

# JOURNAL OF HEPATOLOGY

**EASL Clinical Practice Guidelines:  
HFE Hemochromatosis**

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# EASL Clinical Practice Guidelines for HFE Hemochromatosis

European Association for the Study of the Liver\*

## Preamble

Iron overload in humans is associated with a variety of genetic and acquired conditions. Of these, HFE hemochromatosis (HFE-HC) is by far the most frequent and most well-defined inherited cause when considering epidemiological aspects and risks for iron-related morbidity and mortality. The majority of patients with HFE-HC are homozygotes for the C282Y polymorphism [1]. Without therapeutic intervention, there is a risk that iron overload will occur, with the potential for tissue damage and disease. While a specific genetic test now allows for the diagnosis of HFE-HC, the uncertainty in defining cases and disease burden, as well as the low phenotypic penetrance of C282Y homozygosity poses a number of clinical problems in the management of patients with HC. This Clinical Practice Guideline will therefore focus on HFE-HC, while rarer forms of genetic iron overload recently attributed to pathogenic mutations of transferrin receptor 2, (TFR2), hepcidin (HAMP), hemojuvelin (HJV), or to a sub-type of ferroportin (FPN) mutations, on which limited and sparse clinical and epidemiologic data are available, will not be discussed. We have developed recommendations for the screening, diagnosis, and management of HFE-HC.

## Introduction

This Clinical Practice Guideline (CPG) has been developed to assist physicians and other healthcare providers as well as patients and interested individuals in the clinical decision making process for HFE-HC. The goal is to describe a number of generally accepted approaches for the diagnosis, prevention, and treatment of HFE-HC. To do so, four clinically relevant questions were developed and addressed:

- (1) What is the prevalence of C282Y homozygosity?
- (2) What is the penetrance of C282Y homozygosity?
- (3) How should HFE-HC be diagnosed?
- (4) How should HFE-HC be managed?

Each question has guided a systematic literature review in the Medline (PubMed version), Embase (Dialog version), and the Cochrane Library databases from 1966 through March 2009. The study selection was based on specific inclusion and exclusion criteria (Table 1). The quality of reported evidence has been graded according to the Grades of Recommendation, Assessment, Development, and Evaluation system (GRADE) [2–6]. The GRADE system classifies recommendations as strong or weak, according to the balance of the benefits and downsides (harms, burden, and cost) after considering the quality of evidence (Table 2). The quality of evidence reflects the confidence in estimates of the

true effects of an intervention, and the system classifies quality of evidence as high, moderate, low, or very low according to factors that include the study methodology, the consistency and precision of the results, and the directness of the evidence [2–6]. Every recommendation in this CPG is followed by its GRADE classification in parentheses.

## What is the prevalence of C282Y homozygosity?

*The prevalence of HFE gene polymorphisms in the general population*

The frequency of HC-associated HFE gene polymorphisms in the general population was determined in 36 screening studies, which fulfilled the inclusion criteria (Table 3). The allelic frequency of C282Y was 6.2% in a pooled cohort of 127,613 individuals included in the individual patient meta-analysis from these 36 studies (Table 3).

From this allelic frequency for C282Y, a genotype frequency of 0.38% or 1 in 260 for C282Y homozygosity can be calculated from the Hardy–Weinberg equation. The reported frequency of C282Y homozygosity is 0.41%, which is significantly higher than the expected frequency. This probably reflects a publication or ascertainment bias.

Significant variations in frequencies of the C282Y allele between different geographic regions across Europe have been reported with frequencies ranging from 12.5% in Ireland to 0% in Southern Europe (Fig. 1).

In addition to C282Y, which is also known as the ‘major’ HFE-associated polymorphism, H63D, considered to be the ‘minor’ HFE polymorphism, has been found more frequently in HC patients than in the control population. The frequency of the H63D polymorphism shows less geographic variation, with an average allelic frequency of 14.0% from pooled data (23,733 of 170,066 alleles). An additional HFE polymorphism is S65C, which can be associated with excess iron when inherited *in trans* with C282Y on the other parental allele. The allelic frequency of this polymorphism is ~0.5% and appears to be higher in Brittany, France.

*The prevalence of homozygosity for C282Y in the HFE gene in clinically recognized hemochromatosis*

The prevalence of C282Y homozygosity in clinically recognized individuals with iron overload was assessed in a meta-analysis including 32 studies with a total of 2802 hemochromatosis patients of European ancestry (Table 4). This analysis of pooled data shows that 80.6% (2260 of 2802) of HC patients are homozygous for the C282Y polymorphism in the HFE gene. Compound heterozygosity for C282Y and H63D was found in 5.3% of HC patients (114 of 2117, Table 4). In the control groups, which were reported in 21 of the 32 studies, the frequency of C282Y homozygosity was 0.6% (30 of 4913 control individuals)

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## Clinical Practice Guidelines

**Table 1. Inclusion and exclusion criteria for the literature search.**

Inclusion and exclusion criteria for searching references

### Inclusion criteria

1. Populations: adults age >18 y, population applicable to Europe, North America, Australia, New Zealand, screening population with elevated iron measures, asymptomatic iron overload, or HFE C282Y homozygosity (all ages were included for questions on C282Y prevalence)
2. Disease: symptomatic (liver fibrosis, cirrhosis, hepatic failure, hepatocellular carcinoma, diabetes mellitus, cardiomyopathy, or arthropathy hypogonadism, attributable to iron overload) or asymptomatic with or without C282Y homozygosity
3. Design:
  - a. Questions on prevalence: cohort or cross-sectional studies (also studies in newborns)
  - b. Questions on burden, natural history, penetrance: cross-sectional and longitudinal cohort studies
  - c. Questions on therapeutics: RCTs and large case series
4. Outcomes: incidence, severity, or progression of clinical hemochromatosis or iron measures, nonspecific symptoms (for questions on therapy)

### Exclusion criteria:

1. Nonhuman study
2. Non-English-language
3. Age: <18 y unless adult data are analyzed separately
4. Design: Case-series with <15 patients, editorial, review, letter, congress abstract (except research letters)
5. For questions on epidemiology and diagnosis: does not include HFE genotyping
6. Does not report relevant prevalence or risk factors (for questions on prevalence–penetrance), does not report relevant outcomes (for questions on therapy)
7. Not phlebotomy treatment (for questions on therapy)

**Table 2. Quality of evidence and strength of recommendations according to GRADE.**

	Example	Note	Symbol
<b>Quality of Evidence</b>			
High	Randomized trials that show consistent results, or observational studies with very large treatment effects	Further research is very unlikely to change our confidence in the estimate of effect	A
Moderate	Randomized trials with methodological limitations, or observational studies with large effect	Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate	B
Low and very Low	Observational studies without exceptional strengths, or randomized trials with very serious limitations; unsystematic clinical observations (e.g., case reports and case series; expert opinions) as evidence of very-low-quality evidence	Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate. Any estimate of effect is very uncertain	C
<b>Strength of recommendations*</b>			
Strong	Defined as being 'confident that adherence to the recommendation will do more good than harm or that the net benefits are worth the costs'		1
Weak	Defined as being 'uncertain that adherence to the recommendation will do more good than harm OR that the net benefits are worth the costs.'	The uncertainty associated with weak recommendations follows either from poor-quality evidence, or from closely balanced benefits versus downsides.	2

\*Factors that affect the strength of a recommendation are: (a) quality of evidence, (b) uncertainty about the balance between desirable and undesirable effect; (c) uncertainty or variability in values and preferences; (d) uncertainty about whether the intervention represents a wise use of resources (see refs. [2–6]).

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**Table 3. Prevalence of the common HFE polymorphisms C282Y and H63D in the general population.**

