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Clinical aspects of hemochromatosis

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Abstract

Hemochromatosis is one of the most frequent genetic diseases among the white populations, affecting one in three hundred persons. Its diagnosis has been radically transformed by the discovery of the HFE gene. In a given individual, the diagnosis can, from now on, be ascertained on the sole association of a plasma transferrin saturation (TS) over 45% and homozygosity for the C282Y mutation. Liver biopsy is only required to search for cirrhosis whenever there is hepatomegaly and/or serum ferritin >1000 ng/ml and/or elevated serum AST. Family screening is mandatory, primarily centered on the siblings. The treatment remains based on venesection therapy which improves many features of the disease (one of the most refractory, however, being the joint signs) and permits normal life expectancy provided the diagnosis is established prior to the development of cirrhosis or of insulin-dependent diabetes. In view of the prevalence, the non-invasive diagnosis, the spontaneous severity and the efficacy of a very simple therapy, hemochromatosis should benefit from population screening. This screening could be based, first, on the assessment of transferrin saturation, followed – when elevated – by the search for the C282Y mutation. The discovery of the HFE gene has also paved the road for the individualization of other types of iron overload syndromes which are not HFE-related.

Keywords: Hemochromatosis; HFE gene; Cirrhosis; Venesection

1. Introduction

Hemochromatosis [1] is one of the most frequent genetic diseases in the Caucasian populations, affecting approximately one in three hundred persons of Northern European descent [2]. The discovery in 1996 [3] of the HFE gene has very rapidly provided a genetic test which has radically transformed the diagnostic strategy of the disease, whereas the treatment of hemochromatosis remains based on venesections.

2. The diagnosis of hemochromatosis

Three main situations should be considered: the diagnosis of hemochromatosis in an individual, the diagnosis among family members at risk for hemochromatosis, and finding individuals with hemochromatosis in a population. These situations will be elaborated.

2.1. The individual diagnosis

The diagnostic strategy rests upon the following three successive steps [4].

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2.1.1. First step: to evoke the diagnosis from miscellaneous clinical presentations

2.1.1.1. Diagnosis and typical clinical picture. This evocation is easy when facing the typical clinical picture of the disease – a middle-aged man presenting more or less associated: (i) Diffuse melanoderma, more often metallic grey than brown. (ii) Hepatomegaly. The liver is markedly enlarged, firm and sharp to palpation, and despite its cirrhotic features this hepatomegaly is accompanied neither by signs of hepatocellular insufficiency (no palmar erythema, no spider nevi, no bruises, normal prothrombin time) nor by signs of portal hypertension. (iii) Diabetes mellitus, often requiring insulin. When this classical triad of "bronzed cirrhosis with diabetes" is present, the diagnosis is immediately evoked, especially if there are also signs of cardiomyopathy. However, such a picture corresponds to a stage of irreversible complications compromising of vital prognosis, so that establishing the diagnosis at this stage is far too late and must be considered today as a diagnostic failure.

2.1.1.2. Early diagnosis of hemochromatosis. Therefore, it is essential to pay attention to other possibilities of phenotypic expression of hemochromatosis, hopefully allowing an earlier diagnosis. It should be first remembered that women can be as severely affected as men [5]. Such overt forms may be found in young adults as well as in elderly women [6].

Three types of features, which can be summarized as the "rule of three A's", correspond usually to earlier signs: (i) *Asthenia.* Unexplained chronic fatigue, with sometimes a sexual component in males, can be the only feature of the disease and, paradoxically, iron overload is sometimes discovered while thinking rather of iron deficiency. (ii) *Arthralgia.* Arthropathy is an often misdiagnosed presenting feature of hemochromatosis, the diagnostic delay being estimated between 4 and 10 yr. The most characterisric expression is chronic arthritis of the second and third metacarpophalangeal joints, resulting in a "painful handshake", a symptom which should be highly suggestive of the disease. Other joints can also be affected, especially wrists and knees. Patients can also suffer from attacks of pseudo-gout (by pyrophosphate arthropathy). Radiologically, the most common changes are subchondral arthropathy and chondrocalcinosis. Arthropathy greatly affects the quality of life in hemochromatosis. (iii) *Aminotransferase* (*transaminase*) *increase*. One should keep in mind that any hypertransaminasemia, less than three times the upper normal limit, which is not related to alcohol, non-alcoholic steato-hepatitis (NASH), virus, auto-immunity or drugs may reflect hepatic iron overload. Among other possible, although non-specific, features of hemochromatosis: ichthyosis and nail abnormalities, especially on the first three digits, such as platonychia or true koilonychia, the latter sign being rather paradoxical since it is also present as part of the chronic iron deficiency syndrome.

