’ treatment, that is, those that had received frequent phlebotomy and responded biochemically (12). However, the ‘inadequate’ group would include those with advanced or refractory disease, including those that did indeed receive venesection but did not respond. Finally, it is worth noting that two studies included patients that were diagnosed before the availability of *HFE* testing and thus their cohorts would likely include, not only non-*HFE* hemochromatosis patients, but a higher

proportion with late-presenting, severe phenotypes (12,13). As a further example of suboptimal controlling, another study compared HH patients with a diverse control group consisting of those with differing liver pathologies and varying treatment statuses (11).

Despite the weaknesses in the evidence, the above findings do seem to suggest that phlebotomy improves mortality but particularly in those who show a biochemical response to treatment and achieve iron depletion within a reasonable time frame.

With regards to liver fibrosis, while phlebotomy seems capable of reversing pathology, the evidence is limited. While 2 studies suggested a degree of reversibility with phlebotomy, further supportive data comes from abstracts only (13,18). Similarly, RCT described a reduction in markers of fibrosis with treatment erythrocytapheresis though, it must be noted, there was no fibrosis in the cohort at baseline (20).

Regarding liver cirrhosis, one study reported a higher frequency of cirrhosis in patients inadequately treated with phlebotomy (11). However, adequate treatment could feasibly be precluded by the presence of cirrhosis itself and so cannot simply be attributed to the effects of phlebotomy.

One study's finding that all cases of HCC were found in cirrhotic livers, and remarkably most in those that were depleted of iron, may suggest that phlebotomy does not prevent HCC in the cirrhotic stage (13). However, the fact that those with HCC were found to have the highest amounts of mobilizable iron may paradoxically suggest a role for phlebotomy. Similarly it is accepted that those with high serum ferritin at diagnosis are at a greater risk of HCC risk (41) and, indeed, another paper found that the vast majority of HCC cases were from the untreated pool of patients (9).

As with Cochrane's study, we found that there were no serious adverse events in those receiving phlebotomy or erythrocytapheresis, while there was also no significant improvement in health-related quality of life with either modality (2).

Our findings on the effects of treatment on extrahepatic manifestations, however, are contradictory and sparse. There were discrepancies in outcomes with regards to diabetes, cardiac symptoms and function, mental health, fatigue, ED and arthralgia. For example, interpretation of the already conflicting reports on the effects of iron reduction therapy on joint pain is further limited by the heterogeneous nature of the included cohorts, especially given the variety of arthritic phenotypes in HH.

The discrepancy in outcomes points towards a lack of robust evidence, with many contributing factors. The relevant studies are mainly retrospective cohort studies, with small numbers participating, and are largely unmatched for the presence of cirrhosis, comorbidities or co-interventions. 19 of the 21 (90%) included non-randomised studies of intervention had a serious risk of bias when analysed with the ROBINS-I tool and many of the studies used non-standardised treatment regimens that varied within studies, let alone between studies, which is especially significant given that iron reduction therapy is not standardised. Furthermore, much of the data on the effects of treatment comes from retrospective questionnaires, which is weakened by heavy recall and selection biases.

A further pitfall potentially affecting a significant portion of the studies, as they were performed before the introduction of *HFE* testing, is the probability of secondary iron overload cases being diagnosed as HH. The *HFE* gene was identified as recently as 1996, with homozygosity for a missense mutation in this gene being responsible for the majority of cases of HH (42). Since this point, the diagnosis of HH has largely been defined genotypically whereby, prior to this, there was a greater emphasis on phenotype. Given that a significant proportion of the cohorts across many of the included studies were diagnosed prior to the discovery of *HFE*, there is the probability that cases of secondary iron overload were erroneously diagnosed as HH. This is especially probable given that cirrhosis with secondary iron overload is far more common than cirrhosis secondary to HH and that the two are indistinguishable histologically. These phenomena have likely introduced a bias to reported outcomes in these earlier papers and so must be interpreted cautiously in the context of confirmed HH.

Another noteworthy caveat to the available evidence is the high prevalence of advanced liver disease in many of the included, and particularly earlier, studies. Up to 85% of study participants

were known to have cirrhosis in one study (9), warranting caution when applying findings to a more generally representative HH population with milder disease. Even with inherent selection bias, studies estimate the prevalence of biopsy-proven cirrhosis to be 23-28% in Caucasians with HH, with the vast majority of these also having co-existing contributors to their liver disease (43,44). Cirrhosis solely due to the effects of iron overload was seen in only 3%. It has also been reported that the lifetime incidence of severe liver disease alone appears to be approximately 9% of male HFE C282Y homozygotes of European ancestry based on data from prospective cohort studies (45). Thus, our findings on the efficacy of treatment possibly have less relevance in those with less advanced disease, which is important given that these patients are far more prevalent in clinical practice.

Overall the positive data outlined in this review, though limited, does suggest a benefit of treatment, which may be multi-faceted (Figure 5). The dearth of robust evidence is unlikely to change in the near future, until and when novel therapies which challenge phlebotomy as the gold standard emerge. Indeed, hepcidin agonists are on the horizon for HH (46), and the related clinical trials will provide informative evidence for clinical practice, regardless of the outcomes. For now, phlebotomy remains the mainstay of treatment for HH and will continue to be recommended, given the potential benefits, even in the absence of iron-clad evidence supporting its use.

