

**Worse Outcomes of Patients With HFE
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Transferrin Saturation During Maintenance Therapy**

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Title: Worse Outcomes of Patients With HFE Hemochromatosis With Persistent Increases in Transferrin Saturation During Maintenance Therapy

Short title. Transferrin saturation in treated hemochromatosis

Authors. Bardou-Jacquet Edouard ^{1,3}, Lainé Fabrice ^{1,2}, Guggenbuhl Pascal ^{3,5}, Morcet Jeff ², Jézéquel Caroline ^{1,2}, Guyader Dominique ^{1,3}, Moirand Romain ^{1,3} and Deugnier Yves ^{1,3}

1- CHU Rennes, Service des maladies du foie and Centre national de référence des surcharges en fer rares, F-35033 Rennes, France

2- INSERM, CIC 1414, F-35033 Rennes, France

3- University of Rennes 1, Faculty of Medicine, F-35000 Rennes, France

4- CHU Rennes, Service de rhumatologie, F-35203 Rennes, France

5- INSERM UMR 991, F-35000 Rennes, France

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Abbreviations. ALT: alanine aminotransferase - AST: aspartate aminotransferase – BMI: body mass index - FRT50exp: exposure duration to serum ferritin ≥ 50 $\mu\text{g/l}$ - GGT: gammaglutamyl transferase - NTBI: nontransferrin bound iron - SAT50exp: exposure duration to transferrin saturation $\geq 50\%$.

Corresponding author. Edouard Bardou-Jacquet, MD, PhD, Service des maladies du foie, CHU Pontchaillou, 35033 Rennes – France - Tel: +33 2 99 28 42 97 - Email: edouard.bardou-jacquet@univ-rennes1.fr.

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Contributions of authors. E. Bardou-Jacquet: design of the work, acquisition and interpretation of data, drafting and final approval- F. Lainé: acquisition and interpretation of data, and critical revision- P. Guggenbuhl: acquisition and interpretation of data, and critical revision - J. Morcet: management of database and biostatistical analysis of data - C. Jézéquel: acquisition of data and critical revision- D. Guyader: acquisition of data and critical revision- R. Moirand: acquisition of data and critical revision - Y. Deugnier: conception and design of the work, acquisition and interpretation of data, drafting and final approval.

Abstract:

Background & Aims: Even if patients with hemochromatosis maintain low serum levels of ferritin, they still have an increased risk of general and joint symptoms, which reduce quality of life. This could be related to persistently increased transferrin saturation. We assessed whether duration of exposure to increased transferrin saturation during maintenance therapy is associated with more severe general and joint symptoms.

Methods: We performed a longitudinal cohort study of 266 individuals homozygous for the C282Y substitution in *HFE*, seen at a tertiary reference center in Rennes, France, and followed for 3 or more years after initial iron removal. Serum ferritin and transferrin saturation were measured at the same time points; values were used to calculate duration of exposure to serum ferritin 50 µg/l or more (FRT50exp) and to determine transferrin saturation 50% or greater (SAT50exp). Clinical and biochemical follow-up data were recorded from log books completed during maintenance therapy. The primary outcome was change in general and joint symptoms, determined from answers to a self-administered questionnaire.

Results: Patients were followed for 13.5±5.9 years. FRT50exp (3.2±3.5 years) and SAT50exp (4.5±3.4 years) values correlated ($r=0.38$; $P<.0001$), but each associated with different variables in multivariate analysis. We found independent associations, regardless of follow-up time, between SAT50exp ≥6 years and worsened joint symptoms (odds ratio [OR], 4.19; 95% CI, 1.88–9.31), as well as between SAT50exp ≥6 years and decreased athletic ability (OR, 2.35; 95% CI, 1.16–4.73). SAT50exp ≥8 years associated independently with decreased work ability (OR, 3.20; 95% CI, 1.40–7.30) and decreased libido (OR, 3.49; 95% CI, 1.56–7.80).

Conclusion: In a longitudinal study of patients treated for hemochromatosis, we associated duration of exposure to increased transferrin saturation (longer than 6 years) with more severe general and joint symptoms. Maintenance of serum levels of ferritin at 50 µg/l or less does not indicate control of transferrin saturation, so guidelines on the management of hemochromatosis require revision.

