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Title: Regression of Fibrosis Stage With Treatment Reduces Long-Term Risk of Liver Cancer in Patients With Hemochromatosis Caused by Mutation in HFE

Short Title: Fibrosis regression and primary liver cancer

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Abbreviations:

HC: Hemochromatosis

ALAT: Alanine aminotransferase

GGT: Gamma-glutamyltransferase

HCC: Hepatocellular Carcinoma

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Abstract

Background & Aims: Fibrosis stage can decrease following treatment in patients with hemochromatosis caused by mutations in the homeostatic iron regulator gene (HFE), but the effects on cirrhosis are not clear. We assessed regression of severe fibrosis and the ensuing risk of liver cancer after treatment.

Methods: We performed a retrospective analysis of data from 106 patients in France or Australia who were homozygous for the C282Y mutation in HFE with F3 fibrosis (n=40) or F4 fibrosis (n=66) at diagnosis and from whom at least 1 liver biopsy was collected during follow up. We collected data from the time of first biopsy and during follow-up period on patient demographics, treatment, smoking habits, alcohol consumption, infection with hepatitis B or C viruses, and other diseases. The median time between first and last liver biopsy was 9.5 years (range, 3.5–15.6 years). We collected results of tests for liver function, markers of iron stores, and platelet levels. patients were followed for a median 17.6 years (range, 9.8–24.1 years) for development of liver cancer occurrence.

Results: At last liver biopsy, 41 patients (38.6%) had fibrosis scores of F2 or less. Liver cancer occurred in 34 patients (52.3%) with F3 or F4 fibrosis at last liver biopsy vs 2 patients (4.8%) patients with fibrosis scores of F2 or less at last liver biopsy ($P<.001$). Liver cancer incidences were 32.8 per 1000 person-years (95% CI, 22.7–45.9 per 1000 person-years) in patients with F3 or F4 fibrosis and 2.3 per 1000 person-years (95% CI, 0.2–8.6 per 1000 person-years) in patients with fibrosis scores of F2 or less ($P<.001$). In multivariate analysis, male sex (hazard ratio [HR], 6.09; 95% CI, 1.21–30.4), age at diagnosis (HR, 1.16; 85% CI, 1.09–1.25), presence of diabetes (HR, 3.07; 95% CI, 1.35–6.97), excess alcohol consumption (HR, 3.1; 95% CI, 1.47–6.35), serum level of ferritin at diagnosis ($P<.01$), and regression to fibrosis scores of F2 or less (HR, 0.08; 95% CI, 0.01–0.62) were significantly associated with risk of liver cancer.

Conclusions: In a retrospective analysis of patients with hemochromatosis caused by the C282Y mutation in HFE, we found that severe liver fibrosis can regress with treatment. In patients with fibrosis regression to a stage F2 or less, the long-term risk for liver cancer is significantly reduced.

KEY WORDS: HCC, iron overload, outcome, hepatocellular carcinoma

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Introduction

Liver fibrosis is the hallmark of chronic liver disease, ultimately leading to cirrhosis and its complications. The advent of efficient therapy for liver disease has provided evidence for the regression of liver fibrosis in large numbers of patients with chronic hepatitis C and B ^{1,2}. Resolution of liver fibrosis has also been described in patients with HFE hemochromatosis ^{3,4}, autoimmune hepatitis ⁵, primary biliary cholangitis ⁶ and, non-alcoholic fatty liver disease ⁷. Cirrhosis encompasses not only fibrosis, but also architectural and vascular changes. Thus, its reversibility remains difficult to ascertain on the sole observation of fibrosis regression, and debate persists about reversal of cirrhosis ^{8,9}.

Due to the long-standing availability of an efficient therapy, HFE C282Y hemochromatosis (HC) is a perfect model to assess the long-term course of fibrosis after the removal of the causative agent of hepatic damage. Indeed, the severity of hepatic fibrosis is related to the severity of iron burden ¹⁰ and, without additional factors of liver disease, the normalization of iron stores stops liver damage ^{11,12}, and, over time, has a beneficial effect on survival ^{13,14}.