## TABLES

**Table 1. Search summary**

| Database | Interface | Coverage | Date | Hits |
|---|---|---|---|---|
| Embase | OvidSP | 1974 to 2018 May 30 | 31/05/2018 | 1680 |
| Ovid MEDLINE(R) Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily, Ovid MEDLINE and Versions(R) | OvidSP | 1946 to May 30, 2018 | 31/05/2018 | 1006 |
| Total: | | | | 2686 |
| Duplicates: | | | | 663 |
| Final Total: | | | | 2023 |

**Table 1.** We searched EMBASE (Ovid) (1974 to May 2018) and MEDLINE (Ovid) (1946 to May 2018) on 4th April 2018. Articles from 1950 onwards were included and there were no language restrictions. 2023 records were initially returned and subsequently screened.

**Table 2. Data Extraction from Included Studies**

| | | Reported data | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Paper** | **No.** | **Mortality** | **Cirrhosis** | **HCC** | **Diabetes** | **Cardio-vascular disease** | **Adverse events** | **QoL** | **Fatigue** | **Arthralgia** | **Erectile dysfunction** | **Biochemical markers** |
| (Dymock et al., 1972) | 115 | .. | .. | | Y | .. | .. | .. | .. | .. | .. | .. |
| (Darnis, 1972) | 30 | Y | .. | .. | Y | .. | .. | .. | .. | Y | Y | .. |
| (Niederau et al., 1985) | 163 | Y | Y | Y | Y | .. | .. | .. | Y | Y | Y | .. |
| (Conte et al., 1986) | 67 | Y | .. | Y | Y | .. | .. | .. | .. | Y | .. | .. |
| (Adams et al., 1991) | 85 | Y | Y | Y | .. | .. | .. | .. | .. | .. | .. | .. |
| (Fracanzani et al., 1995) | 120 | Y | Y | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| (McDonnell et al., 1999) | 2851 | .. | .. | .. | .. | Y | .. | Y | Y | Y | Y | .. |
| (Milman et al., 2001) | 158 | Y | Y | .. | Y | .. | .. | .. | Y | .. | .. | Y |
| (Falize et al., 2006) | 36 | .. | Y | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| (Phatak et al., 2010) | 49 | .. | .. | .. | .. | .. | Y | .. | .. | .. | .. | .. |
| (Harty et al., 2011) | 203 | .. | .. | .. | .. | .. | .. | .. | .. | Y | .. | .. |
| (Brissot et al., 2011) | 210 | .. | .. | .. | .. | .. | Y | .. | .. | .. | .. | .. |
| (Rehácek et al., 2012) | 22 | .. | .. | .. | .. | .. | Y | .. | .. | .. | .. | .. |
| (Rombout-Sestrienkova et al., 2012) | 38 | .. | .. | .. | .. | .. | Y | Y | .. | .. | .. | Y |
| (Parra Salinas et al., 2012) | 39 | .. | .. | .. | .. | .. | Y | .. | .. | .. | .. | .. |
| (Lukic et al., 2013) | 29 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | Y |
| (Sundic et al., 2014) | 62 | .. | .. | .. | .. | .. | Y | .. | .. | .. | .. | Y |
| (Bardou-Jacquet et al., 2015) | 1085 | Y | Y | .. | .. | Y | .. | .. | .. | .. | .. | .. |
| (Koutsavlis et al., 2016) | 167 | .. | .. | .. | .. | .. | .. | .. | .. | Y | .. | .. |
| (Brückl et al., 2017) | 20 | .. | .. | .. | .. | .. | Y | .. | .. | .. | .. | .. |
| (Ong et al., 2017) | 104 | .. | Y | .. | .. | .. | Y | Y | Y | Y | .. | Y |
| (Chayanupatkul et al., 2017) | 196 | .. | .. | Y | .. | .. | .. | .. | .. | .. | .. | .. |
| (Jabbour et al., 2018) | 39 | .. | Y | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| (Bardou-Jacquet et al., 2019) | 106 | .. | Y | Y | .. | .. | .. | .. | .. | .. | .. | .. |

Table 2. Included studies and their reported outcomes (marked as ‘Y’).

**Table 3. Quality of evidence**

| Paper | Quality Assessment Tool | Risk of Bias/Assessment |
|---|---|---|
| (Dymock et al., 1972) | Robins-I | Serious |
| (Darnis, 1972) | Robins-I | Serious |
| (Niederau et al., 1985) | Robins-I | Moderate |
| (Conte et al., 1986) | Robins-I | Serious |
| (Adams et al., 1991) | Robins-I | Serious |
| (Fracanzani et al., 1995) | Robins-I | Serious |
| (McDonnell et al., 1999) | Robins-I | Serious |
| (Milman et al., 2001) | Robins-I | Serious |
| (Falize et al., 2006) | Robins-I | Serious |
| (Phatak et al., 2010) | Robins-I | Serious |
| (Harty et al., 2011) | Robins-I | Serious |
| (Brissot et al., 2011) | Robins-I | Serious |
| (Rehácek et al., 2012) | Robins-I | Serious |
| (Rombout-Sestrienkova et al., 2012) | Cochrane Risk of Bias | Poor |
| (Parra Salinas et al., 2012) | Robins-I | Serious |
| (Lukic et al., 2013) | Robins-I | Serious |
| (Sundic et al., 2014) | Cochrane Risk of Bias | Fair |
| (Bardou-Jacquet et al., 2015) | Robins-I | Moderate |
| (Koutsavlis et al., 2016) | Robins-I | Serious |
| (Brückl et al., 2017) | Robins-I | Serious |
| (Ong et al., 2017) | Cochrane Risk of Bias | Good |
| (Chayanupatkul et al., 2017) | Robins-I | Serious |
| (Jabbour et al., 2018) | Robins-I | Serious |
| (Bardou-Jacquet et al., 2019) | Robins-I | Serious |

Table 3. Quality of evidence of included studies

## FIGURE LEGENDS

**Fig. 1. Organs affected by HH.** The hepatic and extrahepatic complications of HH are multisystemic and wide-ranging.

**Fig. 2. PRISMA diagram outlining search strategy.** 2023 studies were returned from a search of MEDLINE and EMBASE. These were independently screened and assessed for eligibility by 2 authors with 24 studies included for review.