Authors	Ref.	Country – Population	Individuals screened	Allele frequency for	
				c.845 C>A (Y282)	c.187 C>G (D63)
Beckman et al. (1997)	[7]	Mordvinia	85	0.0176	
		Finland	173	0.052	
		Sweden – Saamis	151	0.0199	
		Sweden – Saamis	206	0.0752	
Merryweather-Clarke et al. (1997)	[8]	UK	368	0.060	0.12
		Ireland	45	0.1	0.189
		Iceland	90	0.067	0.106
		Norway	94	0.074	0.112
		Former USSR	154	0.010	0.104
		Finland	38	0	0.118
		Denmark	37	0.095	0.22
		Netherlands	39	0.026	0.295
		Germany	115	0.039	0.148
		Ashkenazi	35	0	0.086
		Italy	91	0.005	0.126
		Greece	196	0.013	0.135
		Turkey	70	0	0.136
		Spain	78	0.032	0.263
Datz et al. (1998)	[9]	Austria	271	0.041	0.258
Burt et al. (1998)	[10]	New Zealand of European ancestry	1064	0.070	0.144
Jouanolle et al. (1998)	[11]	France – Brittany	1000	0.065	
Merryweather-Clarke et al. (1999)	[12]	Scandinavia	837	0.051	0.173
Distante et al. (1999)	[13]	Norway	505	0.078	0.229
Olynyk et al. (1999)	[14]	Australia	3011	0.0757	
Marshall et al. (1999)	[15]	USA – non-Hispanic whites	100	0.05	0.24
Beutler et al. (2000)	[16]	USA – whites	7620	0.064	0.154002625
Steinberg et al. (2001)	[17]	USA – non-Hispanic whites	2016	0.0637	0.153769841
Andrikovics et al. (2001)	[18]	Hungarian blood donors	996	0.034	0.014
Pozzato et al. (2001)	[19]	Italy – Celtic populations	149	0.03691	0.144295302
Byrnes et al. (2001)	[20]	Ireland	800	0.1275	0.171875
Beutler et al. (2002)	[21]	USA – non-Hispanic whites	30,672	0.0622	
Guix et al. (2002)	[22]	Spain – Balearic Islands	665	0.0203	0.201503759
Deugnier et al. (2002)	[23]	France	9396	0.07636228	
Cimburova et al. (2002)	[24]	Czech Republic	254	0.03937008	0.142
Van Aken et al. (2002)	[25]	Netherlands	1213	0.06141797	
Phatak et al. (2002)	[26]	USA	3227	0.0507	0.1512
Jones et al. (2002)	[27]	UK	159	0.085	0.173
Candore et al. (2002)	[28]	Italy – five regions	578	0.025	0.147
Salvioni et al. (2003)	[29]	Italy – North	606	0.0470297	0.143564356
Papazoglou et al. (2003)	[30]	Greece	264	0	0.089015152
Sanchez et al. (2003)	[31]	Spain	5370	0.03156425	0.208007449
Mariani et al. (2003)	[32]	Italy – North	1132	0.032	0.134
Altes et al. (2004)	[33]	Spain – Catalonia	1043	0.0282838	0.19894535
Adams et al. (2005)	[34]	USA – whites	44,082	0.06825915	0.153157751
Barry et al. (2005)	[35]	USA – non-Hispanic whites	3532	0.057	0.14
Meier et al. (2005)	[36]	Germany	709	0.044	

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## Clinical Practice Guidelines

Table 3 (continued)

Authors	Ref.	Country – Population	Individuals screened	Allele frequency for	
				c.845 C>A (Y282)	c.187 C>G (D63)
Matas et al. (2006)	[37]	Jewish populations – Chuetas	255	0.00784314	0.123529412
Hoppe et al. (2006)	[38]	USA – non-Hispanic whites	991	0.05499495	0.134207871
Aranda et al. (2007)	[39]	Spain – Northeastern	812	0.03140394	0.219211823
Terzic et al. (2006)	[40]	Bosnia and Herzegovina	200	0.0225	0.115
Floreani et al. (2007)	[41]	Italy – Central	502	0.0189243	0.148406375
Raszeja-Wyszomirska et al. (2008)	[42]	Poland – Northwestern	1517	0.04416612	0.154251813

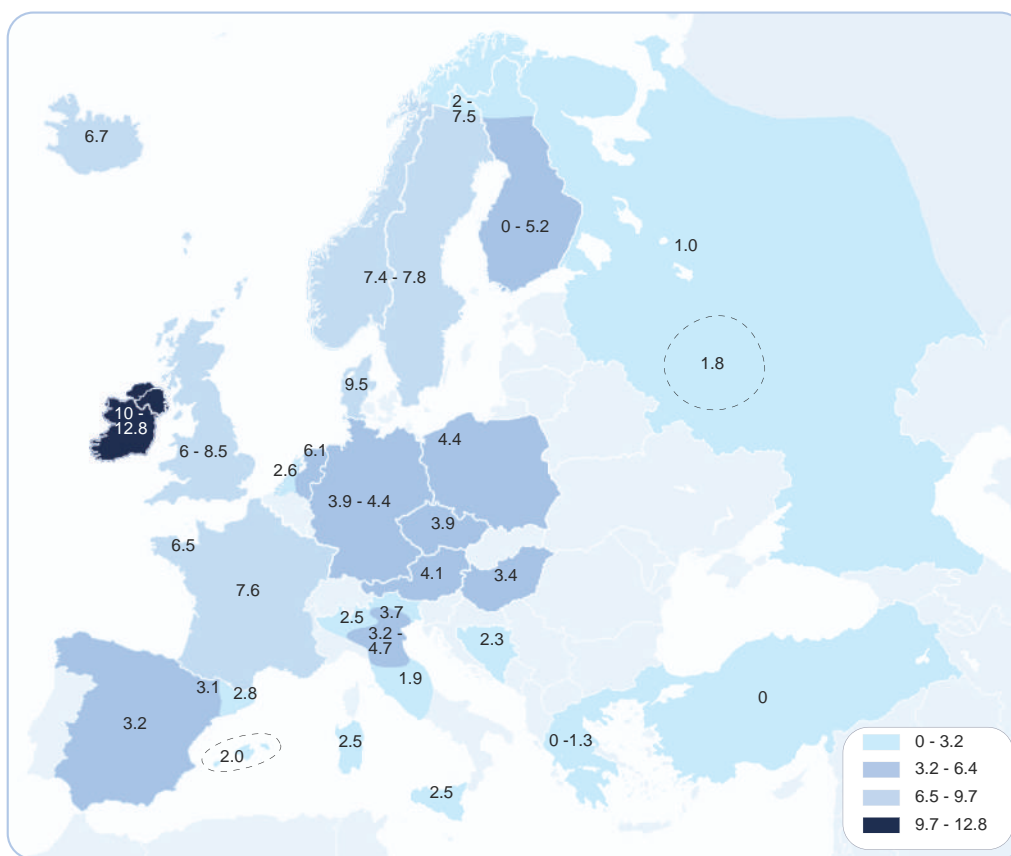


Fig. 1. Frequency of the C282Y allele in different European regions. (For detailed information see Table 3.)

and compound heterozygosity was present in 1.3% (43 of 3190 of the control population).

Hence, 19.4% of clinically characterized HC patients have the disease in the absence of C282Y homozygosity. Although compound heterozygosity (H63D/C282Y) appears to be disease associated, in such individuals with suspected iron overload, cofactors should be considered as a cause [72–74].

#### The prevalence of HFE genotypes in selected patient groups

##### Fatigue

To date, there are only cross-sectional or case-control studies investigating the prevalence of C282Y homozygosity in patients

with fatigue or chronic fatigue syndrome [75–77]. None of the three studies found the prevalence of C282Y homozygosity to be increased.

##### Arthralgia

Most available studies investigated the prevalence of C282Y mutations in patients with inflammatory arthritis [78–80]; there are few studies in patients with non-inflammatory arthralgia or chondrocalcinosis [75,81]. In the majority of studies of patients with undifferentiated osteoarthritis the prevalence of C282Y homozygosity did not exceed that of the control population [3,80]. In patients with osteoarthritis in the 2nd and 3rd metacarpophalangeal joints, higher allele



**Table 4. Prevalence of C282Y homozygosity and C282Y/H63D compound heterozygosity in clinically recognized hemochromatosis.**

Authors	Ref.	Study population	Prevalence of HLA/HFE among clinical hemochromatosis cases			
			No. of cases	C282Y homozygote	C282Y/H63D compound heterozygote	Wild type both alleles
Feder et al. (1996)	[1]	USA – Multicenter	187	148		21
Jazwinska et al. (1996)	[43]	Australia	112	112	0	
Jouanolle et al. (1996)	[44]	France	65	65	3	0
Beutler et al. (1996)	[45]	USA – European origin	147	121		
Borot et al. (1997)	[46]	France – Toulouse	94	68	4	18
Carella et al. (1997)	[47]	Italy – Northern	75	48	5	
Datz et al. (1998)	[9]	Austria	40	31		
Willis et al. (1997)	[48]	UK – Eastern England	18	18		
The UK Haemochromatosis Consortium (1997)	[49]	UK – Consortium	115	105		5
Press et al. (1998)	[50]	USA – Portland	37	12		
Cardoso et al. (1998)	[51]	Sweden	87	80	3	1
Sanchez et al. (1998)	[52]	Spain	31	27	2	1
Ryan et al. (1998)	[53]	Ireland	60	56	1	2
Nielsen et al. (1998)	[54]	Germany – Northern	92	87	4	
Murphy et al. (1998)	[55]	Ireland	30	27		
Mura, et al. (1999)	[56]	France – Brittany	711	570	40	35
Brissot et al. (1999)	[57]	France – Northwest	217	209	4	2
Bacon et al. (1999)	[58]	USA	66	60	2	
Brandhagen et al. (2000)	[59]	USA – Liver transplant recipients	5	4		
Rivard et al. (2000)	[60]	Canada – Quebec	32	14	3	8
Papanikolaou et al. (2000)	[61]	Greece	10	3		5
Guix et al. (2000)	[62]	Spain – Balearic Islands	14	13		
Brandhagen et al. (2000)	[63]	USA	82	70		2
Sham et al. (2000)	[64]	USA – Minnesota	123	74	15	6
Van Vlierberghe et al. (2000)	[65]	Belgium – Flemish	49	46	2	1
Bell et al. (2000)	[66]	Norway	120	92	3	
Hellerbrand et al. (2001)	[67]	Germany – Southern	36	26	3	2
de Juan et al. (2001)	[68]	Spain – Basque population	35	20	4	2
Guix et al. (2002)	[22]	Spain – Balearic Islands	30	27	2	0
De Marco et al. (2004)	[69]	Italy – Southern	46	9	10	11
Bauduer et al. (2005)	[70]	France – Basque population	15	8	2	
Cukjati et al. (2007)	[71]	Slovenia	21	10	2	2

frequencies of the HFE-polymorphisms (C282Y and H63D) were found, although this was not accompanied by an increased frequency of C282Y homozygotes [82,83]. A higher prevalence of C282Y homozygosity was only found in patients with well-characterized chondrocalcinosis [81].