The regression of fibrosis in long-term treated HC patients has been described but results are conflicting in patients with severe liver fibrosis ¹⁵. In a previous study, Falize et al showed that fibrosis regressed in most patients, even those with cirrhosis ³. Conversely, Powell et al, found that fibrosis regressed in all patients that were pre-cirrhotic, but not in those with cirrhosis at diagnosis ⁴. Both studies suffered from a relatively low number of patients with severe fibrosis, which, combined with sampling and interpretation bias of liver biopsy, makes it difficult to draw definitive conclusion.

As in most cirrhotic liver diseases, primary liver cancer (hepatocellular carcinoma (HCC) and cholangiocarcinoma) is a major cause of death in patients with HC complicated by severe fibrosis. In this study, we investigated fibrosis stage regression and the ensuing risk of primary liver cancer development in patients with HFE hemochromatosis on long-term maintenance therapy.

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Patients and method

Inclusion criteria

Patients included in the databases of the University Hospital of Rennes, France and the QIMR Berghofer Medical Research Institute, Brisbane, Australia who fulfilled the following inclusion criteria were selected: homozygosity for the *HFE* C282Y mutation, liver biopsy at diagnosis showing severe fibrosis or cirrhosis, defined as F3 or F4 fibrosis stage, respectively, according to the METAVIR or Scheuer staging systems, and availability of, at least, one additional histological assessment during follow-up. Patients with a diagnosis of liver cancer within 1 year of initial diagnosis were excluded.

Data recorded

The following clinical data were recorded at the time of the first biopsy and during follow-up: age, gender, weight, smoking habits, alcohol consumption (excessive drinking was defined as >21 standard drinks per week in men and >14 in women), presence of viral hepatitis B or C infection, high blood pressure, diabetes or osteoarthritis. The date of the last clinical follow-up was recorded.

Biochemical data collected were liver function tests, markers of iron stores (serum ferritin, transferrin saturation), and platelets. Initial liver samples were obtained at the time of diagnosis by percutaneous needle biopsy. Liver samples were obtained during follow-up either by percutaneous liver biopsy for clinically indicated reasons, or during a surgical procedure or transplantation.

Histological examinations were performed by one expert liver pathologist in each center. Fibrosis was assessed according to the METAVIR (France) or SCHEUER (Australia) grading system. liver biopsy deemed as inappropriate for histological assessment at the time of sampling were not considered. Because almost all liver specimens had been destroyed, central blinded reading of samples was not performed.

All cases of hepatocellular carcinoma and cholangiocarcinoma occurring during follow-up were recorded. Liver cancer diagnosis was based either on radiological findings according to guidelines at the time of HCC diagnosis, or on histological assessment.

Statistical analysis

Qualitative and quantitative data are reported as number (percentage) and median [interquartile range], respectively. To assess factors associated with fibrosis regression, univariate logistic regression was first performed. Then clinically relevant variables and variables with a p value lower than 0.2 were included in multivariate logistic regression with stepwise backward selection according to the likelihood ratio.

Kaplan Meier analysis and Log Rank test were performed to assess the incidence of primary liver cancer according to the regression of fibrosis to a stage \leq F2.

To assess factors associated with liver cancer, univariate Cox proportional hazard analysis was performed. Then, clinically relevant variables and variables with a p value lower than 0.2 were included in a multivariate Cox proportional hazard model with stepwise backward selection according to the likelihood ratio. To avoid potential lead time bias and because the definite date of commencement of fibrosis regression was unknown, we used fibrosis regression as a time-dependent covariate with the date of the last liver biopsy as turning on of exposure in patients with fibrosis regression. Quantitative variables were transformed into qualitative variables (tertile) for logistic regression and Cox model analysis.