**Fig. 3. Fig 3. Survival rates in patients with hereditary hemochromatosis according to phlebotomy treatment. treatment.** Patients adequately treated (n=66) are compared with inadequately treated patients

(n=62). The difference in survival between treated and untreated patients is significant ($p<0.0001$). Reproduced with permission from Milman et al, 2001.

**Fig. 4. Primary liver cancer incidence according to fibrosis stage.** Kaplan Meier analysis of primary liver cancer incidence, according to fibrosis stage at last liver biopsy (stage F2: plain line; stageF3/F4: dashed line). Follow up was limited to 35 years because of low number of patients at risk afterwards. Reproduced with permission from Bardou-Jacquet et al, 2019.

**Fig. 5. Systems/outcomes possibly benefited by phlebotomy treatment.** The possible benefits of phlebotomy treatment in HH are multisystemic.

## References


1. Powell LW, Seckington RC, Deugnier Y. Haemochromatosis. The Lancet. 2016 Aug;388(10045):706–16.

2. Buzzetti E, Kalafateli M, Thorburn D, Davidson BR, Tsochatzis E, Gurusamy KS. Interventions for hereditary haemochromatosis: an attempted network meta-analysis. Cochrane Database Syst Rev. 2017 Mar 8;3:CD011647.

3. Cargill T, Prabu A, Ryan J. CRD42018103247. The clinical outcomes of patients with haemochromatosis undergoing iron reduction therapy: a systematic review of the literature. [Internet]. PROSPERO; 2018. Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42018103247

4. Sterne JA, Hernán MA, Reeves BC, Savović J, Berkman ND, Viswanathan M, et al. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. BMJ. 2016 Oct 12;355:i4919.

5. Higgins JPT, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ. 2011 Oct 18;343:d5928.

6. Niederau C, Fischer R, Pürschel A, Stremmel W, Häussinger D, Strohmeyer G. Long-term survival in patients with hereditary hemochromatosis. Gastroenterology. 1996 Apr;110(4):1107–19.

7. Adams PC, Speechley M, Kertesz AE. Long-term survival analysis in hereditary hemochromatosis. Gastroenterology. 1991 Aug;101(2):368–72.

8. Bardou-Jacquet E, Morcet J, Manet G, Lainé F, Perrin M, Jouanolle A-M, et al. Decreased cardiovascular and extrahepatic cancer-related mortality in treated patients with mild HFE hemochromatosis. J Hepatol. 2015 Mar;62(3):682–9.

9. Conte D, Piperno A, Mandelli C, Fargion S, Cesana M, Brunelli L, et al. Clinical, biochemical and histological features of primary haemochromatosis: a report of 67 cases. Liver. 1986 Oct;6(5):310–5.

10. Darnis F. [Hemochromatosis (apropos of 100 cases)]. Ann Biol Clin (Paris). 1972;30(4):349–78.

11. Fracanzani AL, Fargion S, Romano R, Conte D, Piperno A, D'Alba R, et al. Portal hypertension and iron depletion in patients with genetic hemochromatosis. Hepatol Baltim Md. 1995 Oct;22(4 Pt 1):1127–31.

12. Milman N, Pedersen P, á Steig T, Byg KE, Graudal N, Fenger K. Clinically overt hereditary hemochromatosis in Denmark 1948-1985: epidemiology, factors of significance for long-term survival, and causes of death in 179 patients. Ann Hematol. 2001 Dec;80(12):737–44.

13. Niederau C, Fischer R, Sonnenberg A, Stremmel W, Trampisch HJ, Strohmeyer G. Survival and causes of death in cirrhotic and in noncirrhotic patients with primary hemochromatosis. N Engl J Med. 1985 Nov 14;313(20):1256–62.

14. Powell LW, Dixon JL, Ramm GA, Purdie DM, Lincoln DJ, Anderson GJ, et al. Screening for hemochromatosis in asymptomatic subjects with or without a family history. Arch Intern Med. 2006 Feb 13;166(3):294–301.

15. Bacon BR, Adams PC, Kowdley KV, Powell LW, Tavill AS. Diagnosis and management of hemochromatosis: 2011 Practice Guideline by the American Association for the Study of Liver Diseases. Hepatology. 2011 Jul;54(1):328–43.

16. Guyader D, Jacquelinet C, Moirand R, Turlin B, Mendler MH, Chaperon J, et al. Noninvasive prediction of fibrosis in C282Y homozygous hemochromatosis. Gastroenterology. 1998 Oct;115(4):929–36.