#### Diabetes

Association of the C282Y polymorphism with diabetes mellitus has been mainly evaluated in patients with type 2 diabetes mellitus in cross-sectional and case-control studies [84–95]. Apart from one exception, no association between type 2 diabetes and C282Y homozygosity was found [75]. A higher prevalence of the C282Y allele was found in proliferative diabetic retinopathy and nephropathy complicating type 2 diabetes [96], although the frequency of C282Y homozygosity was not

increased. The prevalence of C282Y homozygotes in patients with type 1 diabetes mellitus has been addressed in only one study where a significantly higher rate of C282Y homozygotes was detected (odds ratio 4.6; prevalence 1.26%) [97].

#### Liver disease

There are a limited number of studies reporting C282Y-homozygosity in unselected patients with liver disease [98–100]. Three to 5.3% of patients were C282Y-homozygous, which is about 10-fold higher than the reported prevalence in the general population. The prevalence of C282Y homozygosity increased to 7.7% if patients were selected on the basis of a transferrin saturation of >45% [98].

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### *Hepatocellular carcinoma*

Hepatocellular carcinoma (HCC) is a recognized complication of HFE-HC. Nevertheless few studies have analyzed the frequency of C282Y homozygosity in patients with HCC and these are limited with respect to their size [101–106]. The etiology of HCC differed significantly between the studies. Patients with clinical HC were specifically excluded in one study [103]. Subgroup analysis for gender specific prevalence and different etiologies were statistically underpowered. However, three studies in HCC reported a frequency of C282Y-homozygotes of 5.5–10% [101,102,106] and three further studies found an increased prevalence of C282Y heterozygosity [103,105,107]. Only one study [104] did not find an association between HCC and the C282Y-polymorphism.

*Hair loss, hyperpigmentation, amenorrhea, loss of libido*  
There were no hits according to the search criteria.

### *Porphyria cutanea tarda*

The prevalence of C282Y homozygosity among patients with porphyria cutanea tarda (PCT) was found to be increased significantly compared with control populations, ranging from 9% to 17% in several studies [108–124]. No association between PCT and the C282Y polymorphism was found in Italian patients [125]. The association between PCT and the common HFE gene polymorphisms C282Y and H63D is illustrated by a recent meta-analysis, where the odds ratios for PCT were 48 (24–95) in C282Y homozygotes, and 8.1 (3.9–17) in C282Y/H63D compound heterozygotes [126].

### *The prevalence of C282Y homozygosity in individuals with biochemical iron abnormalities*

There is considerable variation in the cut-off of ferritin and transferrin saturation used for genetic screening of hereditary hemochromatosis (HH).

### *Serum ferritin*

The prevalence of elevated ferritin varies between 4 and 41% in healthy populations depending on the cut-off and the screening setting (Table 5) [10,13,14,23,84]. The positive predictive value of an elevated ferritin for detection of C282Y-homozygotes was 1.6% to 17.6% (Table 5). The frequency of a ferritin concentration above 1000 µg/L was 0.2% to 1.3% in non-selected populations [34,133].

### *Transferrin saturation*

Elevated transferrin saturation was found in 1.2% to 7% of screened individuals in unselected populations [10,13,14,23,129–131] (Table 5). The positive predictive value of elevated transferrin saturation for the detection of C282Y-homozygotes was 4.3% to 21.7% (Table 5).

### **What is the penetrance of C282Y homozygosity?**

Differences in inclusion criteria and in the definition of biochemical and disease penetrance have produced a range of estimates for the penetrance of C282Y homozygosity. The disease penetrance of C282Y homozygosity was 13.5% (95% confidence interval 13.4–13.6%) when 19 studies were included in the meta-analysis and the results of individual studies weighted on the inverse variance of the results of the individual study (Fig. 2) [134,135].

### *Excess iron*

Although the majority of C282Y homozygotes may have a raised serum ferritin and transferrin saturation, this cannot be relied upon as secure evidence of iron overload. An individual patient data meta-analysis including 1382 C282Y homozygous individuals reported in 16 studies showed that 26% of females and 32% of males have increased serum ferritin concentrations (>200 µg/L for females and >300 µg/L in males) (Table 6). The prevalence of excess tissue iron (>25 µmoles/g liver tissue or increased siderosis score) in 626 C282Y homozygotes who underwent liver biopsy was 52% in females and 75% in males as reported in 13 studies. The higher penetrance of tissue iron overload is due to the selection of patients for liver biopsy, which is more likely to be carried out in patients with clinical or biochemical evidence of iron overload.

When all 1382 patients with reported iron parameters were included in the meta-analysis, the penetrance of excess liver iron was then 19% for females and 42% for males.

### *Clinical penetrance and progression*

Disease penetrance based on symptoms (e.g. fatigue, arthralgia) is difficult to assess due to the non-specific nature and high frequency of such symptoms in control populations [21].

Disease penetrance based on hepatic histology has been studied but is biased by the fact that liver biopsy is usually reserved for patients with a high pre-test likelihood for liver damage. However, these studies give an estimate of disease expression in C282Y homozygotes. Elevated liver enzymes were found in 30% of males in one study [142]. Liver fibrosis was present in 18% of males and 5% in females homozygous for C282Y; cirrhosis was present in 6% of males and 2% of females [66,144]. A recent meta-analysis concludes that 10% to 33% of C282Y homozygotes eventually would develop hemochromatosis-associated morbidity [147].

Penetrance is generally higher in male than in female C282Y homozygotes. C282Y homozygotes identified during family screening have a higher risk of expressing the disease (32–35%) when compared with C282Y homozygotes identified during population based studies (27–29%).

Three longitudinal (population screening) studies are available and show disease progression in only a minority of C282Y homozygotes [140,141,146]. Available data suggest that up to 38% to 50% of C282Y homozygotes may develop iron overload, with (as already stated) 10% to 33% eventually developing hemochromatosis-associated morbidity [147]. The proportion of C282Y homozygotes with iron overload-related disease is substantially higher for men than for women (28% vs. 1%) [146].

### *The prevalence and predictive value of abnormal serum iron indices for C282Y homozygosity in an unselected population*

Serum iron studies are usually used as the first screening test when hemochromatosis is suspected. The predictive value of screening for serum iron parameters in the general population is highlighted by two studies [131,145].

The prevalence of persistently increased serum transferrin saturation upon repeated testing was 1% (622 of over 60,000). Of these individuals ~50% also had hyperferritinemia (342 of 622). Homozygosity for C282Y could be detected in ~90% of men and ~75% of women with a persistently elevated transferrin saturation and increased serum ferritin. From a cross-sectional

Table 5. Prevalence of C282Y homozygosity in patients with elevated serum ferritin and transferrin saturation.