KEY WORDS: arthropathy, iron regulation, hepcidin deficiency, treatment

INTRODUCTION

The p.Cys282Tyr (C282Y) substitution on the *HFE* gene is the main mutation associated with hemochromatosis. At the homozygous state, it is responsible for an inappropriately low production of hepcidin, the hormone regulating systemic iron, which results in increased serum iron levels and then in elevated transferrin saturation, the biochemical hallmark of the disease ¹. Increased transferrin saturation is directly related to the degree of hepcidin deficiency irrespective of total body iron stores, whilst increased serum ferritin levels inform on the amount of iron stored in excess. Once transferrin saturation exceeds 50%, nontransferrin bound iron (NTBI), a toxic form of iron, occurs. NTBI is avidly taken up by parenchymal cells and accounts for the constitution of iron excess in the liver, heart and pancreas ². It is known to enhance the production of reactive oxygen species which, in turn, leads to the damage of lipids, proteins and DNA ². Thus, NTBI is thought to be involved in the occurrence and/or the worsening of iron-related symptoms.

The treatment of hemochromatosis relies upon regular phlebotomies. All guidelines ³⁻⁶ agree to treat patients as soon as serum ferritin levels exceed the upper normal limit. They recommend to obtain and then to maintain “low body iron stores”. However, in the absence of evidence-based data, the definition of “low body iron stores” remains vague consisting of serum ferritin levels $\leq 50 \mu\text{g/l}$ ^{4,5}, comprised between 50 and 100 $\mu\text{g/l}$ ³ or “within the normal range” ⁶. Determination of transferrin saturation is not considered for the monitoring of phlebotomy therapy and no precise data are currently available on the evolution of transferrin saturation in patients on long-term maintenance therapy.

Even when recommended serum ferritin levels are achieved by maintenance therapy, patients remain at risk of developing or worsening general symptoms ⁷ and joint ⁸ symptoms, which greatly affects their quality of life ⁹. We hypothesized that this could be, at least partly, related to sustained exposure to NTBI through long-term elevated transferrin saturation.

The present study was designed (i) to precisely measure the respective variations of serum ferritin and transferrin saturation levels in a large sample of patients on maintenance

therapy and (ii) to determine whether the persistence of elevated transferrin saturation was associated with an increase in general and joint symptoms.

PATIENTS AND METHODS

Inclusion criteria. C282Y homozygotes were identified from LOGIFER, a database launched in 1989 and prospectively documented since then. Patients were eligible when fulfilling the following criteria: (i) age \geq 18 years at diagnosis, (ii) follow-up \geq 3 years after the achievement of low body iron stores defined as the time when serum ferritin was first measured $< 50 \mu\text{g/l}$ and/or the weekly or bimonthly rhythm of phlebotomy therapy was abandoned, (iii) availability of follow-up data (date and volume of phlebotomies, serum ferritin, transferrin saturation and hemoglobin) including one paired measurement of both transferrin saturation and serum ferritin per year at least, and (iv) last vital status as “alive” in December 2010¹⁰.

Data collected

Data recorded at the time of diagnosis were extracted from LOGIFER and included year, sex, age, proband / relative status, living area, alcohol consumption (non excessive [$< 30 \text{ g / day}$ in males and $< 20 \text{ g / day}$ in females] or excessive), tobacco smoking (non smoker or former/current smoker), increased blood pressure (antihypertensive therapy or blood pressure $> 140/90 \text{ mmHg}$), body mass index (BMI – kg/m^2), diabetes (defined according to WHO classification), transferrin saturation, and serum iron, transferrin, ferritin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gammaglutamyl transferase (GGT), and hemoglobin. Hepatic fibrosis was staged on liver biopsy according to the METAVIR scoring system¹¹ from 0 (no fibrosis) to 4 (definite cirrhosis), “severe fibrosis” referring to F3 and F4 stages. According to Guyader et al¹², in the absence of liver biopsy, patients with serum ferritin $< 1000 \mu\text{g/l}$ were considered free of severe fibrosis.

Data recorded at the achievement of low body iron stores were extracted from LOGIFER and from logbooks previously given to patients at the time of diagnosis. They included serum

iron, ferritin and hemoglobin, transferrin saturation, and the number of grams of iron removed calculated as the number of liters of blood removed divided by 2.