17. Bardou-Jacquet E, Morandeau E, Anderson GJ, Ramm GA, Ramm LE, Morcet J, et al. Regression of Fibrosis Stage With Treatment Reduces Long-Term Risk of Liver Cancer in Patients With Hemochromatosis Caused by Mutation in HFE. Clin Gastroenterol Hepatol Off Clin Pract J Am Gastroenterol Assoc. 2019 Oct 14;

18. Falize L, Guillygomarc'h A, Perrin M, Lainé F, Guyader D, Brissot P, et al. Reversibility of hepatic fibrosis in treated genetic hemochromatosis: a study of 36 cases. Hepatol Baltim Md. 2006 Aug;44(2):472–7.

19. Jabbour TE, McHugh K, Patil D, Zuo C, Kim S, Lee H. Histologic Changes of Noncirrhotic Portal Hypertension (NCPH) in Post-Phlebotomy Biopsies from haemochromatosis patients: evidence for regressed fibrosis. In: Laboratory Investigation. 2018. p. 634.

20. Ong SY, Gurrin LC, Dolling L, Dixon J, Nicoll AJ, Wolthuizen M, et al. Reduction of body iron in HFE-related haemochromatosis and moderate iron overload (Mi-Iron): a multicentre, participant-blinded, randomised controlled trial. Lancet Haematol. 2017 Dec;4(12):e607–14.

21. Chayanupatkul M, Mapakshi SR, Richardson P, El-Serag HB, Kanwal F. Risk and risk factors of hepatocellular carcinoma in patients with hfe-related hereditary hemochromatosis. In: Hepatology. United States.; 2017. p. 436A.

22. Barton JC, Acton RT. Diabetes in HFE Hemochromatosis. J Diabetes Res. 2017;2017:9826930.

23. Pilling LC, Tamosauskaite J, Jones G, Wood AR, Jones L, Kuo C-L, et al. Common conditions associated with hereditary haemochromatosis genetic variants: cohort study in UK Biobank. BMJ. 2019 16;364:k5222.

24. Dymock IW, Cassar J, Pyke DA, Oakley WG, Williams R. Observations on the pathogenesis, complications and treatment of diabetes in 115 cases of haemochromatosis. Am J Med. 1972 Feb;52(2):203–10.

25. McDonnell SM, Preston BL, Jewell SA, Barton JC, Edwards CQ, Adams PC, et al. A survey of 2,851 patients with hemochromatosis: symptoms and response to treatment. Am J Med. 1999 Jun;106(6):619–24.

26. Brissot P, Ball S, Rofail D, Cannon H, Jin VW. Hereditary hemochromatosis: patient experiences of the disease and phlebotomy treatment. Transfusion (Paris). 2011 Jun;51(6):1331–8.

27. Rombout-Sestrienkova E, Nieman FHM, Essers BAB, van Noord PAH, Janssen MCH, van Deursen CTBM, et al. Erythrocytapheresis versus phlebotomy in the initial treatment of HFE hemochromatosis patients: results from a randomized trial. Transfusion (Paris). 2012 Mar;52(3):470–7.

28. Sundic T, Hervig T, Hannisdal S, Assmus J, Ulvik RJ, Olaussen RW, et al. Erythrocytapheresis compared with whole blood phlebotomy for the treatment of hereditary haemochromatosis. In: Blood Transfusion. 2014. p. s84–9.

29. Brückl D, Kamhieh-Milz S, Kamhieh-Milz J, Salama A. Efficacy and safety of erythrocytapheresis and low-dose erythropoietin for treatment of hemochromatosis. J Clin Apheresis. 2017 Jun;32(3):170–4.

30. Parra Salinas I, Montes Limon A, Andrade M, Recasens Flores V, Garcia-Erce J, Gonzalez Rodriguez V, et al. Therapeutic isovolemic erythrocytoapheresis for iron overload in hereditary hemochromatosis, an efficacy and safety assessment. In: Haematologica. Amsterdam Netherlands: Conference Publication: (var.pagings); 2012. p. 710.

31. Rehácek V, Bláha M, Jirousová H, Cernohorská J, Papousek P. Therapeutic erythrocytapheresis in the initial treatment of hereditary hemochromatosis. Acta Medica (Hradec Kralove). 2012;55(4):180–5.

32. Phatak P, Brissot P, Wurster M, Adams PC, Bonkovsky HL, Gross J, et al. A phase 1/2, dose-escalation trial of deferasirox for the treatment of iron overload in HFE-related hereditary hemochromatosis. Hepatol Baltim Md. 2010 Nov;52(5):1671–779.

33. European Association For The Study Of The Liver. EASL clinical practice guidelines for HFE hemochromatosis. J Hepatol. 2010 Jul;53(1):3–22.

34. Smith K, Fife-Schaw C, Dibb B, Griffiths W. Living with the Impact of Iron Overload. [Internet]. Haemochromatosis UK.; 2018 [cited 2019 Nov 4]. Available from: http://haemochromatosis.org.uk/wp-content/uploads/2018/10/Impact-of-iron-overload-no-bleed.pdf

35. Harty LC, Lai D, Connor S, Dunne A, Ali M, Ryan J, et al. Prevalence and progress of joint symptoms in hereditary hemochromatosis and symptomatic response to venesection. J Clin Rheumatol Pract Rep Rheum Musculoskelet Dis. 2011 Jun;17(4):220–2.

36. Koutsavlis I, Mitchell S, MacGilchrist A. Management of patients with Hereditary Haemochromatosis (HH) a single institution's experience. In: Gut. United Kingdom; 2016. p. A290.