Person-year incidence rate and their 95% confidence interval (CI) were determined and compared by Poisson distribution using MedCalc Statistical Software version 18.2.1 (MedCalc Software bvba, Ostend, Belgium).

For both Cox regression analysis and Person-year incidence rate, the start of the time interval was the time of the first liver biopsy.

A p value of <0.05 was considered as significant. Statistical analyses were performed using IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, N.Y., USA)

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Results

Population characteristics

Of the 112 HFE C282Y homozygous patients with severe (F3-F4) liver biopsy-proven fibrosis at diagnosis and at least one follow-up liver biopsy, five were excluded because of liver cancer within the first year of diagnosis and one because of liver cancer before the last liver biopsy. The final cohort thus included 106 patients. Patient characteristics at diagnosis are described in Table 1. Two patients had associated chronic hepatitis C. Most patients (n=98(92.5%)) were successfully venesected and exhibited normal body iron stores during follow-up according to serum ferritin. Treatment data were lacking for the remaining 8(7.5%) patients.

The main reasons for repeated liver biopsy were fibrosis evaluation (n=53(50%)) (half of them to clarify fibrosis stage because of borderline cirrhosis at diagnosis), suspected liver tumor (n=17(16%)), surgical resection or liver transplantation (n=13(12%)), associated chronic liver disease (n=4(3%)), and unknown causes (n=19(17%)).

Regression of liver fibrosis

Fibrosis stage improved in 44(41.5%) patients after a median time of 9.5[3.5-15.6] years between the first and last liver biopsy. At the last liver biopsy, 61.3% of patients had F3F4 fibrosis, and 41(38.6%) had a stage \leq F2 fibrosis.

Among the 66 patients with cirrhosis at diagnosis (Table 2), cirrhosis was still found in the last biopsy in 51(77.3%) and fibrosis stage decreased in 15(22.7%) with regression to stage \leq F2 in 12(18.2%). Of the 40 patients with F3 fibrosis at diagnosis, 29(72.5%) had fibrosis regression and 2(5%) worsened to F4. Fibrosis stage regression to \leq F2 was more likely to occur in F3 patients ($p<0.001$).

In F3-F4 patients, univariate logistic regression showed that age at diagnosis, diabetes, serum ferritin, ALAT, and GGT were associated with fibrosis regression. Multivariate logistic regression analysis showed that older age at diagnosis (OR:0.9(0.88-0.96) $p=0.015$), presence of diabetes (OR:0.22(0.06-0.77) $p=0.019$) and higher GGT (OR:0.15(0.03-0.66) $p=0.012$) were all significantly and negatively associated with fibrosis regression to stage \leq F2.

Occurrence of liver cancer

During a median follow-up duration of 17[9.8-23.5] years, liver cancer occurred in 36(34%) patients (Table 3). Of these, 3 had F3 fibrosis and 33 had cirrhosis at diagnosis. At the time of the follow-up liver biopsy, 30(83.3%) still had cirrhosis, 4 had F3 fibrosis and 2 had F2 fibrosis. Kaplan Meier analysis of primary liver cancer incidence according to regression of liver fibrosis is showed in Figure 1. Cholangiocarcinoma was diagnosed in 7 patients, and combined HCC cholangiocarcinoma in three patients.

Two patients with F2 fibrosis at last liver biopsy had primary liver cancer. One had liver cancer ten years after the first liver biopsy. He had chronic excessive

alcohol consumption without other known causes of liver disease. He underwent right hepatectomy (diagnostic liver biopsy initially described HCC) yielding a large tissue sample (20x15x13cm, diagnosis of cholangiocarcinoma was then established). At that time he had normal body iron stores but increased GGT (311UI/L). The second patient had HCC diagnosed 38 years after the initial diagnosis. He was overweight (BMI 27, waist circumference 103cm), and at that time had normal body iron stores. He underwent surgical resection (8x5x3cm of liver was resected with an HCC of 3.5cm).