Data recorded at inclusion were gathered from self-administered questionnaires and from patient logbooks. Questionnaires included the following questions regarding the period of maintenance therapy: Do you consider that your sport ability, your work ability, your libido decreased, improved or is stable? Do you consider that pain in your joints (shoulders, elbows, wrists, metacarpophalangeal and proximal interphalangeal (MCP-PIP) joints, knees, ankles, and tarsus and metatarsophalangeal joints) decreased, improved or is stable? To answer these questions, patients were guided by a schematic representation of joints. Other questions were: Have you experienced joint replacement? Have you been diagnosed with cancer (hepatic / extra-hepatic), diabetes or cardiovascular disease? What is your current weight? Dates and volumes of phlebotomies and corresponding values of transferrin saturation and serum iron, ferritin and hemoglobin measured on samples drawn just before phlebotomies were collected from logbooks.

Calculation of exposure duration to transferrin saturation $\geq 50\%$ (SAT50exp) and to serum ferritin $\geq 50 \mu\text{g/l}$ (FRT50exp). Curves of all available results of paired transferrin saturation and serum ferritin values were drawn according to time. SAT50exp and FRT50exp since the first achievement of low body iron stores were calculated as the number of years during which the curve had been above the transferrin saturation threshold of 50% and the serum ferritin level of 50 $\mu\text{g/l}$ respectively.

Statistical analysis.

First, SAT50exp and FRT50exp were analyzed as continuous variables. Then, due to the detection of threshold effects, they were transformed into categorical variables using ROC curve analysis to determine their best cut-off value in predicting symptoms. All univariate and multivariate analyses were adjusted on follow-up duration. Logistic models were used to identify initial and follow-up variables independently associated with clinical outcomes as reported by questionnaires and to identify the variables independently associated with SAT50exp and to FRT50exp among those collected at the time of diagnosis and at the initial

achievement of low body iron stores. All variables for which statistical significance was < 0.2 were introduced into models. P values < 0.05 were considered statistically significant. Quantitative variables were expressed as mean \pm standard deviation (SD) and qualitative variables as n (%). Statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC)

LOGIFER database was declared at the CNIL authority (National Committee for Informatics and Freedom). The study was authorized by the ethics committee of Rennes (n° 11-27). All patients gave informed written consent.

RESULTS

Study population

From the 1932 C282Y homozygous patients prospectively recorded in LOGIFER at the date of study, 497 (25.7%) fulfilled eligibility criteria. Of these, 209 (42%) either no longer resided at the indicated address (n = 47) or did not respond (n = 162). Among the 288 (58%) responders, 22 were not included because of an insufficient number of transferrin saturation measurements. Finally, 266 / 497 persons (53.5%) were available for analysis. They had been on maintenance therapy for 13.5 ± 5.9 years. Their main characteristics are presented in table 1. With respect to initial clinical and biochemical presentation, the 162 patients who received but did not complete the questionnaire did not significantly differ from responders (Table 1).

Relationship between paired serum ferritin and transferrin saturation levels

A total of 7125 paired measurements of serum ferritin and transferrin saturation had been performed during follow-up, which corresponded to a mean number of 27.2 ± 15 samples per patient (range: 4 – 90) and to a mean number of 2.6 ± 9 samples per patient per year. When considering the threshold of 50 for the two tests, both were low in 49% of samples, and increased transferrin saturation was found in 41% of samples irrespective of serum ferritin levels and in 26% of samples with low serum ferritin levels.

Relationship between FRT50exp and SAT50exp, and symptoms

The best cut-off values for the prediction of symptoms was 2.5 years with respect to FRT50exp and either 6 or 8 years with respect to SAT50exp (Table 2). As shown in table 3, a significant association was found between increased joint symptoms and SAT50exp \geq 6 years, decreased libido and SAT50exp \geq 8 years, and decreased work ability and both FRT50exp \geq 2.5 years and SAT50exp \geq 8 years. There was only a trend for an association between decreased sport ability and SAT50exp \geq 6 years.