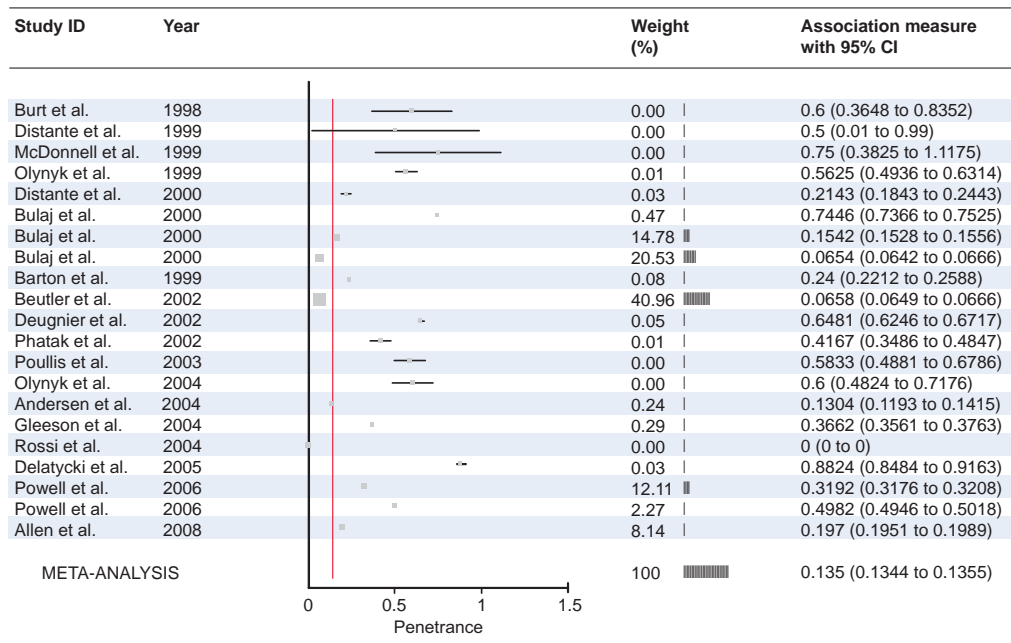
Authors	Ref.	Study population	Prevalence of C282Y homozygotes among patients with elevated serum ferritin		Prevalence of C282Y homozygotes among patients with elevated transferrin saturation (TS)		Comments
			Prevalence of elevated serum ferritin	Prevalence of C282Y	Prevalence of TS elevation	Prevalence of C282Y	
Deugnier et al.	[23]	Cross-sectional, n = 9396 <sup>e,m</sup>	76 of 981 (7.5%)	21 of 76 (17.6%)	70 of 993 (7%)	26 of 70 (18%)	Health care, young patients; ferritin available for a subgroup only
Olynyk et al.	[14]	Cross-sectional, n = 3011; <sup>f,n</sup>	405 of 3011 (13.5%)	8 of 405 (2%)	202 of 3011 (6.7%)	15 of 202 (7.4%)	Patient selection included persistently elevated TS (45% or higher) or homozygosity for the C282Y mutation
Burt et al.	[10]	Cross-sectional, n = 1064 <sup>g,l</sup>	42 of 1040 (4.0%)	2 of 42 (4.8%)	46 of 1040 (4.4%)	5 of 46 (10.9%)	Voters
Distante et al.	[13]	Cross-sectional, n = 505 <sup>h,l</sup>	23 of 505 (4.6%)	2 of 23 (8.7%)	25 of 505 pts (5%)	2 of 25 (8%)	Health care
McDonnell et al.	[127]	Cross-sectional, n = 1450 <sup>l,o</sup>	No data	No data	60 of 1640 (3.7%)	13 of 60 (21.7%)	HMO employees; only data for TS
Delatycki et al.	[128]	Cross-sectional, n = 11,307	No data	No data	No data	No data	2 of 47 pts (biopsy in 6 pts) had precirrhotic fibrosis
Adams et al.	[129]	Cross-sectional, n = 5211 <sup>p</sup>	No data	No data	60 of 5211 (1.2%)	4 of 60 (6.7%)	Blood donors
Adams et al.	[34]	Cross-sectional, n = 99,711 <sup>a,q</sup>	No data	No data	150 of 5211 (2.9%)	9 of 150 (6%)	
Beutler et al.	[16]	Cross-sectional, n = 9650 <sup>b,r</sup>	No data	No data	278 of 5211 (5.3%)	12 of 278 (4.3%)	
Barton et al.	[130]	Cross-sectional, n = 43,453 caucasian <sup>a,r</sup>	9299 whites (21.4%)	147 of 9299 (1.6%)	2976 of 43,453 (6.8%)	166 of 2976 (5.6%)	
Asberg et al.	[131]	Cross-sectional, n = 65,238 <sup>m</sup>	No data	No data	2.7% of males, 2.5% of females	269 of 1698 (15.8%)	
Gordeuk et al.	[132]	Cross-sectional, n = 101,168 <sup>a,r</sup>	2253 of 101,168 (2.2%)	No data	2253 of 101,168 (2.2%)	155 of 2253 (6.9%)	Primary care combination of TS and ferritin

**Ferritin [ $\mu\text{g/L}$ ] cutoffs:** <sup>a</sup> >300 males and postmenopausal females, >200 females, <sup>b</sup> >250 males and >200 females, <sup>c</sup> >300 males and females, <sup>d</sup> >250 males and >200 females, <sup>e</sup> >280 males >130 females, <sup>f</sup> >300 males and females, <sup>g</sup> >428 males >302 females, <sup>h</sup> >200 males and females, <sup>i</sup> 95% percentile **transferrin saturation [%] cutoff:** <sup>k</sup> >55: males >45: females, <sup>l</sup> >50, <sup>m</sup> >55: males and >50: females, <sup>n</sup> >45, <sup>o</sup> >55: males, >60: females, <sup>p</sup> >54 or >49 or >45, <sup>q</sup> >55: males >45: females, <sup>r</sup> >50 overall >45 overall.

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**Fig. 2. Forest plot of studies on the penetrance of hemochromatosis.** Studies are weighted on the inverse of the confidence interval. (For detailed information see Table 6.)

point of view, the disease penetrance of the C282Y/C282Y genotype in this study cohort, defined as the prevalence of liver cirrhosis, was ~5.0% in men and <0.5% in women [145].

### Recommendations for genetic testing:

#### General population:

- Genetic screening for HFE-HC is not recommended, because disease penetrance is low and only in few C282Y homozygotes will iron overload progress (1 B).

#### Patient populations:

- HFE testing should be considered in patients with unexplained chronic liver disease pre-selected for increased transferrin saturation (1 C).
- HFE testing could be considered in patients with:
  - porphyria cutanea tarda (1 B).
  - well-defined chondrocalcinosis (2 C).
  - hepatocellular carcinoma (2 C).
  - type 1 diabetes (2 C).
- HFE testing is not recommended in patients with
  - unexplained arthritis or arthralgia (1 C).
  - Type 2 diabetes (1 B).

### How should HFE-HC be diagnosed?

The EASL CPG panel agreed on the following case definition for diagnosis of HFE-HC:

*C282Y homozygosity and increased body iron stores with or without clinical symptoms.*

The following section will address the genetic tests and tools for assessing body iron stores.

#### Genetic testing – Methodology

C282Y homozygosity is required for the diagnosis of HFE-HC, when iron stores are increased (see diagnostic algorithms). Any

other HFE genotype must be interpreted with caution. The available methods are reported in Table 7. The intronic variant c.892+48 G>A may complicate amplification refractory mutation system (ARMS) – PCR for genetic testing [183]. The common S65C polymorphism may complicate interpretation of real-time PCR and melting curve analysis tests [184]. Finally, *in cis* inheritance of rare genetic variants [185] must be considered when gene tests are interpreted.

Sequencing of the HFE gene in C282Y heterozygotes presenting with a phenotype compatible with hemochromatosis has revealed the existence of other rare HFE mutations. Among these, the S65C mutation has been more intensively studied [56]. It may contribute – but only when inherited *in trans* with the C282Y mutation – to the development of mild iron overload with no clinical expression in the absence of co-morbid factors.

Homozygosity for H63D is not a sufficient genetic cause of iron overload and when H63D homozygosity is found in association with hyperferritinemia, co-morbid factors are usually present and do not reflect true iron overload [186]. In a population based study of blood donors, homozygosity for H63D was associated with higher transferrin saturation [187].

In rare selected pedigrees, private mutations have also been reported (V59M [188], R66C [163], G93R, I105T [154,188], E168Q [181], R224G [163], E277K & V212V [189], and V295A [27]) as well as intronic HFE variant frame shift mutations c.340+4 T>C (also referred to as IVS2, T-C+4) [190], c.1008+1 G>A (also referred to as IVS5+1G/A) [153], and c.471del [152]. Some of these may result in a severe HC phenotype when present in the homozygous state [153] or in the compound heterozygote state with C282Y [191,192].

In C282Y heterozygotes with mildly increased iron stores, compound heterozygosity with other HFE variants including H63D and S65C have been reported [56,193–195].

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Table 6. Data from studies addressing the penetrance of C282Y homozygotes.

Authors	Ref.	Study type	C282Y homozygotes (females)	Definition of penetrant disease	Affected individuals	Penetrance	Comments
Burt et al. (1998)	[10]	Cross-sectional	5 (4)	Hepatic iron index >1.9 upon liver biopsy	3	60%	No liver biopsy in unaffected individuals because of normal serum iron parameters
Distante et al. (1999)	[13]	Cross-sectional	2 (1)	Iron removed >5 g or HII >1.9 or histological iron grade >2+	1	50%	Unaffected patient had Pearl's stain Grade 2 and HII of 1.7
McDonnell et al. (1999)	[127]	Cross-sectional	4 (3)	Iron removed >5 g or HII >1.9 or histological iron grade >2+	3	75%	One unaffected patient had elevated serum iron parameters
Olynyk et al. (1999)	[14]	Cross-sectional	16 (9)	HII >1.9 or histological iron grade >2	9	56.3%	Two additional patients had serum ferritin of 1200 µg/L and 805 µg/L respectively, but did not undergo liver biopsy. Cirrhosis was found in 1 patient, fibrosis in 3 patients, and arthritis in 6 patients
Distante et al. (2000)	[136]	Cross-sectional & short term follow up	14 (9)	HII >1.9 or histological iron grade >2 or congestive heart failure + marked and persistent hyperferritinemia and TS > 55%	3	21.4%	Liver biopsy available only in 5 patients: a total of 5 patients of whom 4 had no biopsy had persistent hyperferritinemia
Bulaj et al. (2000)	[137]	Cross-sectional – affected individuals	184 (48)	At least one disease-related condition (cirrhosis, fibrosis, elevated ALT or AST, arthropathy)	137	74.5%	
		Cross-sectional – family members	214 (101)		33	15.4%	
		Cross-sectional – unselected	107 (41)		7	6.5%	
Barton et al. (1999)	[138]	Cross-sectional – family based	25 (n.d.)	Cirrhosis or diabetes attributable to iron overload	6–23	24–79%	III-defined HC phenotype was present in a total of 23 patients
Beutler et al. (2002)	[21]	Cross-sectional	152 (79)	'liver problems' (assessed in 124)	10	8.1%	Signs and symptoms that would suggest a diagnosis of HC in only one patient
Waalén et al. (2002)	[139]	Cross-sectional	141 (80)	Only symptoms and serum iron parameters reported			92 patients had elevated serum ferritin concentrations, disease-associated symptoms were equal in control group and C282Y homozygotes
Deugnier et al. (2002)	[23]	Cross-sectional	54 (44)	At least one disease-related symptom (fatigue, arthralgia, diabetes, increased ALT)	35	64.8%	21 patients had increased serum iron parameters
Phatak et al. (2002)	[26]	Cross-sectional	12 (8)	Iron removed >5 g for males and >3 g for females	5	42%	Increased serum ferritin in 50% of patients
Poullis et al. (2003)	[98]	Cross-sectional	12 (5)	Histological iron grade >2	7	58%	Increased serum ferritin in 11 out of 12 patients, but coincidence of significant co-morbidities (HCV and iron in 5 patients)
Olynyk et al. (2004)	[140]	Longitudinal	10 (6)	Hepatic iron >25 µmol/g	6	60%	Gradual increase in TS over 10 year observation – no biopsy in 4 patients