Thus, liver cancer occurred in 34(52.3%) patients with F3F4 fibrosis at last liver biopsy compared to 2(4.8%) patients with fibrosis \leq F2 ($p < 0,001$). The incidence rates in patients with F3F4 and \leq F2 fibrosis stage at last liver biopsy were 32.8(95%CI:22.7-45.9) and 2.3(95%CI:0.2-8.6) per 1000 person-years, respectively ($p < 0.001$). Among the 25 patients who had F0 or F1 fibrosis at their last liver biopsy, none had liver cancer during a median follow-up of 19.1[14.8-25.2] years.

Of the patients who had F4 fibrosis at diagnosis, liver cancer occurred in 32(56.1%) patients with F3F4 fibrosis at last liver biopsy versus 1(7.1%) patients with fibrosis \leq F2 at the last biopsy ($p < 0.01$). Incidence rates were respectively 37.2(95CI:25.5-52.7) and 4.1(95%CI: 0.05-22.9) per 1000 person-years ($p < 0.01$).

Because of the potential indication bias induced by follow-up liver biopsy performed for the diagnosis or treatment of HCC, we performed a subgroup

analysis restricted to patients for whom follow-up liver biopsy was performed for fibrosis assessment or non-liver-related surgery. Fifty nine patients (of whom 32 had F4 fibrosis at diagnosis) with a median follow-up of 19.1[14.8-24.3] years were included in this subgroup analysis. Fibrosis regression to a stage \leq F2 occurred in 32(54.2%) patients (including 9 who had F4 fibrosis at diagnosis). Primary liver cancer occurred in 8 of the 27 patients with F3F4 fibrosis at last liver biopsy and in none of the 32 patients with fibrosis \leq F2 at last liver biopsy. Incidence rates were respectively 14.8(95%CI: 6.4-29.3) and 0(95%CI: 0.0-5.8) per 1000 person years (p=0.002).

Risk of liver cancer and fibrosis regression

In univariate Cox regression analysis, age at diagnosis, sex, excessive alcohol consumption, diabetes, serum ferritin, GGT, fibrosis stage at diagnosis, and fibrosis regression to a stage \leq F2 at the last liver biopsy were associated with liver cancer. Using multivariate analysis (Table 4), regression of fibrosis to a stage \leq F2 was associated with a significant reduction of liver cancer risk (HR:0.081[0.011-0.623]).

Discussion

The present study, based on a large population with sequential liver biopsy and long term follow-up, demonstrates that, in HFE hemochromatosis, fibrosis can regress even in patients with severe fibrosis or cirrhosis at diagnosis. Moreover, it indicates that regression of severe fibrosis is associated with a striking reduction in the risk of liver cancer compared to patients without fibrosis regression. Because severe hepatic fibrosis and its inherent cancer risk are hallmarks of chronic liver disease irrespective of its underlying cause, our results may be extended to other liver diseases.

Although it is well documented for mild-moderate stages of fibrosis, the regression of fibrosis remains controversial for established cirrhosis ¹⁶. In hemochromatosis, severe fibrosis and cirrhosis regression has been documented histologically in some case reports ^{15,17} and in a retrospective French study of 36 patients.³ The small numbers of patients studied reflects the fact that follow-up liver biopsy are rarely performed in patients after treatment. Combining the databases from two large centers and extending the inclusion period allowed us to study a larger population over an extended period, with a long time (9.5[3.5-15.6] years) between the first and last liver biopsy. Hemochromatosis is a unique disorder due to its curability by a life-long treatment, allowing a prolonged clinical follow-up. This is not the case in other curable chronic liver diseases ^{18,19}, which limits their usefulness for observing the long-term process of fibrosis regression ⁸.