To assess whether SAT50exp and FRT50exp were independently associated with general and joint symptoms, regression analysis was performed for each symptom associated with SAT50exp / FRT50exp at $p < 0.20$ at univariate analysis after adjustment for follow-up duration. All variables collected at diagnosis and at the achievement of low body iron stores, the duration and location of follow-up and answers to questionnaires were introduced into models together with SAT50exp and FRT50exp, when associated to SAT50exp / FRT50exp at $p < 0.20$. SAT50exp remained significantly associated with all symptoms and FRT50exp with decreased work ability only (Table 4).

The same analyses were applied to exposure duration to transferrin saturation \geq 75% (SAT75exp). The best cut-off value was 8 months. An independent association was found between SAT75exp and all clinical outcomes as was seen for SAT50exp (data not shown).

Relationship between FRT50exp and SAT50exp

Mean FRT50exp was 3.2 ± 3.5 years and mean SAT50exp was 4.5 ± 3.4 years. As shown in figure 1, they were positively but weakly correlated ($r = 0.38$ [95% CI, 0.28-0.48]; $p < 0.0001$). Mean SAT50exp was 2.4 ± 2.3 years in the 34 patients with serum ferritin levels constantly $< 50 \mu\text{g/l}$ and then progressively increased parallel to FRT50exp (Figure 1).

Explanatory factors of FRT50exp and SAT50exp

To identify, among variables collected at diagnosis, at the achievement of low body iron stores and during follow-up, those associated with FRT50exp and SAT50exp, logistic regression analysis was performed. As indicated in table 5, FRT50exp and SAT50exp were not explained by the same independent variables, except for follow-up duration (positive association) and BMI (positive association for FRT50exp and negative association for SAT50exp). Neither FRT50exp nor SAT50exp depended on the number of measurements of serum ferritin and transferrin saturation per year.

DISCUSSION

The present study is the first to document the course of both serum ferritin and transferrin saturation levels in a large series of C282Y homozygotes on long-term maintenance therapy. Despite the fact there was an overall correlation between transferrin saturation and serum ferritin, the two variables could not be considered as collinear, i.e. one could not be deduced from the other. The same was true for SAT50exp and FRT50exp. SAT50exp was found to be elevated (2.4 ± 2.3 years) in the 34 patients with serum ferritin levels constantly $< 50 \mu\text{g/l}$ and steadily increased parallel to FRT50exp (Figure 1). Moreover, multivariate analyses indicated that FRT50exp and SAT50exp were not explained by the same variables (Table 5). After adjustment for follow-up duration, SAT50exp was negatively associated with BMI and positively associated with tobacco smoking and older age at diagnosis. Decrease in serum transferrin levels, a common feature in hemochromatosis, is related to persisting down-regulation of transferrin synthesis by iron stores. Its extent depends on the rs3811647 polymorphism in the transferrin gene which was identified as a significant modifier of iron metabolism in hemochromatosis¹³. Overweight was shown to be associated with lower transferrin saturation, especially in female C282Y homozygotes¹⁴, possibly through the production of hepcidin by visceral adipose tissue^{15, 16}. Tobacco-related chronic hypoxia could explain increased transferrin saturation due to the negative effect of hypoxia-inducible factors on hepcidin synthesis¹⁷. After adjustment for follow-up duration, FRT50exp was positively associated with BMI, serum ferritin level at the end of initial achievement of low body iron stores and follow-up outside the Liver Unit. The last two variables likely reflect different therapeutic habits. In contrast to SAT50exp, BMI was positively associated with FRT50exp. This is in line with the previous demonstration of increased serum ferritin levels in

insulin-resistance syndrome¹⁸ for which BMI is a surrogate marker. These findings demonstrate that, in patients on maintenance therapy, transferrin saturation and serum ferritin measurements provide different information and that low serum ferritin levels do not guarantee normalization of transferrin saturation.