continued on next page

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Table 6 (continued)

Authors	Ref.	Study type	C282Y homozygotes (females)	Definition of penetrant disease	Affected individuals	Penetrance	Comments
Andersen et al. (2004)	[141]	Longitudinal	23 (16)	At least one disease-related condition (cirrhosis, fibrosis, elevated ALT or AST, arthropathy)	3	13.0%	Increased serum ferritin in 16 patients
Gleeson et al. (2004)	[142]	Family based study	71 (25)	Histological iron grade >3+	26	36.6%	Only 71 out of 209 C282Y homozygote patients who underwent liver biopsy were included
Rossi et al. (2004)	[143]	Cross-sectional	2		0	0%	No clinical symptoms
Delatycki et al. (2005)	[128]	Cross-sectional	51 (26)	Disease-associated symptoms	45	88%	45 patients had disease-associated symptoms (tiredness, abdominal pain, joint pain)
Powell et al. (2006)	[144]	Cross-sectional – family based	401 (201)	Histological iron grade >2	128	32%	At least one disease related condition 17%
		Cross-sectional – population based	271 (112)	Histological iron grade >2	135	50%	At least one disease related condition 27%
Asberg et al. (2007)	[145]	Cross-sectional	319 (0)	Cirrhosis	11–16	3.4–5%	Predicted/calculated penetrance
Allen et al. (2008)	[146]	Longitudinal	203 (108)	Serum ferritin >1000 µg/L	40	19.7%	In persons homozygous for the C282Y mutation, iron overload-related disease developed in a substantial proportion of men but in a small proportion of women

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Table 7. Methods for HFE genotyping.

Method		Simultaneous detection of multiple mutations	Detection of novel/rare genetic variations	Specialized equipment required	Amenable for high throughput	References(s)
RFLP	PCR amplification followed by restriction fragment length polymorphism	-	-	-	+/-	[148–150]
Direct sequencing	PCR amplification followed by direct sequencing	+	+	-	-	[151–154]
Allelic discrimination PCR	Real time PCR (TaqMan®) with displacing probes and modifications	-	-	+/-	+/-	[155–160]
Melting curve analysis	(Light Cycler®)	+	+	+/-	+/-	[161,162]
D-HPLC	Denaturing HPLC	+	+	+/-	+	[163]
SSP	Sequence specific priming PCR	-	-	-	+	[164–170]
SPA	Solid-phase amplification	-	-	-	+	[171]
SSCP	Single strand conformation polymorphism analysis	+	+	-	+/-	[172,173]
OLA	Oligonucleotide ligation assay	-	-	-	+	[148]
SCAIP	Single-condition amplification with internal primer	+/-	+/-	-	+	[151]
Advanced read-out	Mass spectrometry based, capillary electrophoresis, chip based	n/a	n/a	n/a	+	[174–179]
Reverse hybridization assay	Multiplex PCR amplification followed by reverse hybridization	n/a	n/a	n/a	+	[21,150,180,181]
Novel extraction methods	Dried blood spots, whole-blood PCR	n/a	n/a	n/a	++	[38,158,182]

#### Increased body iron stores

##### Serum ferritin

The most widely used biochemical surrogate for iron overload is serum ferritin. According to validation studies where body iron stores were assessed by phlebotomy, serum ferritin is a highly sensitive test for iron overload in hemochromatosis [21]. Thus, normal serum concentrations essentially rule out iron overload. However, ferritin suffers from low specificity as elevated values can be the result of a range of inflammatory, metabolic, and neoplastic conditions such as diabetes mellitus, alcohol consumption, and hepatocellular or other cell necrosis.

Serum iron concentration and transferrin saturation do not quantitatively reflect body iron stores and should therefore not be used as surrogate markers of tissue iron overload.

Therefore, in clinical practice, hyperferritinemia may be considered as indicative of iron overload in C282Y homozygotes in the absence of the confounding factors listed above.

##### Imaging

**Magnetic resonance imaging (MRI):** The paramagnetic properties of iron have been exploited to detect and quantify iron by MRI. The 'gradient recalled echo techniques' are sensitive when using a well-calibrated 1.5 Tesla device. There is an excellent inverse correlation between MRI signal and biochemical hepatic iron concentration (HIC) (correlation coefficient: -0.74 to -0.98) allowing for the detection of hepatic iron excess within the range 50–350 μmol/g with a 84–91% sensitivity and a 80–100% specificity according to cut-off levels of HIC ranging from 37 to 60 μmol/g wt [196–198]. MRI may also help to (i) identify heterogeneous distribution of iron within the liver, (ii) differentiate parenchymal (normal splenic signal and low

hepatic, pancreatic, and cardiac signals) from mesenchymal (decreased splenic signal) iron overload, and (iii) detect small iron-free neoplastic lesions. However, only a few patients with HFE-proven HC were studied [197].

**Superconducting quantum interference device (SQUID) susceptometer:** The SQUID susceptometer allows for *in vivo* measurement of the amount of magnetization due to hepatic iron. Results are quantitatively equivalent to biochemical determination on tissue obtained by biopsy. However, the device was not specifically validated in HFE-HC patients. In addition, it is not widely available, which restricts its use in clinical routine [199–201].

##### Liver biopsy

Liver biopsy used to be the gold standard for the diagnosis of HC before HFE genotyping became available. Now that this is readily available, homozygosity for C282Y in patients with increased body iron stores with or without clinical symptoms is sufficient to make a diagnosis of HFE-HC.

Where there is hyperferritinemia with confounding cofactors, liver biopsy may still be necessary to show whether iron stores are increased or not [98]. Liver biopsy still has a role in assessing liver fibrosis. The negative predictive value of serum ferritin <1000 μg/L and normal AST in absence of hepatomegaly for the presence of severe fibrosis or cirrhosis averaged 95% [202,203].

Serum hyaluronic acid is reported to correlate with the degree of hepatic fibrosis in HC, and if validated may provide an alternative approach to liver biopsy for the diagnosis of advanced fibrosis [204]. Transient elastography can also be helpful for determination of advanced fibrosis and cirrhosis [205].

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### Amount of iron removed

The total number of phlebotomies required to achieve low concentrations of serum ferritin may be a useful retrospective surrogate marker for the excess body iron stores in HFE-HC. The assumption that one liter of blood contains 0.5 g of iron allows for an estimate of the amount of iron removed by phlebotomies. This broadly correlates with pre-therapeutic hepatic iron concentration. Allowing for the amount of absorbed iron during therapy and taking into account the initial and post-therapeutic haemoglobin levels improves the reliability of the calculation, especially when the interval between phlebotomies exceeds one week [203].

### Family screening

Siblings of patients with HFE-related HC must undergo screening, since they have a 25% chance of being susceptible. Serum ferritin, and transferrin saturation should be assessed. Ideally HFE mutation analysis should be encouraged after appropriate counseling with regard to the pros and cons of testing (mortgage, insurance issues).

Whether they are screened with the above procedure depends upon their age, health status, and the attitude of the family.

Individuals who are C282Y homozygotes, or have HFE-related HC, frequently ask for advice on the evaluation of the susceptibility of their children who are often younger than the age of consent. In this situation, HFE genotyping of the unaffected spouse is valuable [206], so that the likelihood of genetic susceptibility and thus the need for testing of children later in life can be established.

