



HEPATITIS C VIRUS INFECTION, IRON METABOLISM, LIVER FIBROSIS AND RESPONSE TO DIRECT ACTING ANTIVIRALS (DAAS)

Joana Ferreira^{1,2}, Paula Faustino^{2,3}, Manuel Bicho^{1,2}, Fátima Serejo^{2,4}

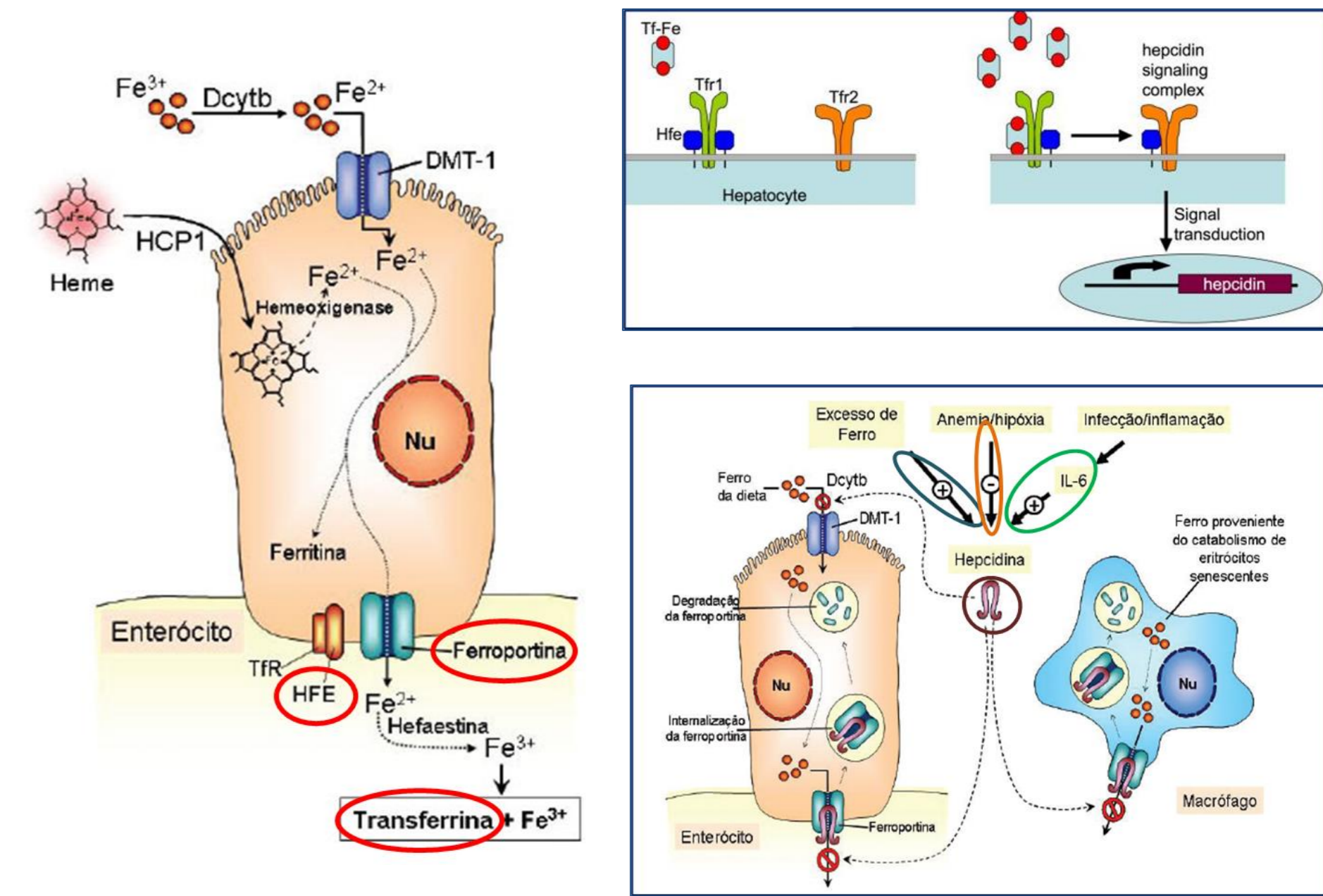
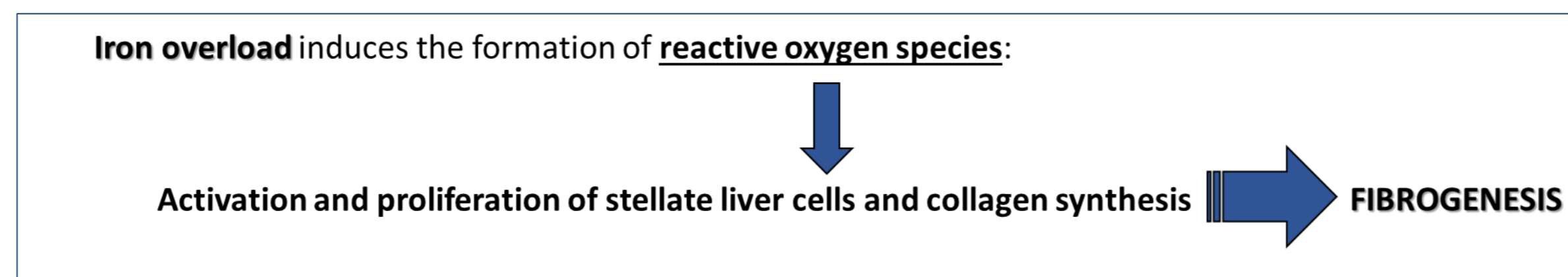
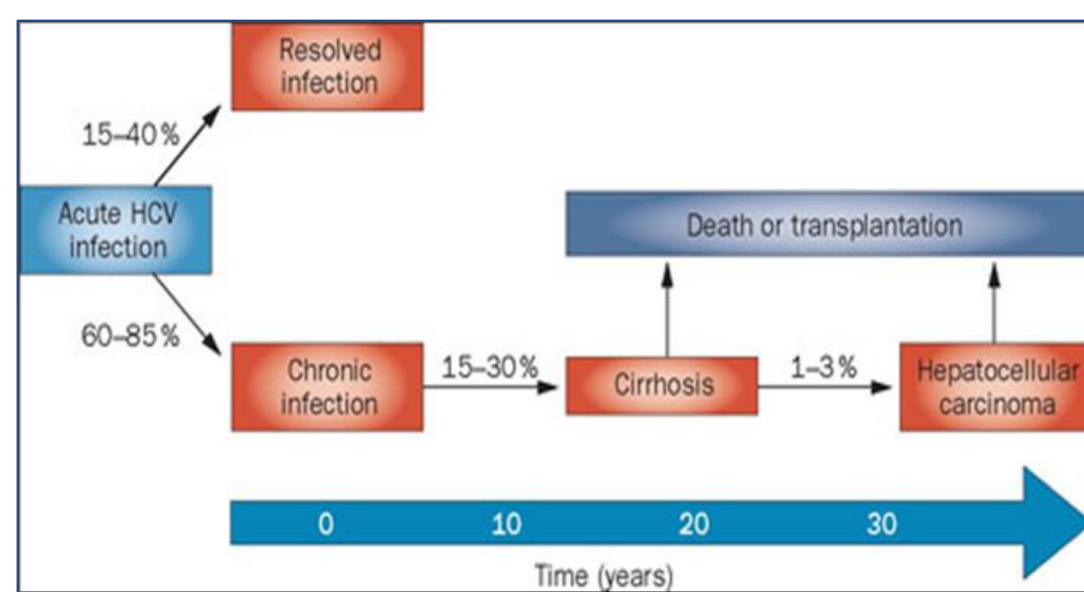