The present study also showed that, unlike FRT50exp, SAT50exp was independently associated with all types of morbidity events, especially the worsening of MCP and PIP joint symptoms which are considered as the most specifically related to hemochromatosis⁸. This is a key issue since, nowadays, hemochromatosis much more impairs the functional outcome, through general and joint symptoms, than survival^{1,10}. Hemochromatosis arthropathy is only partly related to the severity of iron burden¹⁹, since it may develop even when serum ferritin levels are moderately increased²⁰. In addition, as previously stressed^{8, 21, 22} and confirmed by the present study, iron-related joint disease does not consistently respond to phlebotomy therapy. In the study from Sahinbegovic et al, only 13.6% of patients had improvement of their joint symptoms after iron removal²³. Even more, in a series of 18 regularly phlebotomized patients, Richette et al found a paradoxical increase of a marker of cartilage degradation, CTXII, which could even suggest a negative impact of iron removal on cartilage metabolism²⁴. Whether this could be related to persisting elevated transferrin saturation levels despite low serum ferritin levels as suggested by the present study deserves attention. Hemochromatosis rheumatism mimicks crystal arthropathies which are mediated by inflammasome activation²⁵ and responsive to Interleukine-1 inhibitor therapy²⁶. Recently, a link between NTBI and inflammasome was suggested by the demonstration of an activation of the NLRP3 inflammasome through the induction of the cellular labile iron pool by ferric ammonium citrate in human monocytes. This was associated with a dose dependent increase in interleukin-1 β and inhibited by iron chelation²⁷. Unfortunately, for technical reasons, specific markers such as NTBI and interleukins were not available in the present series, but should be part of forthcoming studies assessing the putative role of persistently elevated transferrin saturation in the clinical outcome of hemochromatosis patients on long-term maintenance therapy.

The present results strongly suggest that, if maintenance therapy is performed, long-term exposure to elevated levels of transferrin saturation is associated with an increased

occurrence of general and joint complications. This is not contrary to the hypothesis raised in the forthcoming Mi-Iron Melbourne study (Clinicaltrials.gov - NCT01631708) that it could be safer not to treat homozygotes with mild iron burden. Indeed, phlebotomies induce recurrent phases of iron hyper-absorption with increased transferrin saturation through the decrease of hepcidin production secondary to bursts of erythropoiesis. Therefore, it cannot be excluded that the cure could be worse than the disease in patients with mild iron burden.

Several issues about the present study have to be discussed. First, the study relied on a self-administered questionnaire, which forced us to resort to a few simple specific questions. All other data, including initial clinical presentation and biochemical follow-up data, were prospectively recorded from either LOGIFER or patient logbooks. Second, only 497 of 1932 patients (25.7%) were eligible. This was mainly explained by eligibility criteria including one paired measurement of serum ferritin and transferrin saturation per year at least. Requiring a higher number of transferrin saturation determinations per year would have been methodologically more relevant but would have resulted in a dramatic decrease of the population studied. Indeed, more and more patients have only serum ferritin determined because repeated transferrin saturation measurements are no longer reimbursed by the French national health system since 2000 and are not considered useful to manage therapeutic follow-up since the publication of the French⁴ and international^{3,5,6} guidelines. However, it is important to point out that FRT50exp and SAT50exp were not correlated with the number of measurements per year, but because they were, as logically expected, correlated with follow-up duration, all comparisons were made with adjustment on follow-up. Third, the marked biological variability of transferrin saturation could have introduced a bias. However, assessment of SAT50exp and FRT50exp relied on paired determinations made on samples drawn usually at the same time of day in a given subject, just before iron removal, and a long time after the previous phlebotomy, which should have likely minimized transferrin saturation variability. Fourth, the threshold of 50 µg/l for serum ferritin was chosen because it represents the ideal goal assigned to patients. That of 50% for transferrin saturation was proposed following the study of Loréal et al²⁸ who, in C282Y patients serially followed during phlebotomy therapy, showed that serum NTBI was found in almost all subjects when transferrin saturation exceeded 50% with a good correlation between transferrin saturation and NTBI levels. Similar data were found in patients with iron-loading

anæmias²⁹, especially myelodysplastic syndromes³⁰. Then, it can be assumed that most patients with continual SAT50exp were continuously exposed to NTBI. The threshold of 75% could also have been chosen in agreement with the study of Le Lan et al³¹ and with clinical and experimental data collected in iron-loading anæmias showing that labile plasma iron, the most redox active component of NTBI, occurs when transferrin saturation exceeds 75%^{29,30}, but this would have introduced too large a disequilibrium in sample size between the groups with short and long exposure to elevated transferrin saturation impairing statistical power. It is noteworthy that, when performing such an analysis, a similar association was found between SAT75exp and clinical outcomes. Interestingly, the best SAT75exp cut-off value was 8 months compared to 6 or 8 years for SAT50exp, which supports a dose-response relationship. Fifth, the response rate was 53%. This was not due to a high number of deaths since a study of the vital status of all subjects recorded in the database was performed one year before launching the present study¹⁰. A significant number of patients were lost to follow-up (47/497) and 162 did not respond. These patients did not differ from those who completed the questionnaire with respect to disease severity at diagnosis, to follow-up duration or to age at inclusion (Table 1). Then, even if the most worsened patients had been more prone to fill out questionnaires, this should have not influenced the association we found between the worsening of general and joint symptoms and SAT50exp.