2.1.2. Second step: to ascertain biochemical abnormalities of iron metabolism

The key point in the diagnosis of hereditary hemochromatosis is to check the serum transferrin saturation (TS).

Normal transferrin saturation rules out hemochromatosis. It is admitted that normal TS levels (i.e., $<45\%$) exclude the iron overload related to hemochromatosis, provided a coexisting inflammatory syndrome (as reflected for instance by increased serum CRP) is not confounding the interpretation. However, a normal transferrin saturation remains compatible with the presence of two types of non-hemochromatosis-related iron overload syndromes: (i) One is frequent – corresponding to the recently described insulin resistance–associated liver siderosis [7]. This syndrome, also named dysmetabolic hepatosiderosis, corresponds to mild or moderate iron excess occurring especially in men with features of insulin resistance (increased body mass index, diabetes, hyperlipidemia). TS is normal, contrasting with high levels of serum ferritin [8]. This situation is often mistaken for hemochromatosis and today represents certainly one of the most confounding clinical settings. (ii) The other condition is exceptional and corresponds to hereditary aceruloplasminemia [9]. This disease is due to a mutation in the ceruloplasmin gene located on chromosome 3. It mimics hemochromatosis in that it is familial and can be

associated with major hepatocytic iron overload and diabetes mellitus. However, besides the low TS value, two main arguments orientate towards this disease: (i) The contrast, quite unexpected in the absence of an inflammatory syndrome, between on the one hand low TS (and sometimes anemia) and on the other hand marked hyperferritinemia. (ii) The neurological "atmosphere" (extrapyramidal syndrome, cerebellar ataxia, dementia) which is never present in hemochromatosis. The proof of the diagnosis rests upon an undetectable serum concentration of serum ceruloplasmin.

Increased TS reflects the basic metabolic abnormality of hemochromatosis and is acknowledged as the most sensitive single test for phenotypic identification of the disease. Edwards and Kushner [10] have shown that TS is usually above 60% in men and 50% in women. Furthermore, TS remains high throughout the day [11] in those patients. However, although a sensitive marker, elevated TS is not specific of hemochromatosis. It is found in other iron overload syndromes of hematological origin or sideroblastic anemia, the major mechanisms accounting for iron excess being dyserythropoiesis and/or transfusions. But, under these conditions, the key differential data as compared to hemochromatosis are the presence of chronic anemia. TS can also be elevated in the absence of any iron excess in case of hepatic cytolysis (as expressed by increased levels of serum transaminases) especially when associated with hepatic failure (resulting in decreased transferrin synthesis) and with excessive alcohol consumption.

In summary, when the clinical data suggest hemochromatosis, a normal serum TS value excludes the diagnosis provided CRP is normal, and an increased TS is highly indicative of the disease provided recent excessive alcohol consumption is absent, and hemoglobin concentration, transaminase levels and prothrombin time are normal.

2.1.3. Third step: to prove hereditary hemochromatosis

This confirmative pathway is based today on a single blood test searching for the HFE mutation C282Y. Three situations occur: (a) the patient is C282Y +/+ (he/she is homozygous for this mutation); (b) the patient is C282Y +/− (he/she is heterozygous for the mutation); or (c) the patient, despite a phenotypic picture of hemochromatosis, is C282Y −/−.

2.1.3.1. The patient is C282Y +/+ (he/she is homozygous for this mutation). Then, hemochromatosis is ascertained and no further investigations are needed to confirm the diagnosis. At this point, the problem is to start a work-up in order to evaluate the degree of iron overload and the possible visceral and/or metabolic consequences of the disease.