1Institute for Scientific Research Bento Rocha Cabral; 2ISAMB, Genetics Laboratory, Lisbon Medical School, University of Lisbon, 3National Institute of Health Dr. Ricardo Jorge, Human Genetics Department, 4Gastroenterology and Hepatology Department, Hospital de Santa Maria, CHLN.



Introduction and Aims

Chronic Hepatitis C (CHC) is characterized by hepatic and extra-hepatic manifestation. Among hepatic manifestations, liver fibrosis is probably the most significant as it can lead to cirrhosis, hepatocellular carcinoma and death. Iron overload is one of the extra-hepatic manifestations induced by Hepatitis C Virus (HCV) infection. It can induce fibrosis-promoting signals in the parenchymal and non-parenchymal cells as well as the formation of reactive oxygen species, which accelerate disease progression and exacerbate liver pathology.



Aims

- Evaluate changes in iron metabolism induced by HCV elimination.
- Study the association of liver fibrosis with biochemical and genetic parameters of iron metabolism before and after DAAs treatment.
- Identify baseline parameters that could predict the improvement of liver fibrosis after DAAs treatment.

Results

A. Comparison of biochemical parameters before and after DAAs treatment

- Patients showed lower values of Fe, TIBC and FT and higher values of Hp after treatment.

Parameter	Before DAAs treatment		After DAAs treatment		p value*
	Median	[min-max]	Median	[min-max]	
Fe (µg/dl)	119	[36-254]	111	[32-288]	0.003
TIBC (µg/dl)	324	[119-479]	317	[90-471]	0.012
TS (%)	38	[5-325]	34	[8-110]	0.135
FT (µg/dl)	201	[8-1263]	132	[9-834]	<0.001
Hp (mg/dl)	107	[3-241]	116	[7-294]	<0.001

*Two related samples tests

B. Association of biochemical parameters with fibrosis stage before and after DAAs treatment

- Before treatment:** Patients F3/4 showed higher values of Fe, TIBC, TS and FT and lower values of Hp comparing to those F1/2, before and after treatment.