The main pitfalls that foster controversy about the regression of cirrhosis are the sampling variability of liver biopsy and the poor inter- and intra-observer reproducibility in distinguishing severe fibrosis from cirrhosis.²⁰ This is particularly so after fibrosis regression, when the parenchyma regenerates and the fibrous septa become progressively finer and resorb.⁸ The present study does not escape this limitation. Unfortunately, central double-blinded reading of liver biopsy was not available due to the destruction of many biopsy blocks for exceeding the legal retention limit or for determining hepatic iron concentration. These are the reason why fibrosis stages were considered in 2 groups (F0-F2 versus F3-F4) to study fibrosis regression.

The present results are consistent with data from studies in other liver diseases. First, rates of cirrhosis and fibrosis regression were much higher than would be expected from sampling bias. In our study, fibrosis regressed to \leq F2 fibrosis in 18.2% of patients with cirrhosis and in 72.5% of patients with F3 fibrosis. This is much higher than the 9.7% discrepancy rate found between F0-F2 and F3-F4 stages in a large study in HCV.²¹ Second, the rate of cirrhosis regression was comparable to that observed in a large study of follow-up liver biopsy in patients with cirrhosis related to various causes where, among 113 patients with cirrhosis, 14(12.4%) had cirrhosis regression between the first and follow-up biopsies 4.3 \pm 2.1 years apart²². The slightly higher rate of fibrosis regression found in our study might be due to a longer time interval between liver biopsy. Third, factors that we found associated with the regression of fibrosis were consistent with literature data. Younger age at diagnosis suggests that more recent fibrosis is more likely to regress than long standing fibrosis.²³ A normal

GGT can be considered as a surrogate marker of the absence of an ongoing liver disease ²⁴. The contribution of diabetes to the severity of liver fibrosis is well documented ²⁵, including in HC ²⁶. This suggests that factors associated with fibrosis regression actually reflect the absence of other liver insults, thus permitting liver wound healing following removal of iron via venesection. ⁸

Nevertheless, sampling variability may still cast doubt on the observed regression of severe fibrosis. Due to the lack of large autopsy studies in patients with treated HC, the next best method to investigate the potential for F3F4 fibrosis to regress is to compare pathological data to a hard clinical endpoint. To this end, we chose to focus on liver cancer which is the hallmark of severe liver fibrosis and is relevant to any chronic liver disease.⁹ Interestingly, the risk of liver cancer in patients with fibrosis regression to \leq F2 was strikingly low, which is consistent with the fibrosis regression hypothesis. Moreover, the liver cancer rate of 32.8(95%CI:22.7-45.9) per 1000 person-years for patients without fibrosis regression was similar to that observed in other studies. In a large cohort, the incidence rates of hepatocellular carcinoma were 24 and 36 per 1000 person-years in patients with cirrhosis due to chronic hepatitis C and B, respectively. ²⁷ Our results are also in line with the 14.4% 5-year cumulative incidence of primary liver cancer in a large prospective cohort of patients with hepatitis C-related cirrhosis in France. ²⁸ No data are available to determine the relative contributions of virus eradication and fibrosis regression on the reduction of liver cancer incidence after virus eradication in patients with cirrhosis related to hepatitis C ^{28, 29}. In HCV patients with a sustained viral response, the liver cancer incidence rates found by Cardoso et al. (12.4 per 1000

person-years) and Nahon et al. (6.7% 5-year cumulative incidence) were much higher than the incidence rate found in our study (2.3(95%CI: 0.2-8.6) per 1000 person-year).^{29 28} This suggests that patients with fibrosis regression to \leq F2 correspond to a specific subpopulation in which the risk of liver cancer is much lower than in those for which the cause of cirrhosis has been removed. This is supported by multivariate analysis showing that even after adjustment for other risk factors of liver cancer, fibrosis regression to \leq F2 was associated with a 90% reduction of liver cancer risk.

Overall our results show that in patients with severe fibrosis or cirrhosis at diagnosis, fibrosis regression is associated with a marked decrease in liver cancer risk, allowing us to conclude that the regression of fibrosis stage observed is not due to pathological assessment bias.