For evaluating iron excess, two main investigations are valuable: (i) One is biochemical and widely accessible – the concentration of serum ferritin which, in hemochromatosis, is in good correlation with the total iron burden. Several precautions must, however, guide the interpretation of serum ferritin values: (i) The risk for overestimating iron overload by some confounding factors such as inflammation, cytolysis or a dysmetabolic syndrome. (ii) The risk for underestimation whenever the data are interpreted only by reference to the upper normal limits of this parameter. Indeed, there is a wide range of normal values (usually 10–300 μg/l) and the pitfall is for instance to consider a normal level of 200 μg/l in a young adult woman whereas the normal expected value approximates 30! A multicenter American study [12] reported that the median serum ferritin concentration increased from 23 μg/l for age 12–16 yr and reached a plateau of 120–130 μg/l after 32 yr. In women, values were in the range of 30 μg/l until menopause after which values rose to approximately 80 μg/l. (ii) The other investigation to quantify iron excess non-invasively is hepatic magnetic resonance imaging (MRI) provided it is accessible and adequately calibrated for this purpose. This technique permits the determination of a reliable "MRI hepatic iron concentration" [13].

For evaluating the visceral and/or metabolic consequences, a general work-up is engaged including especially serum transaminases, glucose studies and, depending on the clinical context, electro-echocardiogram, joint and bone X-rays, and hormonal tests. In fact, one of the major difficulties for the clinician is to decide whether a liver biopsy should be performed or not in order to

appreciate the possible development of severe fibrosis. This decision constitutes a real dilemma in so far as on one hand liver biopsy remains an invasive procedure which should not be performed if the probability for fibrosis is only minimal and on the other hand it is essential not to miss marked hepatic fibrosis (grade 3 -bridging fibrosis- or grade 4 -cirrhosis) due to its high risk for subsequent hepatocellular carcinoma development. Guyader et al. [14] have recently helped to define the criteria which, in practice, allow in a C282Y +/+ patient to avoid a liver biopsy because they mean the absence of risk for hepatic fibrosis. These criteria are the absence of hepatomegaly and normal serum aspartate aminotransferase and serum ferritin <1000 μg/l.

In summary, when, in a given patient with increased transferrin saturation, the HFE mutation C282Y +/+ is found, the diagnosis of hemochromatosis is established. A liver biopsy is only performed in case of suspicion of fibrosis, namely from a prognostic view and no more for a diagnostic purpose.

2.1.3.2. The patient is C282Y +/− (he/she is heterozygous for the mutation). The most likely genetic status is compound heterozygosity which corresponds to various genotypic situations: (i) The commonest expected genotypic profile is C282Y/H63D compound heterozygosity (the patient is C282Y +/− and H63D +/−). In this setting, however, iron overload remains generally mild so that possible associated co-factors of iron overload must always be searched for (such as excessive alcohol consumption, dysmetabolic hepatosiderosis or porphyria cutanea tarda) [15]. (ii) Exceptionally, other profiles of compound heterozygosity (i.e., not involving H63D), which for most of them still belong to the field of clinical research, might be involved: IVS3 + 1G,→,T [16], which has been reported in association with severe phenotypic expression of hemochromatosis; the S65C mutation [17] associated with a mild phenotype; the I105T mutation [18].

In practice, when iron overload is associated with heterozygosity for the C282Y mutation, it is reasonable to search for the H63D mutation, but even if compound heterozygoty is found, one must not forget to look for possible co-factors of iron overload. It should also be kept in mind that simplex heterozygosity for C282Y (i.e., C282Y +/−and H63D −/−) is in the large majority of the cases unable to explain by itself the development of iron excess and that other factors need to be found.

2.1.3.3. The patient, despite a phenotypic picture of hemochromatosis, is C282Y −/−. Two situations are then possible: (i) Juvenile hemochromatosis. This exceptional disease is now recognized as being genetically independent of hemochromatosis since the causal mutation is located on chromosome 1 [19]. The diagnosis should be evoked in subjects less than 30 yr of age and with heart failure and/or endocrine disorders (hypogonadotropic hypogonadism). (ii) Inherited non-HFE-related hemochromatosis, an entity especially described in Italian families [20,21], with the possibility, however, that some of the reported cases may in fact be related to the juvenile form.