### Recommendations for the diagnosis of HFE-HC:

- Patients with suspected iron overload should first receive measurement of fasting transferrin saturation and serum ferritin (1 B), and HFE testing should be performed only in those with increased transferrin saturation (1 A).
- Patients from liver clinics should be screened for fasting transferrin saturation and serum ferritin (1 C) and offered genetic HFE testing if transferrin saturation is increased (1 B).
- HFE testing for the C282Y and H63D polymorphism should be carried out in all patients with otherwise unexplained increased serum ferritin and increased transferrin saturation (1 B).
- Diagnosis of HFE hemochromatosis should not be based on C282Y homozygosity alone, but requires evidence of increased iron stores (1 B).
- C282Y/H63D compound heterozygotes and H63D homozygotes presenting with increased serum ferritin (>200 µg/L in females, >300 µg/L in males), increased transferrin saturation (>45% in females, >50% in males) or increased liver iron should first be investigated for other causes of hyperferritinemia (1 C).
- In C282Y homozygote patients with increased iron stores, liver biopsy is no longer necessary to diagnose hemochromatosis. Liver biopsy could be offered to C282Y homozygous patients with serum ferritin above 1000 µg/L, elevated AST, hepatomegaly, or age over 40 years (1 C).
- Genetic testing of 'other hemochromatosis genes' (TFR2, SLC40A1, HAMP, HJV) could be considered in patients with increased iron stores after exclusion of C282Y homozygosity if (i) iron excess has been proven by direct assessment, i.e. by MRI or liver biopsy, and (ii) other hepatic and haematological disorders have been ruled out (2 C).

- According to the autosomal recessive transmission of HFE-HC, genetic testing of siblings of individuals with HFE-HC should be carried out. Genetic testing of other 1st degree relatives should be considered (1 B). (Practical and cost effective strategies for family screening have been published [206].)

### Which strategy should be used to diagnose HFE-HC?

To outline a diagnostic strategy in patients with suspected HC, several clinical scenarios for patients who should be investigated for HFE-HC have been selected. The following section will discuss a practical diagnostic approach to patients with suspected iron overload.

In contrast to the previous sections, where evidence based recommendations were made, this section is based on the expert opinion of the EASL CPG panelists (Y.D., J.D., A.E., A.P., R.S., H.Z.).

### Suggestive symptoms and signs

In patients with symptoms or signs suggestive of HC (unexplained liver disease, chondrocalcinosis, type 1 diabetes, arthralgia, HCC, cardiomyopathy, or porphyria cutanea tarda) serum iron parameters should be determined. If any of these symptoms is related to HC or iron overload, they will be associated with increased serum ferritin concentrations and diagnostic work-up should be carried out as described below.

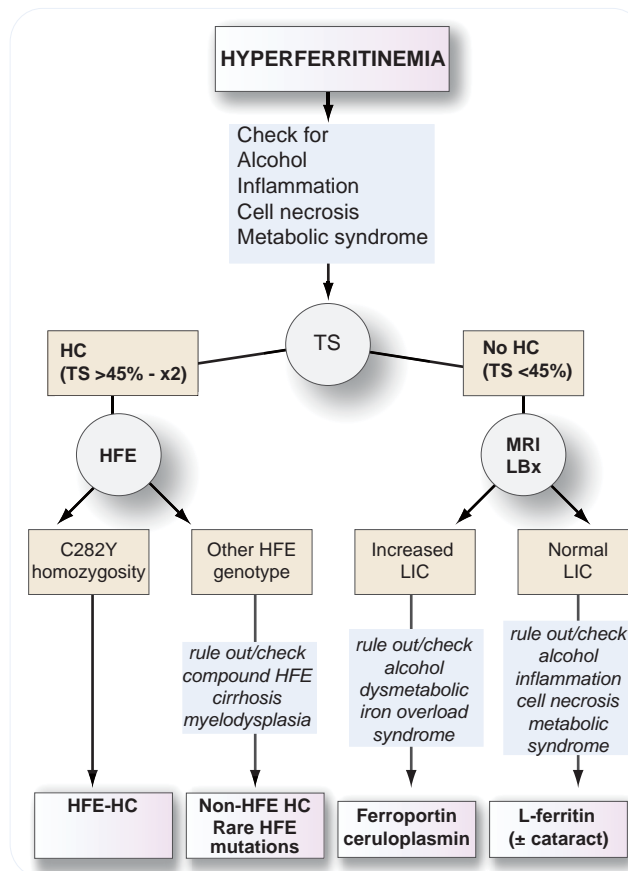
### Hyperferritinemia

In patients presenting with increased serum ferritin concentrations, it is mandatory to search for common causes of hyperferritinemia before genetic tests are carried out (Fig. 3). It is estimated that in over 90% of outpatients with hyperferritinemia, one of the following causes can be identified: chronic alcohol consumption, inflammation (check for CRP), cell necrosis (check for AST, ALT and CK), tumors (ESR, CT scan), and non-alcoholic fatty liver disease (NAFLD) and/or the metabolic syndrome (check for blood pressure, BMI, cholesterol, triglycerides, and serum glucose). In the absence of such conditions or when hyperferritinemia persists despite treatment of another potential underlying cause, transferrin saturation (TS) should be determined. After confirmation of TS elevation, HFE genotyping should be done.

If the patient is a C282Y homozygote, the diagnosis of HFE-HC can be established. For all other genotypes, confounding cofactors, compensated iron loading anemia, or non-HFE hemochromatosis should be considered. If other factors are suspected, molecular analysis for rare HFE, HJV, HAMP, and TFR2 mutations can be undertaken, with the genetic focus selected according to the clinical, laboratory, and pathological features. Patients with compound heterozygosity for the C282Y and the H63D usually present with mild iron overload, which is associated with comorbid factors such as obesity, NAFLD, chronic alcohol consumption, and end-stage cirrhosis.

If the transferrin saturation is either normal or low, the presence or absence of iron overload will guide further diagnostic work-up. Assessment of liver iron stores by direct means (i.e. MRI or liver biopsy) is recommended. If liver iron concentration is increased, iron overload related to alcohol consumption or to metabolic abnormalities should be considered before genetic testing for non-hemochromatotic genetic iron overload diseases is carried out (ferroportin disease, aceruloplasminemia).

If liver iron concentration is normal, the common causes of hyperferritinemia should be reconsidered before genetic



**Fig. 3. Proposed algorithm for the diagnosis of genetic causes of hyperferritinemia.**

testing for L-ferritin gene mutations (to investigate the hyperferritinemia-cataract syndrome).

In patients with an unclear presentation, family members should be evaluated for the evidence of iron overload, and/or the exact amount of iron removed by phlebotomy should be calculated before rare genetic disorders are tested for by candidate gene sequencing and linkage analysis by a research laboratory.

#### *C282Y homozygosity*

If an individual is found to be homozygous for C282Y, management is guided by the serum ferritin concentration (Fig. 4). If the serum ferritin concentration is normal, follow-up once a year is proposed. If the serum ferritin is elevated, initial evaluation should include fasting blood glucose, serum AST, and ALT activity. Further tests should be ordered according to the clinical features (liver scanning, ECG, echocardiography, gonadotropic hormones). For the staging of liver fibrosis, liver biopsy should be considered in patients with serum ferritin >1000 µg/L, unless cirrhosis is obvious upon scanning.

#### *Documented tissue iron overload (liver biopsy or MRI)*

In patients displaying hepatic iron deposition in their liver biopsy, further diagnostic considerations depend on the cellular and lobular distribution of iron and on the presence or absence of associated findings including fibrosis, steatosis, steatohepatitis, abnormal crystal inclusions, and chronic hepatitis (Fig. 5). In patients with pure parenchymal (i.e. hepatocellular) iron

overload, the two main differential diagnoses are: (i) early HC in the absence of cirrhosis after excluding compensated iron loading anemia; and (ii) end-stage cirrhosis in which iron distribution is heterogeneous from one nodule to the next, and there are no iron deposits in fibrous tissues, biliary walls, or vascular walls. In patients with mesenchymal or mixed iron overload, the correct diagnosis can be suggested according to the type of associated lesions.

#### **How should HFE-HC be managed?**

There are very few data on the threshold of tissue iron excess at which tissue damage is seen. A study of the degree of lipid peroxidation has been done in treated and untreated HC patients, as well as in heterozygotes, suggesting changes at low levels of iron loading [207]; however, this study has not been confirmed. The relationship between liver iron concentration [208], serum ferritin (>1000 µg/L) [202], and hepatic damage do not help define when the treatment of iron overload should begin. Another marker of toxicity and tissue damage may be non-transferrin bound (ie. free or labile) plasma iron because of its potential for catalyzing the generation of reactive oxygen species *in vivo* [209].