One potential bias in this study is the reason for follow-up biopsy, which may lead to overestimate the incidence of cancer in the setting of HCC or suspected HCC. To address this, we isolated a subgroup for whom follow-up liver biopsy was performed in the absence of HCC or suspected HCC. Interestingly, a similar major reduction of cancer incidence was found among patients with fibrosis regression. This strongly reinforces the conclusion that regression of fibrosis is associated with a much lower risk of cancer, if any. Indeed, it is unclear whether the 2 cases of liver cancer after fibrosis regression reported in our study correspond to a true, albeit minimal, residual risk of liver cancer related to persisting vascular and architectural changes despite fibrosis regression, or whether they relate to the misdiagnosis of persisting cirrhosis in these patients.

Cirrhosis encompasses both fibrosis and architectural changes. It was not possible to adequately address the question of whether the reduced incidence of primary liver cancer is not only due to fibrosis regression but also to the improvement of architectural change. This remains an important question as architectural changes may play a role in carcinogenesis. However, the pathological features associated with treatment-induced architectural change during follow-up are poorly characterized and there is no scoring/staging system available for its assessment.

Because primary liver cancer incidence in patients with fibrosis regression falls below cost efficacy cut-offs for screening, our results advocate for fibrosis assessment after eradication of the underlying liver disease in patients with severe fibrosis at diagnosis. However the time required for adequate assessment remains to be determined. Because liver biopsy is an invasive procedure, non-invasive tests are likely to be the most relevant means of assessing liver fibrosis regression during follow-up. However, there are very few data regarding the use of noninvasive tests in HC and we do not have sufficient data in our study cohort that would be relevant to the follow-up of patients. Therefore liver biopsy remains the gold standard to assess the presence of cirrhosis and more studies are required before recommendations can be made on their use to assess fibrosis regression and guide the clinical management of patients.

In conclusion our study shows that severe liver fibrosis can regress to milder stage after efficient treatment of HC, and that fibrosis regression to a stage \leq F2 is associated with a major reduction of the long-term liver cancer risk. These

results raise two questions concerning the management of patients with severe liver fibrosis at diagnosis who are successfully treated. Firstly, when and by what means, is it best to assess fibrosis regression in these patients? Secondly, is cancer screening still relevant in these patients? While the results presented in this study clearly demonstrate a reduced risk of liver cancer with fibrosis regression, the retrospective design did not enable us to answer these two outstanding questions definitively. Screening for HCC in patients in which fibrosis has regressed should be continued, although its relevance could be discussed on a case-by-case basis according to the associated HCC risk factors.

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Figure Legend

Figure 1

Primary Liver Cancer incidence according to fibrosis stage at last liver biopsy. Kaplan Meier analysis of primary liver cancer incidence, according to fibrosis stage at last liver biopsy (stage \leq F2: plain line; stage F3F4: dashed line). Follow-up was limited to 35 years because of the low number of patients at risk afterward.

Tables

Table 1.**Characteristics of patients.**

Results are expressed as N(%) or Median[interquartile range]

<i>At diagnosis</i>	<i>Total</i>	<i>≤F2 at last biopsy</i>	<i>F3F4 at last biopsy</i>
Age (year)	46[39-52]	41[36-48]	48[43-53]
Sex Male/Female	94(88.7) / 12(11.3)	36(87.8)/5(12.2)	58(89.2)/7(10.8)
Alcohol excess Yes/No	38(36.2) / 67(63.8)	12(29.3)/29(70.7)	26(40.6)/38(59.4)
Diabetes Yes/No/Missing	33(31.1) / 67(63.2) / 6(5.7)	4(9.8)/34(82.9)/3(7.3)	29(44.6)/33(50.8)/3(4.6)
Body Mass Index (kg/m ²)	24.2 [22.6-26.3]	24.1 [22.3-27.0]	24.2 [22.6-26.0]
Transferrin Saturation (%)	87 [80.9-95.0]	86 [81.0-90.7]	87.3 [80.8-97.0]
Serum ferritin (µg/L)	2940 [2005-4090]	2139 [1700-3690]	3094 [2310-4378]
ASAT (IU/L)	50 [39-70]	47.5 [37.15-59.0]	56.5 [40.0-78.0]
ALAT (IU/L)	80 [54-104]	80.0 [55.5-121.0]	79.0 [50.0-99.0]
GGT (IU/L)	44 [30-87]	33.0 [29.0-74.0]	56.1 [34.0-110.0]
Fibrosis F3/F4	40(37.7) / 66(62.3)	29(70)/12(29.3)	11(16.9)/54(83.1)
<i>Follow-up</i>			
Duration (years)	17.6 [9.8-24.1]	19.4 [16.0-25.2]	15.2 [9.1-20.6]
Time between first and last liver biopsy (years)	9.5 [3.5-15.6]	10.0[5.7-14.7]	9.1 [3.3-15.5]