37. Lin E, Adams PC. Biochemical liver profile in hemochromatosis. A survey of 100 patients. J Clin Gastroenterol. 1991 Jun;13(3):316–20.

38. Adams PC, Speechley M, Barton JC, McLaren CE, McLaren GD, Eckfeldt JH. Probability of C282Y homozygosity decreases as liver transaminase activities increase in participants with hyperferritinemia in the hemochromatosis and iron overload screening study. Hepatol Baltim Md. 2012 Jun;55(6):1722–6.

39. Lukic M, Bojanic I, Mazic S, Baricevic M, Gugic DM, Matic I, et al. Our experience in treating hereditary hemochromatosis: Erythrocytapheresis vs phlebotomy. In: Vox Sanguinis. Amsterdam Netherlands.: Conference Publication: (var.pagings).; 2013. p. 259.

40. Adams PC, Deugnier Y, Moirand R, Brissot P. The relationship between iron overload, clinical symptoms, and age in 410 patients with genetic hemochromatosis. Hepatol Baltim Md. 1997 Jan;25(1):162–6.

41. Selby JV, Friedman GD. Epidemiologic evidence of an association between body iron stores and risk of cancer. Int J Cancer. 1988 May 15;41(5):677–82.

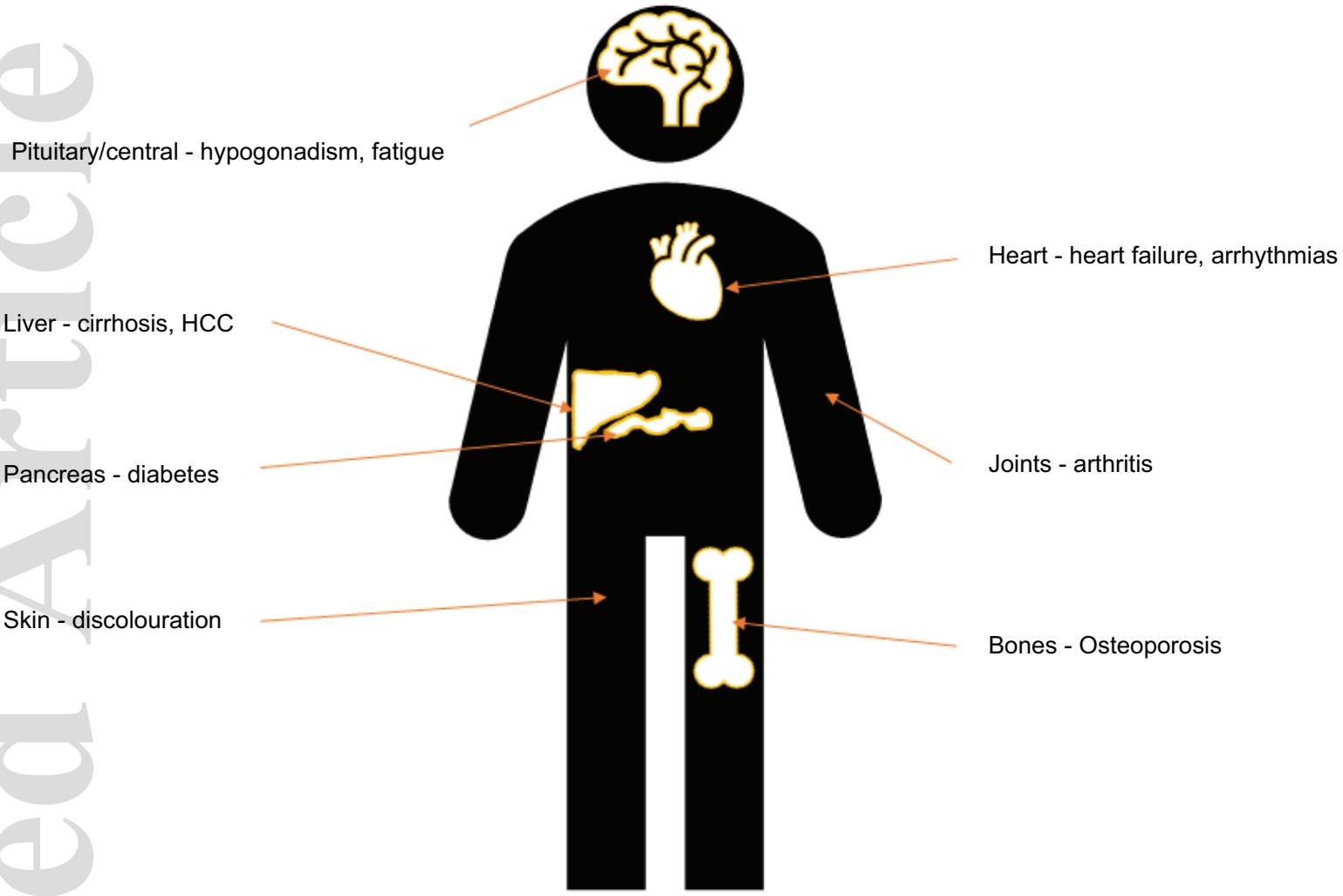
42. Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet. 1996 Aug;13(4):399–408.

43. Sánchez-Luna SA, Brown KE. Clinical burden of liver disease from hemochromatosis at an academic medical center. Hepatol Commun. 2017;1(5):453–9.

44. Barton JC, McLaren CE, Chen W-P, Ramm GA, Anderson GJ, Powell LW, et al. Cirrhosis in Hemochromatosis: Independent Risk Factors in 368 HFE p.C282Y Homozygotes. Ann Hepatol. 2018 Aug 24;17(5):871–9.