Parameter (before DAAs treatment)	F _{1/2} (before DAAs treatment)		F _{3/4} (before DAAs treatment)		p value*
	Median	[min-max]	Median	[min-max]	
Fe (µg/dl)	111	[36-349]	137	[30-317]	<0.001
TIBC (µg/dl)	318	[67-490]	340	[114-496]	0.002
TS (%)	36	[5-325]	40	[8-109]	0.028
FT (µg/dl)	157	[8-774]	257	[12-2479]	<0.001
Hp (mg/dl)	110	[8-241]	30	[3-195]	<0.001

*Mann-Whitney Test

- After treatment:** Patients F3/4 showed higher baseline and after treatment values of Fe, TIBC and FT and lower baseline values of Hp comparing to those F1/2.

Parameter	F _{1/2} (after DAAs treatment)		F _{3/4} (after DAAs treatment)		p value*
	Median	[min-max]	Median	[min-max]	
Fe (µg/dl) _{before}	116	[36-288]	136	[40-279]	0.008
Fe (µg/dl) _{after}	106	[29-264]	119	[34-288]	0.014
TIBC (µg/dl) _{before}	321	[67-479]	339	[119-479]	0.031
TIBC (µg/dl) _{after}	307	[90-843]	335	[134-512]	0.011
TS (%) _{before}	37	[5-325]	42	[8-92]	0.112
TS (%) _{after}	34	[8-110]	36	[10-107]	0.222
FT (µg/dl) _{before}	167	[8-1263]	290	[12-911]	<0.001
FT (µg/dl) _{after}	118	[9-832]	148	[13-5390]	0.024
Hp (mg/dl) _{before}	111	[3-241]	83	[7-216]	0.004
Hp (mg/dl) _{after}	116	[7-360]	99	[7-258]	0.080

*Mann-Whitney Test

Conclusions

- Antiviral treatment with DAAs seems to decrease iron overload associated to HCV infection.
- Biochemical parameters and polymorphism of iron metabolism seems to be associated with liver fibrosis before and after treatment as well as with the improvement of liver fibrosis or with the regression of cirrhosis after antiviral treatment.

Materials and Methods

- 329 patients with CHC (age: 49.93 [45.57-50.28] years; 124 female and 205 male). 134 treated with DAAs with sustained response (age: 53.42 [51.47-55.36]; 58 female and 56 male).
- Liver fibrosis stage was assessed by transient elastography using a Fibrosan and patients were divided in two groups (F1/2_mild and moderate fibrosis and F3/4_severe fibrosis and cirrhosis).
- Biochemical parameters of iron metabolism (Total Iron_Fe, Total Iron Binding Capacity_TIBC, Transferrin Saturation_TS, Ferritin_FT and Haptoglobin_Hp) were evaluated by standard methods.
- Genetic polymorphisms within Transferrin_TF, HFE, Bone Morphogenetic Protein 2_BMP2, Hepcidin_HAMP and Ferroportin_SLC40A1 genes were analyzed by PCR, Sequencing or NGS.
- Statistical analysis was performed using SPSS 26.0 for windows.

C. Association of baseline biochemical parameters with the improvement of fibrosis stage after DAAs treatment

- Within DAAs treated patients, 16.1% showed an improvement of liver fibrosis as they pass from higher fibrosis stages (F3 or F4) to lower fibrosis stages (F1 or F2).
- Comparing baseline biochemical parameters of those who improved liver fibrosis with those who did not, we found increased values of Haptoglobin (Hp) for the first ones

Parameter (before DAAs treatment)	F _{3/4} (before DAAs treatment) to F _{1/2} (after DAAs treatment)		F _{3/4} (before DAAs treatment) to F _{3/4} (after DAAs treatment)		p value*
	Median	[min-max]	Median	[min-max]	
Hp (mg/dl)	114	[3-108]	77	[7-195]	0.039

*Mann-Whitney Test

D. Association genetic polymorphisms with the biochemical parameters before and after DAAs treatment

- Before treatment:**
 - Higher values of Fe and TS were found in heterozygous CY for HFE_C282Y.
 - TIBC was higher in patients carrying allele A (GA or AA) of TF_rs3811647.
 - FT was higher in patients' heterozygous CY for HFE_C282Y and in those carrying allele T (TT or TA) of BMP2_rs235768.
- After treatment:**
 - Fe and TS were found higher in carriers of allele D (HD or DD) of HFE_H63D.
 - FT was higher in patients carrying allele C (GC or CC) of SLC40A1_rs1439816.
 - Higher values of TIBC were associated with seven of the studied SLC40A1 polymorphisms: rs75162690_GG, rs73980217_CC, rs4508635_TT, rs111803199_GG, rs73980218_TT, rs1439816_GG and rs11568351_GC or CC.

E. Association of genetic polymorphisms with fibrosis stage before and after DAAs treatment and with the improvement of fibrosis stage after DAAs treatment

- Before and after treatment:**
 - Patients with GG genotype of SLC40A1_rs1439816 have 3.937 and 4.271 risk, respectively, for being F3/4.
 - Patients with GC or CC genotypes of SLC40A1_rs13008848 had 10.000 risk of staying cirrhotic after treatment.