In practice, whenever there is a strong suspicion of pronounced iron overload and that the patient is not C282Y +/+, one must remain a ''clinician'' and resort to a liver biopsy for a *diagnostic* purpose, accordingly to the ''pre-HFE'' diagnostic strategy. Indeed, in this type of situation, hepatic histology is essential in many diagnostic aspects: (i) It confirms iron overload. (ii) It identifies its predominantly periportal and hepatocytic distribution. (iii) It provides a semiquantitative evaluation of iron excess using a special grading system [22]. (iv) It permits the determination of hepatic iron concentration (HIC), which is closely correlated with the level of iron stores, [23] and can be performed in deparaffinized liver biopsy specimens [24]. Furthermore, when related to the age of the patient, it is possible to determine the hepatic iron index (ratio HIC/age) which, prior to the HFE era, was highly suggestive of homozygous hemochromatosis [25] when >1.9 provided other kinds of iron overload (especially of hematological origin) had been excluded. (v) Finally, liver biopsy is able to detect associated lesions (steatosis for instance).

2.2. Family diagnosis

The preventive strategy has been considerably modified and simplified by HFE testing. Starting from a C282Y proband, it is now possible to evaluate "immediately" the hemochromatosis risk among the family members.

Schematically: (i) C282Y +/+ subjects are homozygous for the HFE gene and either already expressing the disease or are at high risk of developing it. (ii) C282Y +/−individuals are heterozygous for the HFE gene. They will not develop the disease but can transmit the gene to their offspring.

This schematic interpretation needs, however, to be modulated in three main aspects:

(i) The first aspect concerns the risk of clinical expression, i.e., penetrance of C282Y homozygosity. It is increasingly acknowledged that a significant proportion of these individuals will never develop problematical iron overload throughout their life. A recent Australian study [26] reported that only half of the homozygous subjects had clinical features of the disease and that one quarter maintained normal serum ferritin values over a 4 yr follow-up period.

(ii) The second issue relates to the putative risk of heterozygosity: (a) As to the heterozygote individual himself (herself), the risk for developing iron excess seems absent or limited to compound heterozygosity (confined, in clinical practice, to the profile C282Y +/− and H63D +/−) and/or to the presence of co-factors such as alcoholism or dysmetabolic features. Whether heterozygosity for the HFE gene confers upon HFE individuals an increased risk to develop cancer [27] or cardiovascular disease [28,29] requires further confirmation. (b) Concerning his (her) offspring, the risk of homozygosity does exist despite the fact that hemochromatosis is a recessive disease. Indeed, due to the high prevalence of the HFE gene in the general population, the probability for an heterozygote to marry another heterozygote is approximately 10%. It is therefore important to inform the family of this possibility, in a "smooth and positive" way.

(iii) The third comment relates to young offspring. In so far as no treatment is indicated during infancy and adolescence, specific screening has no justification. It can be proposed to perform a phenotypic evaluation (including clinical examination, serum transferrin saturation and ferritin) at 15 yr of age and to postpone genetic testing at 18. It is, however, sometimes difficult to resist the parental pressure to "know" about their children's status. An indirect way to cope with this demand is to perform the genetic test in the spouse. If the spouse does not carry the C282Y mutation (and provided, for the father, that there is no problem of "biological" paternity) it can be deduced that the maximal genetic risk for the offsprings is only heterozygosity for the HFE gene.

2.3. Population diagnosis

Several arguments can be put forward in favor of general screening in Caucasian populations: (i) The high frequency of the disease. (ii) Its severity both in terms of morbidity and mortality. (iii) The possibility, from now on, of establishing the diagnosis on the basis of non-invasive investigations. (iv) The efficacy and simplicity of the treatment (venesections), which not only improves the quality of life but restores normal life expectancy provided the diagnosis is sufficiently early in the course of the disease.

The screening strategy could be based on the assessment of serum TS in adults aged 18 or more. Then, genetic testing for C282Y would be confined to individuals with transferrin saturation >45%. This strategy would avoid the ethical, logistic, and financial problems raised by systematic genetic testing as well as the societal impact of discovering a genetic mutation in asymptomatic persons without a disease. It is in fact essential that major changes occur in the psychological perception of unexpressed or slightly expressed HFE homozygosity, especially by insurers and health care administrators, in order to avoid any adverse genetic discrimination.