Table 2.
Fibrosis stage at last follow-up liver biopsy according to the initial fibrosis stage. Results are presented as N (%)

<i>Last fibrosis stage</i>	<i>Initial fibrosis stage</i>		
	F3	F4	Total
F0	6 (15.0%)	1 (1.5%)	7 (6.6%)
F1	14 (35.0%)	4 (6.1%)	18 (17.0%)
F2	9 (22.5%)	7 (10.6%)	16 (15.1%)
F3	9 (22.5%)	3 (4.5%)	12 (11.3%)
F4	2 (5.0%)	51 (77.3%)	53 (50%)

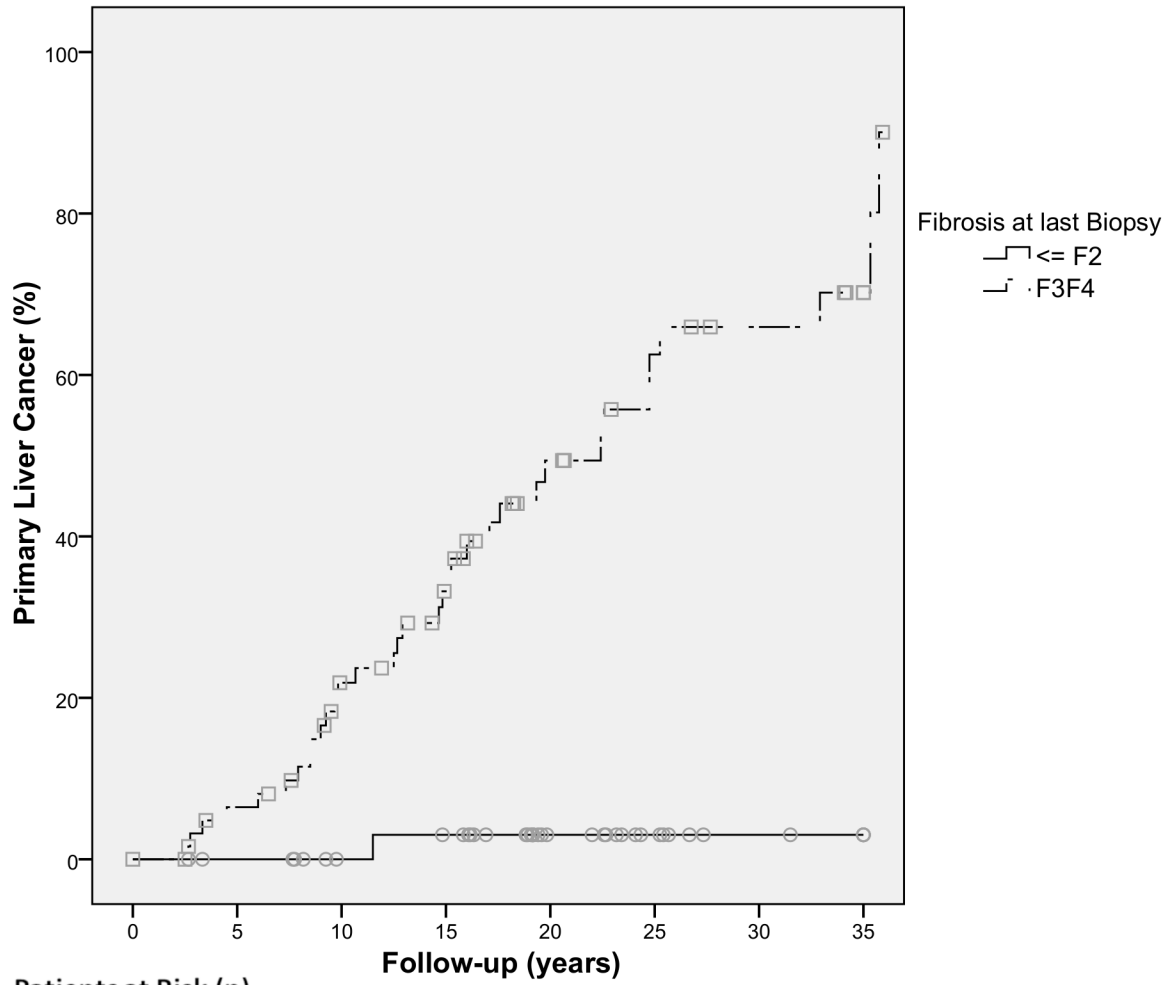
Table 3.
Number of primary liver cancers according to the final fibrosis stage. Results are presented as N(%)