#### *How to manage iron overload in HFE-HC*

#### *How should HFE-HC be treated?*

Three approaches have been used to remove excess iron. None have undergone randomized controlled trials. Phlebotomy is the

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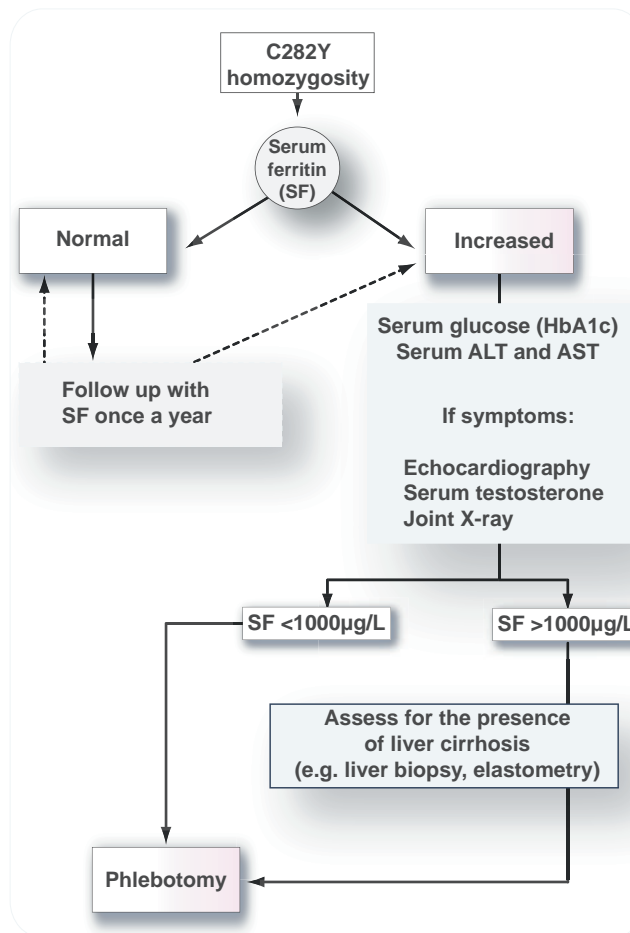


Fig. 4. Proposed algorithm for the diagnostic management of patients with C282Y homozygosity.

mainstay of treatment. Iron chelators are available and can be an option in patients who are intolerant or when phlebotomy is contraindicated. Erythrocytapheresis has been reported in treatment of HC, but is not widely practiced.

There are no studies addressing survival in genotyped C282Y homozygous HC patients. The benefit of phlebotomy has been demonstrated by case series of clinically diagnosed HC, and benefit shown by comparison with historical groups of patients not treated with phlebotomy [210], or inadequately treated with phlebotomy [211], based on measures of iron depletion. In the latter study, Kaplan–Meier analysis of survival at 5 years was 93% for adequately phlebotomized patients, compared to 48% for inadequately phlebotomized patients (10 year survival 78% v 32%).

There are studies on clinical and histopathological improvement by phlebotomy: two of these studies included HFE genotyped patients [212,213]. Fatigue, elevated transaminases, and skin pigmentation improved [214]. Milman et al. [211] reported improvement in the stage of fibrosis on repeat liver biopsy in 15–50% of patients. In another study this was found in all cases (except when cirrhosis was present) [213]. Falize et al. [212] reported improvement in the METAVIR fibrosis score in 35–69% of cases depending upon the initial fibrosis score. In cirrhotic patients, improvement in or resolution of esophageal varices has also been reported [215].

It is recognized, however, that several clinical features are unlikely to improve with iron depletion, in particular

arthralgia [211,214]. Improvement in endocrinological disorders, including diabetes mellitus, and cardiological abnormalities varies, likely related to the degree of tissue/organ damage at the start of treatment.

The benefit of iron depletion by phlebotomy has therefore been established, despite the absence of randomized controlled trials, and is the accepted standard of care. Phlebotomy is well tolerated by patients [216] and the majority of patients comply with treatment [217]. Long-term unwanted effects of venesection have not been reported.

There are no studies providing data to direct the optimal time at which to start venesection. Current recommendations of when to initiate treatment are empirical. Survival of treated patients without cirrhosis and diabetes has been found to be equivalent to that of the normal population, whereas those with these complications have a significantly reduced survival [211,214]. These data emphasize the early initiation of iron removal. The threshold of serum ferritin at which to start treatment is currently taken as above the normal range. There are no studies from which to give an evidence base to the protocol of therapeutic venesection (i.e. frequency, endpoint).

#### How to monitor HFE-HC:

Based on empirical and clinical experience, haemoglobin and haematocrit should be monitored at the time of each

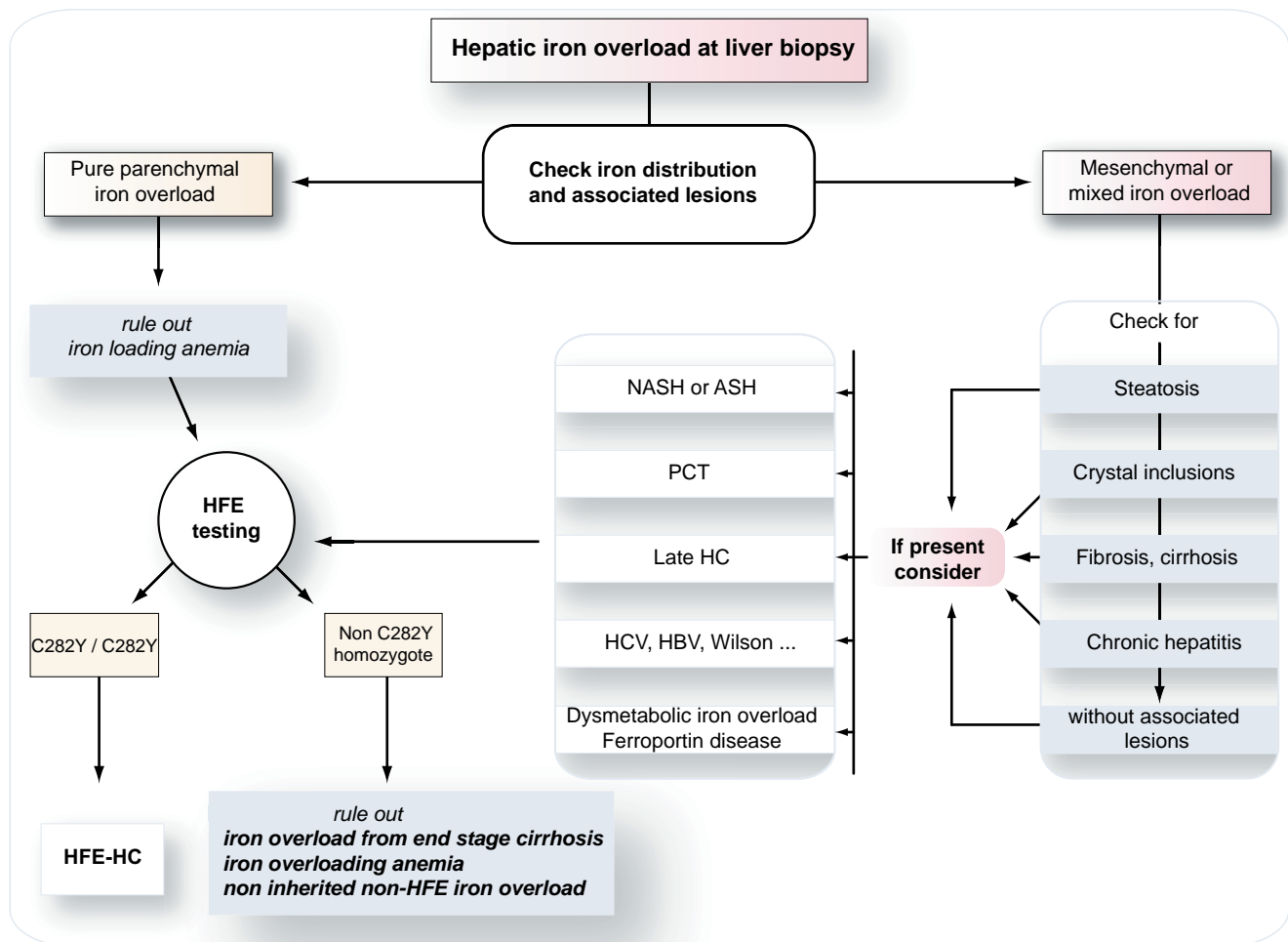


Fig. 5. Proposed algorithm for the diagnostic management of tissue iron overload.

venesection. If anemia is detected, phlebotomy should be postponed until the anemia is resolved.

Serum ferritin is measured and is sufficient to monitor iron depletion. The frequency of measurements depends upon the absolute concentration. When ferritin levels are high, measurement is required less frequently (every 3 months or so); however, as ferritin approaches the normal range, measurements should become more frequent.

#### Endpoint of therapeutic phlebotomy:

There is no evidence base on which to direct the endpoint of therapeutic phlebotomy. The recommendations that exist are based upon (i) a theoretical argument that maintains it is necessary to achieve iron deficiency in order to lower tissue iron levels to normal, and (ii) that a stated target is better than a statement of 'to normal', which would likely lead to variable interpretation and practice. The standard clinical practice is to achieve a target of serum ferritin that is less than 50 µg/L.

#### Maintenance therapy

There are no data from which to base the optimal treatment regimen and target serum iron indices. Once iron depletion has been achieved, the aim is to prevent re-accumulation. The advocated standard practice is to maintain the serum ferritin

at 50–100 µg/L. This is usually achieved with 3–6 months of venesection.

Patients may be offered the alternative approach of ceasing venesection with monitoring of serum ferritin, with the reinstatement of a short therapeutic program when the serum ferritin reaches the upper limit of the normal range [218].