45. Grosse SD, Gurrin LC, Bertalli NA, Allen KJ. Clinical penetrance in hereditary hemochromatosis: estimates of the cumulative incidence of severe liver disease among HFE C282Y homozygotes. Genet Med Off J Am Coll Med Genet. 2018 Apr;20(4):383–9.

46. La Jolla Pharmaceutical Company. A Study of LJPC-401 for the Treatment of Iron Overload in Adult Patients With Hereditary Hemochromatosis [Internet]. NIH ClinicalTrials.gov; 2018. Available from: https://clinicaltrials.gov/ct2/show/NCT03395704

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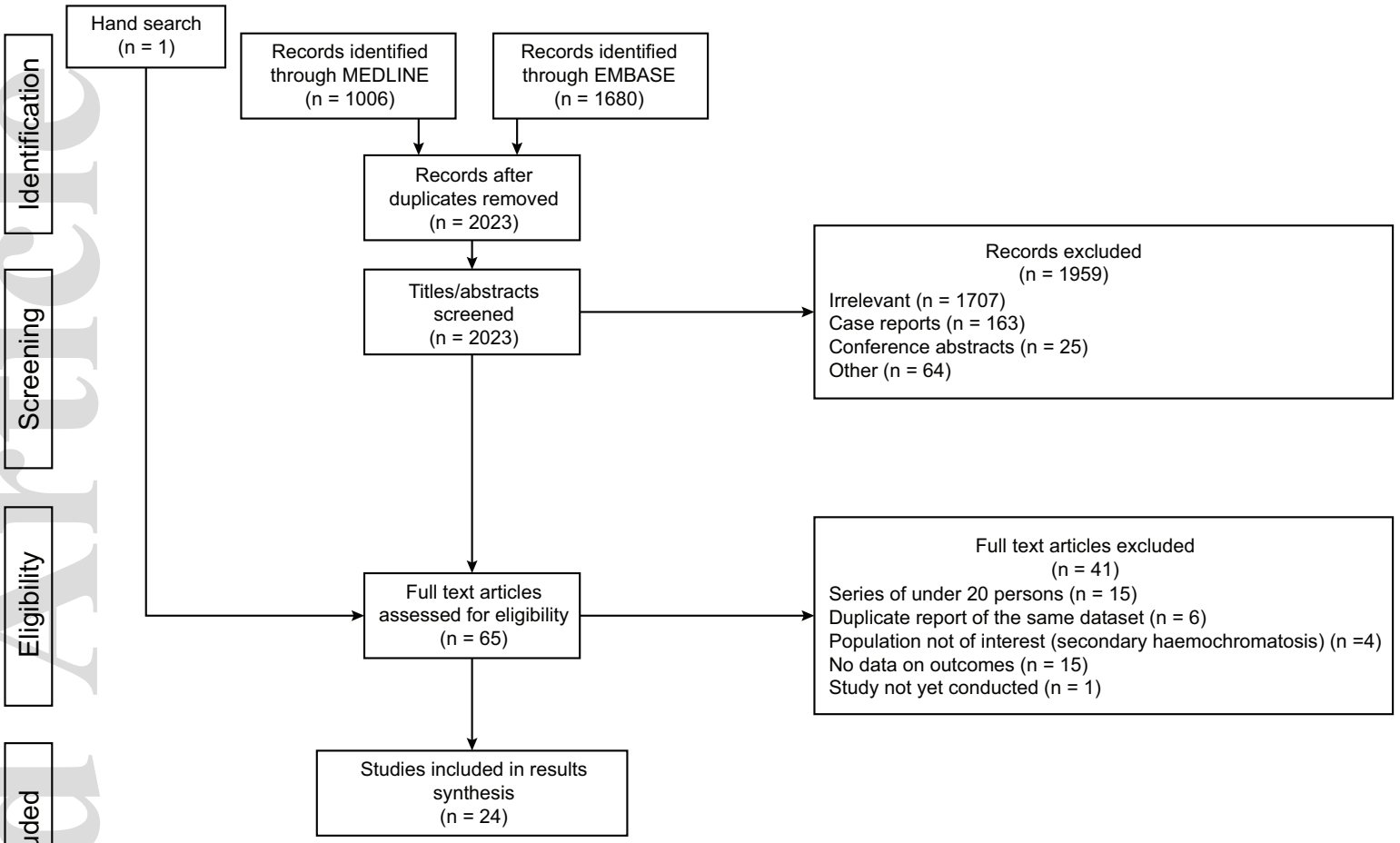