3. Treatment of hemochromatosis [30]

Beside the symptomatic treatment of visceral and metabolic complications for the disease, which

will not be reviewed here due to their lack of specificity, the major curative challenge is to eliminate iron excess.

3.1. Admitted data

Low iron diet is generally considered as useless since a 1 yr diet is equivalent to only two or three venesections. However, supplemental iron and supplemental vitamin C (which increases intestinal absorption of iron) are contraindicated. Tea (which decreases iron absorption) may be beneficial [31].

Chelation therapy is reserved for rare contraindications to venesection such as anaemia, hepatocellular insufficiency, or associated general disease (e.g., arteriosclerosis). It is then based on prolonged subcutaneous desferrioxamine infusion. The putative place of oral chelators such as hydroxypyridinones remains to be defined [32]. Erythrocytapheresis may be an interesting method in those cases contraindicating venesections [33].

Venesection therapy represents, of course, the ideal means to eliminate iron overload, according to a two-phase protocol.

1. The initial phase consists of one 400–500 ml venesection per week (corresponding to the removal of 200–250 mg of iron); the follow-up is based on hemoglobin values for tolerance, and on serum ferritin levels for efficacy. The duration of this phase depends on the degree of iron overload. The initial schedule is stopped as soon as the various serum iron load parameters reach the appropriate levels (<50 μg/l for ferritin and $\leqslant 20\%$ for transferrin saturation, provided hemoglobin levels do not drop below 110 g/l). For heavily iron-loaded patients a period of 2 yr of weekly venesections may be necessary, but more and more, due to moderately iron overloaded forms, several weeks or a few months are sufficient in many patients.
2. Maintenance therapy consists of 400–500 ml venesection every 1–3 months. Its goal is to keep iron parameters in plasma within the normal range (in practice, serum ferritin $\leqslant 50$ μg/l and TS $\leqslant 35\%$; this level usually corresponds with the disappearance of non-transferrin bound iron [34], which is a potentially toxic iron species [35]). The efficacy of venesections is excellent for a survival rate which returns to normal provided neither cirrhosis nor diabetes were present at the time of the diagnosis [36]. But, even in case of cirrhosis, the prognosis is far better than for other types of cirrhosis, especially of alcoholic origin [36]. With regard to various syndromes of the disease, the efficacy of phlebotomies is variable: (i) Good for asthenia, skin pigmentation, and hypertransaminasemia. (ii) Inconstant for arthralgia which may even worsen during (and sometimes after) the depletive treatment, for glucose abnormalities, and for non-cirrhotic fibrosis (which can be steady or decrease). (iii) The results are poor for impotence. (iv) The treatment is inefficient for two types of lesions: (a) cirrhosis, which is -in hemochromatosis as in other etiologies- an irreversible process, and (b) hepatocellular carcinoma which may develop in cirrhotic patients despite adequate iron elimination by phlebotomies.

3.2. Special issues

The cases of mild iron overload. A 400–500 ml weekly regimen may be unnecessary and/or imperfectly tolerated. A pragmatic attitude consists of testing the tolerance of phlebotomies while starting for instance with 200–300 ml per week and to progressively increase the volume possibly up to the "standard" schedule.

Clinically asymptomatic and young individuals. It seems reasonable to propose venesection therapy on and after 18 yr of age, considering on the one hand that iron needs are important during infancy and adolescence, on the other hand that in two large series of asymptomatic patients [36,37] the youngest subjects were 18 and 19 yr old.

Use for transfusion of the blood from hemochromatosis patients. It remains a matter of debate [38–40]. However, there is an increasing demand [41] for a re-evaluation of the present policy which in many countries does not allow healthy hemochromatosis patients to become voluntary blood donors.

4. Conclusion

Hemochromatosis is a remarkable illustration of a disease in which very quick clinical benefit has been obtained from a basic discovery at the molecular level. It is, indeed, a paradoxical disease, its treatment being worthy of the old ages and its diagnostic procedure worthy of the 21st century.

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