<i>Fibrosis stage last liver biopsy</i>	<i>Liver Cancer</i>	<i>No Liver Cancer</i>
F0 (n=7)	0 (0.0%)	7 (100%)
F1 (n=18)	0 (0.0%)	18 (100%)
F2 (n=16)	2 (12.5%)	14 (87.5%)
F3 (n=12)	4 (33.3%)	8 (66.7%)
F4 (n=53)	30 (56.6%)	23 (43.4%)
Total (n=106)	36 (34%)	70 (66%)

Table 4.**Risks factors for liver cancer.**

Variables associated with liver cancer in univariate analysis or clinically relevant, were included in a multivariate Cox regression analysis. Fibrosis regression was included as a time-dependent covariate. * at diagnosis

	<i>Hazard Ratio</i>	<i>95% Confidence Interval</i>	<i>p</i>
<i>Sex (Male)</i>	6.09	1.21-30.4	0.028
<i>Age* (Years)</i>	1.16	1.09-1.25	<0.001
<i>Alcohol* (Yes/No)</i>	3.10	1.47-6.35	0.003
<i>Diabetes* (No)</i>			0.026
<i>Yes</i>	3.07	1.35-6.97	0.007
<i>Missing</i>	1.77	0.34-9.22	0.49
<i>Ferritin* (<2300 µg/L)</i>			0.004
<i>2300-3600 µg/l</i>	11.23	3.00-41.99	<0.001
<i>> 3600 µg/L</i>	4.29	1.30-14.10	0.01
<i>Missing</i>	3.64	0.75-17.66	0.10
<i>Regression to ≤F2 fibrosis stage</i>	0.081	0.011-0.623	0.016



Patients at Risk (n)

≤F2	40	38	33	31	17	10	5	4
F3F4	65	57	43	33	19	11	8	5

Need to Know

Background: Fibrosis can decrease with treatment in patients with hemochromatosis caused by mutations in the homeostatic iron regulator gene (*HFE*). We assessed regression of severe fibrosis and the risk of liver cancer after treatment.

Findings: In a retrospective analysis of patients with hemochromatosis caused by the C282Y mutation in *HFE*, we found that severe liver fibrosis can regress with treatment. Fibrosis regression to a stage F2 or less significantly reduces the long-term risk for liver cancer.

Implications for patient care: Our findings support long-term assessment of liver fibrosis after treatment.

Journal Pre-proof