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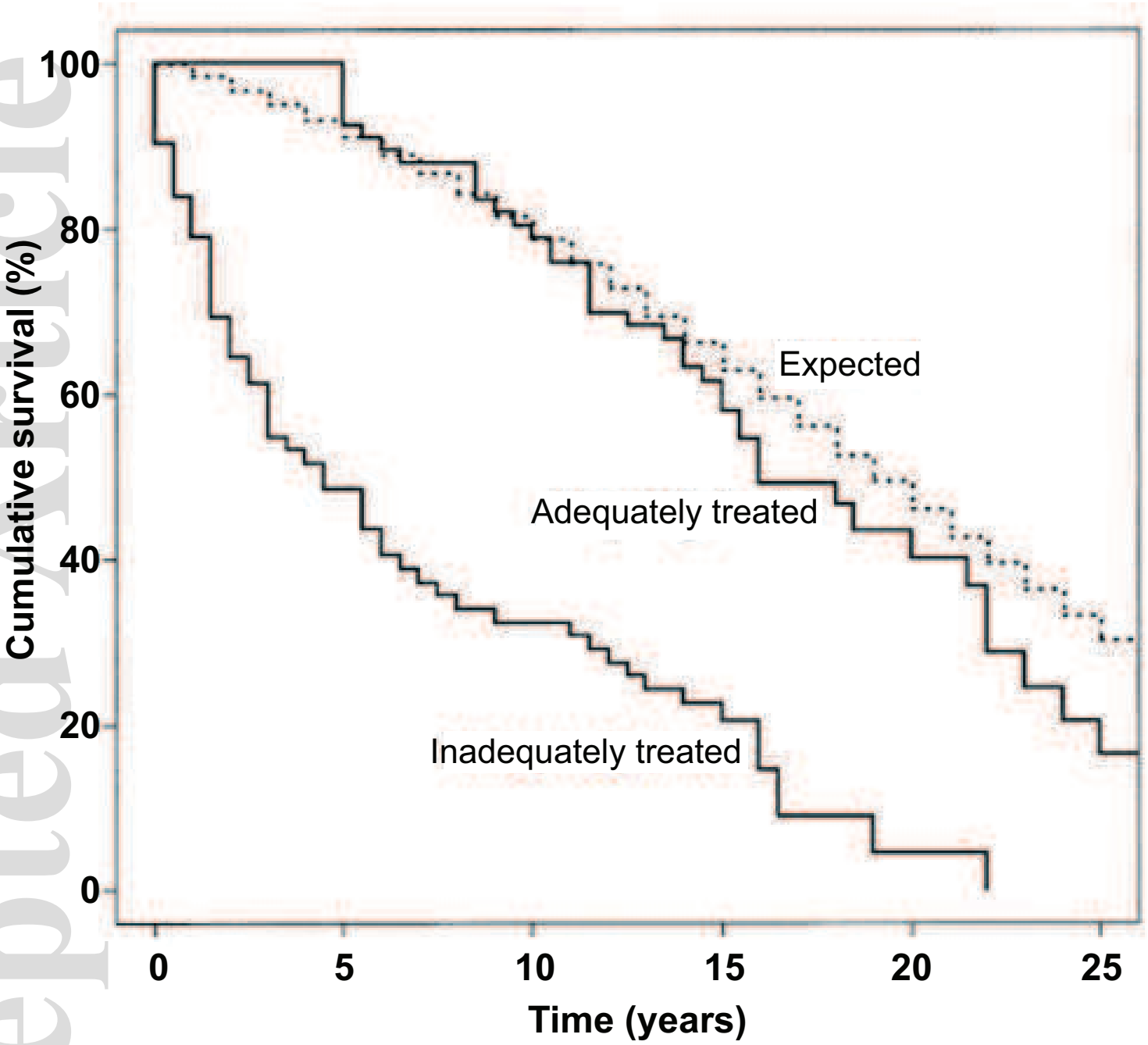


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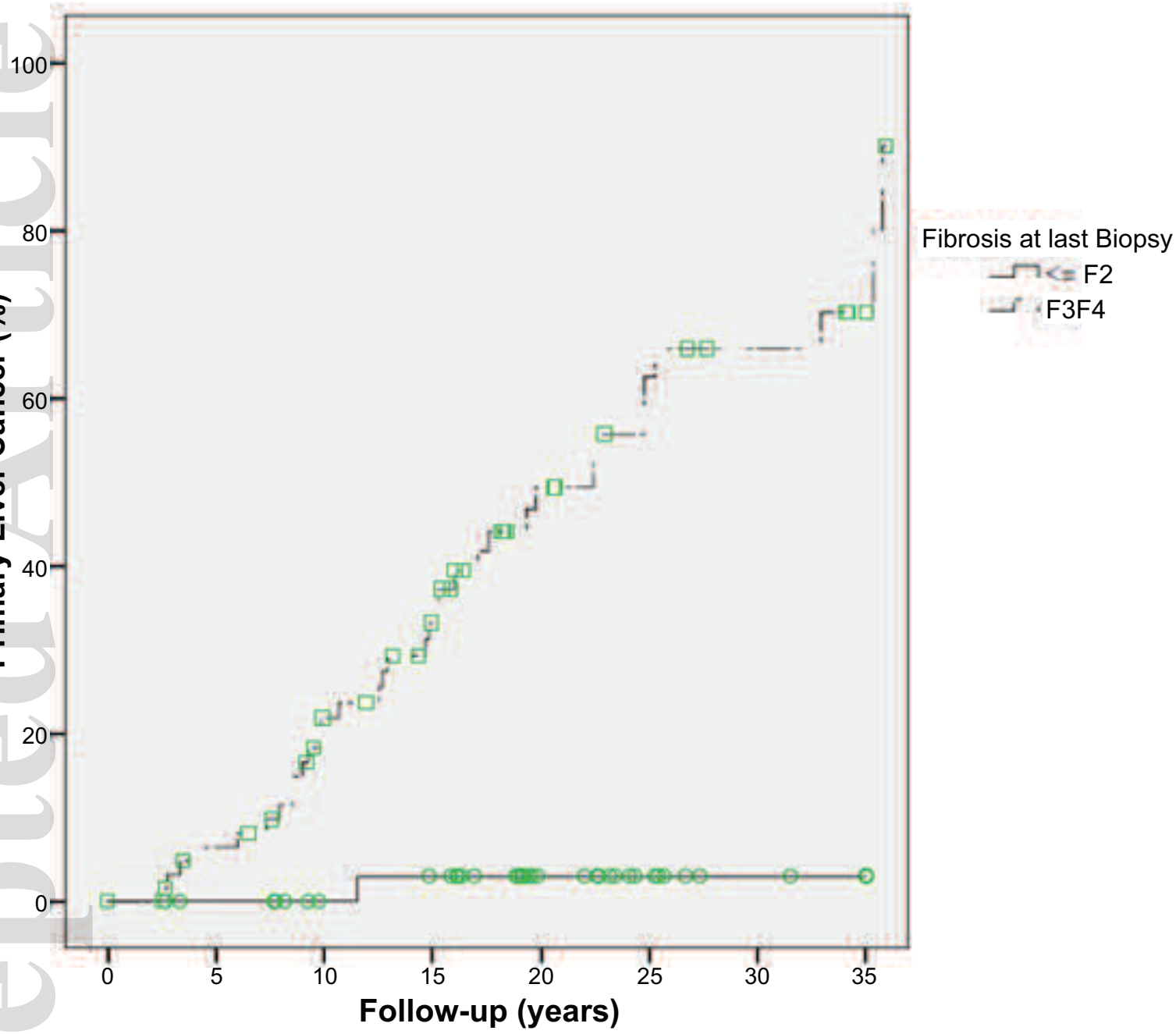