CONCLUSION

The present study brings to light two original facts. First, it demonstrates that exposure duration to transferrin saturation $\geq 50\%$ is independently associated with an increased risk of worsening of general and joint symptoms regardless of follow-up duration and of exposure duration to serum ferritin, except for decreased work ability. Second, it clearly indicates that maintaining serum ferritin levels below the threshold of 50 $\mu\text{g/l}$ does not imply that transferrin saturation is under control. Because the current clinical outcome of treated hemochromatosis is mainly determined by general and joint symptoms, the present findings put into question the recommendations on the management of hemochromatosis.

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LEGENDS OF TABLES

Table 1. Main characteristics of the 266 patients who responded to questionnaire compared to the 162 who received it but did not respond.

Table 2. Best cut-off values of exposure duration to serum ferritin ≥ 50 $\mu\text{g/l}$ and to transferrin saturation $\geq 50\%$ in predicting general and joint symptoms as determined by ROC curve analysis.

Table 3. Frequency of general and joint symptoms according to exposure duration to serum ferritin ≥ 50 $\mu\text{g/l}$ (FRT50exp) and to transferrin saturation $\geq 50\%$ (SAT50exp) after adjustment on follow-up duration. CI = confidence interval. Bold characters show significance at $p < 0.05$.

Table 4. Variables independently associated with morbidity events.

Table 5. Variables independently associated with exposure duration to serum ferritin ≥ 50 $\mu\text{g/l}$ (FRT50exp) and with exposure duration to transferrin saturation $\geq 50\%$ (SAT50exp) at multivariate logistic regression analysis. * All at diagnosis unless specified.

LEGEND OF FIGURE

Figure 1 – Exposure duration to transferrin saturation $\geq 50\%$ (SAT50exp) as years according to exposure duration to serum ferritin ≥ 50 $\mu\text{g/l}$ (FRT50exp) as years (log) in the 266 C282Y homozygous patients.

Table 1

		Responders to questionnaire (n = 266)		Nonresponders to questionnaire (n = 162)		p
At the time of diagnosis						
Gender	Sex ratio [M/F]	1.31	[151/115]	1.03	[82/80]	p=0.14
Age (years)	mean ± sd [n tested]	44.4 ± 11.5	[266]	44.6 ± 14.1	[162]	p=0.86
Year of diagnosis	mean ± sd [n tested]	1997 ± 6	[266]	1998 ± 6	[162]	p=0.15
Body mass index (kg/m ²)	mean ± sd [n tested]	24.3 ± 3.3	[221]	24.1 ± 4.1	[133]	p=0.65
Chronic fatigue	% [n/n tested]	37.0%	[91/246]	41.5%	[63/152]	p=0.38
MCP and IPP joint symptoms	% [n/n tested]	16.5%	[33/200]	18.8%	[24/128]	p=0.60
Diabetes	% [n/n tested]	1.8%	[4/223]	2.9%	[4/137]	p=0.49
Severe fibrosis	% [n/n tested]	6.1%	[15/245]	10.7%	[16/150]	p=0.10
Serum ferritin (µg/l)	mean ± sd [n tested]	1067 ± 1259	[265]	1017 ± 1059	[162]	p=0.60
Transferrin saturation (%)	mean ± sd [n tested]	74.9 ± 17.3	[266]	75.2 ± 16.6	[162]	p=0.88
Iron removed	mean ± sd [n tested]	5.35 ± 4.85	[255]	5.45 ± 5.52	[154]	p=0.85
At the time of study						
Age (years)	mean ± sd [n tested]	59.2 ± 11.9	[266]	58.6 ± 14.4	[162]	p=0.63
Follow-up duration	mean ± sd [n tested]	13.4 ± 5.9	[266]	Not available		