After therapeutic phlebotomy, some patients may not show re-accumulation of iron at the expected rate. Some are taking proton pump inhibitors, which have been reported to be associated with reduced iron absorption and a reduced requirement for venesection [219]. Others may be on prescribed non-steroidal anti-inflammatory drugs. However, in older patients it is necessary to be alert to conditions that may lead to iron loss, such as peptic ulcers, colonic disease, and hematuria, which will need appropriate investigation.

#### Diet

There are no studies proving that dietary interventions and avoidance of dietary iron have an additional beneficial effect on the outcome in patients undergoing venesection. Although diets avoiding excess iron have been discussed, this panel considers that the important issue is maintaining a broadly healthy diet. Iron containing vitamin preparations and iron supplemented foods such as breakfast cereals should be avoided. Compliance with phlebotomy will prevent iron overload.



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Tea drinking has been reported as possibly reducing the increase in iron stores in HC patients [220], but this finding was not confirmed in a subsequent study [221]. Non-citrus fruit intake has also been reported to be associated with a lower serum ferritin, but whether this truly reflects a biological effect on iron stores has not been shown [221].

Vitamin C has been reported to be potentially toxic in patients with iron overload [222]. However there are no articles on the effect of vitamin C on iron absorption or iron stores in HFE-HC. A single case report in a genetically uncharacterized HC patient in whom vitamin C could have had a negative effect on cardiac function [223], has led to the recommendation that it is prudent to limit ingestion of vitamin C supplements to 500 mg/day [224].

As in many liver diseases, excess alcohol ingestion leads to increased hepatic damage in HFE-HC [225]. In addition, recent experimental studies show suppression of hepatic hepcidin expression by alcohol in experimental models [226]. This could account for the observation that there is a linear correlation between alcohol intake and serum iron indices and increased iron absorption in alcoholics [227–229].

### Pregnancy

A normal full term pregnancy removes around 1 g of iron from the mother [230]. Iron supplements should not be given routinely to pregnant women with HFE-related HC. Serum ferritin should be monitored. Iron deficiency should be treated according to the usual guidelines applied to pregnancy. If the ferritin is high, therapeutic phlebotomy should be deferred until the end of pregnancy unless there are cardiac or hepatic issues, in which case the appropriate specialist should be involved in the discussion of the positive and negative effects of treatment.

### How to manage tissue/organ damage

#### Cirrhosis (US, AFP, transplant):

It is important to define whether or not the patient with HFE-HC has cirrhosis. In newly recognized affected patients liver biopsy is recommended in order to assess liver architecture when serum ferritin >1000 µg/L. Transient elastography is a non-invasive tool that can be helpful for the determination of advanced fibrosis and liver cirrhosis [205].

HFE-HC patients with cirrhosis have a 100-fold greater chance of developing HCC than the normal population [214]. As in cases of cirrhosis from other causes (eg. hepatitis C and B), screening to detect an early tumor is recommended using ultrasound examination and serum alpha fetoprotein measurement every six months. Despite some case reports of HCC in non-cirrhotic HC patients, this is very rare, and screening for HCC is not considered necessary in this group.

Hepatic decompensation with ascites, spontaneous bacterial peritonitis, encephalopathy, variceal haemorrhage, and early small tumor formation may require assessment for liver transplantation.

Early reports on the outcome of HFE-HC after liver transplantation for HFE-HC [59,231,232] have found that survival may be lower than in other groups. Survival for transplant patients is around 64% after one year, and 34% after 5 years [231]. Reduced survival compared to other aetiological groups was considered to be related to iron overload; few patients had had iron depletion prior to transplantation. Causes of death were heart disease, infection, and malignancy [231].

**Diabetes mellitus:** Improvement in glucose control may occur during phlebotomy treatment, but insulin dependency is not reversed [214]. Diabetes mellitus is managed in the same way as for other patients with diabetes.

**Arthralgia, arthritis:** Physical and radiological evaluation is necessary. Unfortunately it is unusual for symptoms to be alleviated by phlebotomy treatment. Symptoms, such as joint destruction, often progress.

Anti-inflammatory agents are often ineffective but can be used. Podiatric assessment is valuable with use of insoles in shoes to help with foot pain. Joint replacement (hip and knee) may be necessary.

**Cardiac disease:** Although cardiac failure is a recognized complication of severe iron overload, it is clinically unusual (except in patients with juvenile HC). Electrocardiographic abnormalities have been reported in one third of patients [214], and in one third of these, there is improvement with phlebotomy.

However, any cardiac symptoms should be investigated by the cardiologist, if needed by electrocardiogram (ECG), echocardiography, and 24 h ambulatory ECG monitoring. There is no recognized ferritin level above which cardiac assessment is recommended.

**Endocrine disease:** Hypothyroidism has been reported in 10% of males with HC [233]. Hypogonadism with loss of potency is a recognized complication [214]. Thus the clinical history of patients with these symptoms should be obtained, and thyroid function tests and serum testosterone levels monitored.

**Osteoporosis:** Patients with HC are at risk of osteoporosis, and should undergo a DEXA scan and receive appropriate routine advice or treatment for osteoporosis if diagnosed [234].

### Recommendations for the management of HFE-HC:

- Patients with HFE-HC and evidence of excess iron should be treated with phlebotomy (1 C).
- C282Y homozygotes without evidence for iron overload could be monitored annually and treatment instituted when the ferritin rises above normal (2 C).
- Phlebotomy should be carried out by removing 400–500 ml of blood (200–250 mg iron) weekly or every two weeks. Adequate hydration before and after treatment, and avoidance of vigorous physical activity for 24 h after phlebotomy is recommended (1 C).
- Phlebotomy can be carried out also in patients with advanced fibrosis or cirrhosis (2 C).
- Before the initiation of phlebotomy, patients with HFE-HC should be assessed for complications including diabetes mellitus, joint disease, endocrine deficiency (hypothyroidism), cardiac disease, porphyria cutanea tarda, and osteoporosis (1 C).
- Complications of HFE-HC (liver cirrhosis, diabetes, arthropathy, hypogonadism, PCT) should be managed regardless of whether or not HC is the underlying cause and whether there is symptomatic relief or improvement during phlebotomy (1 C).
- To minimize the risk of additional complications, patients with HFE-HC could be immunized against hepatitis A and B while iron overloaded (2 C).

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## Patient organizations, use of blood from phlebotomy, reimbursement policies and fee exemptions

### Patient organizations

The European Federation of Associations of Patients with Hemochromatosis (EFAPH) federates national European patient organizations. Its mission is to provide information for HC patients and their relatives, to raise public awareness, and to improve the quality of care for HC patients through the support of basic and clinical research. (<http://www.european-haemochromatosis.eu/index2.html>)

### Genetic testing

Measures must be put in place to avoid discrimination of HC patients. In accordance with legal regulations in most countries, genetic testing for HFE-HC should only be carried out after informed consent has been obtained and the results should be made available only to the patient and physicians involved in the management of HFE-HC.

### The use of blood

Blood taken from patients with HFE-HC at phlebotomy should be made available for national blood transfusion services for the public good, if there is no medical contraindication and the patient has given consent. It is recognized that many patients with HFE-HC will have clinical features that exclude them from being accepted as donors (elevated liver function tests, diabetes, medications). But in the absence of these, there appears to be no medical reason, other than administrative and bureaucratic, for why the blood taken may not be used. In Europe, the fact that the blood is being taken for therapeutic reasons should not be a hindrance to its utilization.

A recent survey of EFAPH has shown that regulations for the use of blood obtained from venesection vary within Europe and even within some countries (Germany, Portugal, UK, Norway, and Italy). In Ireland and France, blood from patients with HFE-HC can be used for transfusion purposes under the appropriate medical circumstances. In France, blood donation is not forbidden in patients with HC although not explicitly permitted. According to this survey of the EFAPH, which only covered some parts of Europe, the use of blood from therapeutic venesection of HC patients is explicitly forbidden in some countries (Austria, Hungary, Iceland, Italy, Netherlands, and Spain). The EASL CPG board for HFE-HC advocates the use of blood for therapeutic phlebotomy (where there are no medical contraindications) for transfusion.

### Fee exemptions and reimbursement policies

HFE-HC is a significant cause of liver disease and phenotypic testing for HC should be offered to all individuals suspected to suffer from iron overload or patients who are at risk for the development of the disease. Genetic testing for HFE-HC is not paid for in most countries; however in some, such as France, it is reimbursed. The EASL CPG board on HC advocates full reimbursement of phenotypic and, where indicated, genetic testing for HFE-HC.

According to the EFAPH survey, reimbursement for the treatment is also highly variable across Europe and even varies within countries, where reimbursement may depend on where the treatment is carried out. The EASL CPG board on HC

advocates full reimbursement for treatment of HFE-HC both in the therapeutic and the maintenance phase of therapy.

### Contributors

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Heinz Zoller has received lecture fees from Novartis. Claus Niederau has received research funding and consultancy fees from Novartis. All other contributors and reviewers declare they have nothing to disclose.

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