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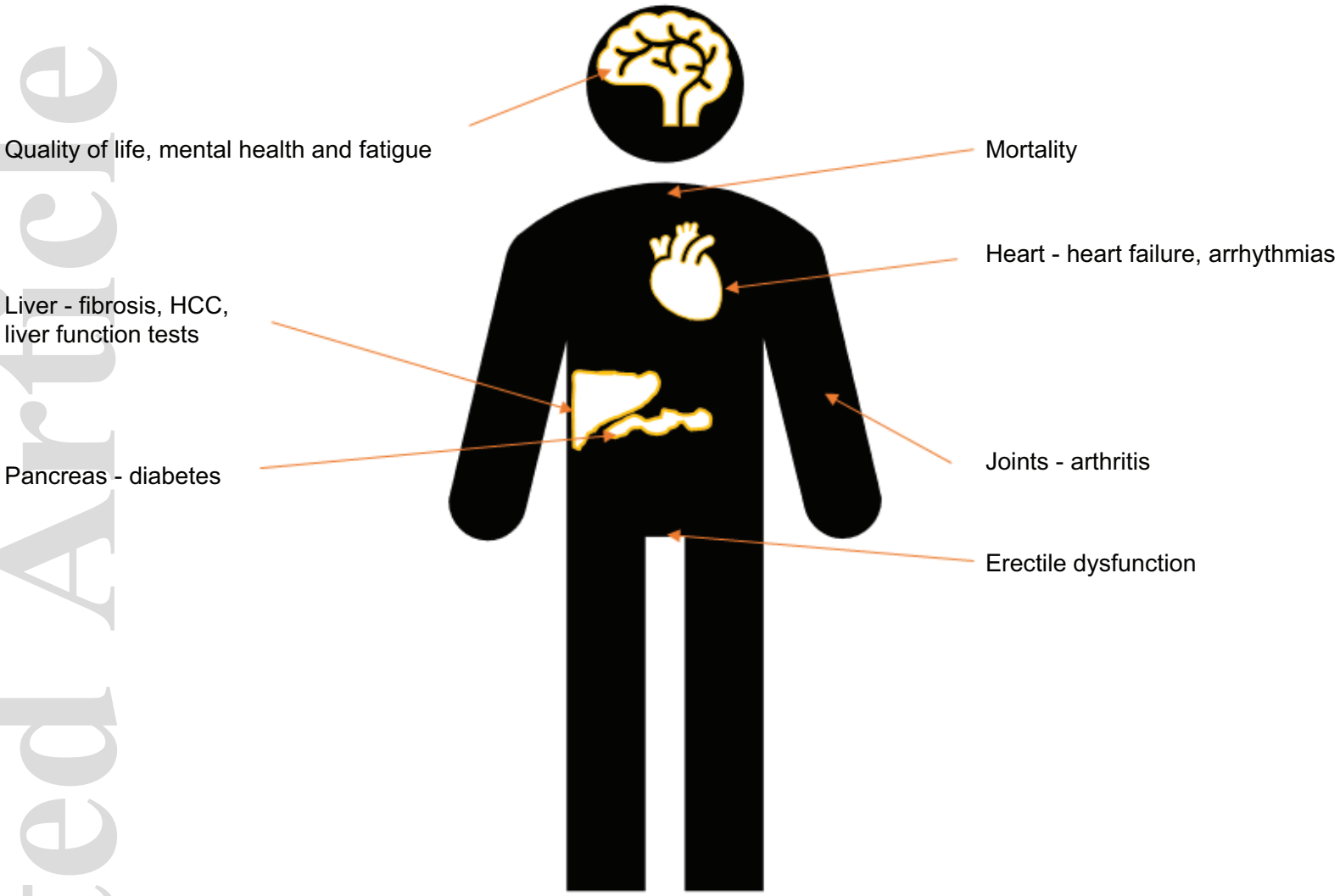


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