Table 2

Symptoms	FRT50exp			SAT50exp		
	Cut-off (years)	Sensitivity*	Specificity*	Cut-off (years)	Sensitivity*	Specificity*
Increased joint symptoms	2.5	59 [53-66]	56 [41-70]	6	70 [64-76]	56 [41-70]
Decreased sport ability	2.5	65 [56-73]	52 [43-61]	6	42 [33-51]	70 [62-79]
Decreased work ability	2.5	67 [57-77]	55 [47-63]	8	64 [53-74]	56 [49-64]
Decreased libido	2.5	62 [53-72]	55 [46-63]	8	55 [46-65]	60 [52-68]

* % [95% confidence interval]

Table 3

	Increased joint symptoms		Decreased sport ability		Decreased work ability		Decreased libido	
	% (n/N)	Odd ratio * [95%CI]	% (n/N)	Odd ratio * [95%CI]	% (n/N)	Odd ratio * [95%CI]	% (n/N)	Odd ratio * [95%CI]
FRT50exp < 2.5 years	13.7 (20/146)	1	47.7 (62/130)	1	26.5 (36/136)	1	38.5 (52/135)	1
FRT50 exp ≥ 2.5 years	20.8 (25/120)	1.63 [0.85-3.12]	56.9 (62/109)	1.42 [0.85-2.38]	45.8 (49/107)	2.28 [1.32-3.91]	45.8 (49/107)	1.31 [0.78-2.20]
SAT50exp < 6 or < 8 years †	11.5 (21/182)	1	47.6 (78/164)	1	30.4 (62/204)	1	37.4 (76/203)	1
SAT50exp ≥ 6 or ≥ 8 years †	28.6 (24/84)	3.61 [1.71-7.66]	61.3 (46/75)	1.73 [0.95-3.16]	59.0 (23/39)	3.12 [1.47-6.63]	64.1 (25/39)	2.98 [1.38-6.46]
FRT50exp < 2.5 and SAT50exp < 6 or < 8 years †	10.6 (12/113)	1	43.6 (44/101)	1	24.8 (31/125)	1	34.7 (43/124)	1
FRT50exp ≥ 2.5 and SAT50exp < 6 or < 8 years †	13.0 (9/69)	1.27 [0.51-3.20]	54.0 (34/63)	1.52 [0.81-2.86]	39.2 (31/79)	1.96 [1.07-3.59]	41.8 (33/79)	1.35 [0.76-2.41]
FRT50exp < 2.5 and SAT50exp ≥ 6 or ≥ 8 years †	24.2 (8/33)	3.18 [1.10-9.18]	62.1 (18/29)	2.10 [0.87-5.06]	45.5 (5/11)	2.42 [0.67-8.70]	81.8 (9/11)	8.44 [1.70-41.8]
FRT50exp ≥ 2.5 and SAT50exp ≥ 6 or 8 ≥ years †	31.4 (16/51)	4.58 [1.82-11.5]	60.9 (28/46)	2.00 [0.94-4.23]	64.3 (18/28)	5.19 [2.07-13.0]	57.1 (16/28)	2.50 [1.03-6.10]

† The best cut-off value differed according to symptoms: 6 years for joint symptoms and decreased sport ability and 8 years for decreased work ability and decreased libido. * CI = confidence interval.

Variables independently associated with worsening of MCP-PIP joints (n = 45/266)

	Odd ratio [95%CI]	
Follow-up duration (years)	0.95	[0.88-1.01]
SAT50exp ≥ 6 years	4.19	[1.88-9.31]
Iron removed (g)	1.10	[1.04-1.17]

Variables independently associated with decreased sport ability (n = 124/239)

	Odd ratio [95%CI]	
Follow-up duration (years)	0.99	[0.94-1.04]
SAT50exp ≥ 6 years	2,05	[1.04-4.04]
Joint symptoms*	3,61	[1.62-8.05]
Body mass index > 27 kg/m²*	2,55	[1.34-4.86]
Cardiovascular disease*	3,70	[1.77-7.76]
Occurrence of cancer	5,57	[1.38-22.47]

Variables independently associated with decreased work ability (n = 85/243)

	Odd ratio [95%CI]	
Follow-up duration (years)	1.01	[0.96-1.07]
SATexp ≥ 8 years	3.20	[1.40-7.30]
FRTexp ≥ 2.5 years	1.91	[1.08-3.38]
Age at diagnosis > 60 years	3.40	[1.60-7.24]

Variables independently associated with decreased libido (n = 101/242)

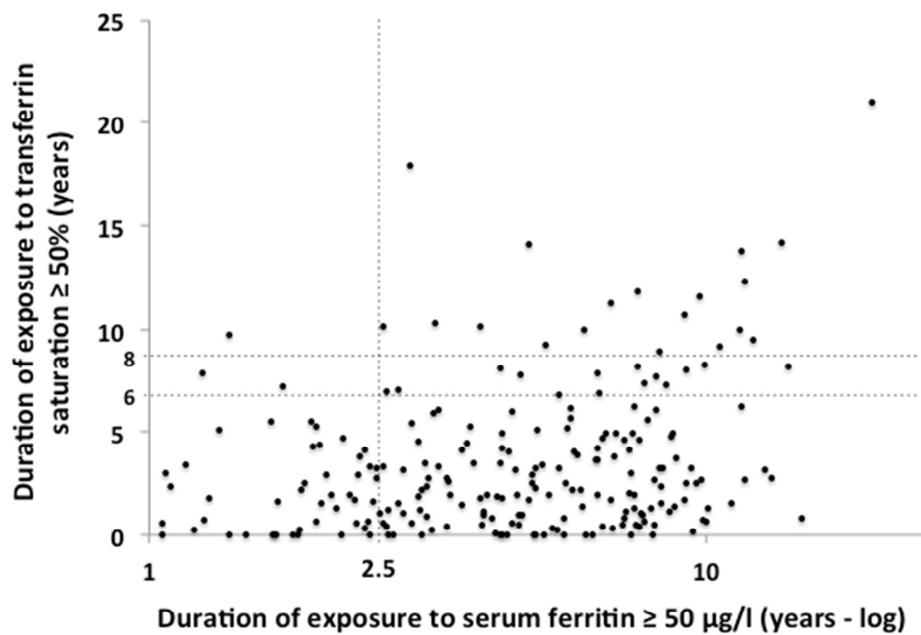
	Odd ratio [95%CI]	
Follow-up duration (years)	0.98	[0.93-1.03]
SAT50exp ≥ 8 years	3.49	[1.56-7.80]
Age at inclusion	3.02	[1.74-5.26]

* At the time of either diagnosis or inclusion

Table 5

Factors independently associated with FRT50exp ≥ 2.5 years (n=99/218)		
	n	Odd ratio [95% CI]
Follow-up duration (years)	218	1.04 [0.98-1.10]
Follow-up location		
Liver Unit	113	1
Elsewhere	105	1.97 [1.10-3.53]
Body mass index at diagnosis		
≤ 25 kg/m ²	140	1
> 25 kg/m ²	78	1.89 [1.05-3.40]
Serum ferritin at the end of initial treatment		
≤ 50 μ g/l	155	1
50-60 μ g/l	31	0.70 [0.31-1.61]
> 60 μ g/l	32	2.59 [1.14-5.88]
Factors independently associated with SAT50exp ≥ 6 years (n=84/234) *		
	n	Odd ratio [95% CI]
Follow-up duration (years)	234	1.24 [1.15-1.34]
Transferrin saturation at the end of initial treatment		
$\leq 45\%$	111	1
$> 45\%$	28	6.88 [2.17-21.8]
Missing	95	1.66 [0.72-3.83]
FRT50exp		
< 2.5 years	130	1
≥ 2.5 years	104	4.11 [1.90-8.90]
Age at diagnosis		
18-45 years	127	1
≥ 46 years	107	2.55 [1.14-5.68]
Tabacco smoking at diagnosis		
Non smoker	170	1
Current Smoker	64	2.26 [1.04-4.91]
Serum transferrin at diagnosis		
< 1.70 g/l	72	1
≥ 1.70 g/l	162	4.86 [2.03-11.7]
Body Mass Index at diagnosis		
< 22 kg/m ²	68	5.10 [1.87-13.9]
22-25 kg/m ²	85	1.21 [0.48-3.10]
> 25 kg/m ²	81	1

* The same factors were associated with SAT50exp ≥ 8 years except for BMI and serum transferrin at diagnosis.



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