Canadian Hemochromatosis Membership Lecture Series

With Guest Leccturer Professor Pierre Brissot
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Transcript

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My city is Rennes. Rennes is the capital of Brittany. It's in the western part of France about 300 miles west of Paris and it's a medium sized city with about 400,000 inhabitants. It is very close to the Channel. The closest port is Saint Malo, and you can see here the port of St. Malo. As you know it's from St. Malo that Jacques Cartier in 1534 embarked to discover Canada.

1:01

I propose that we consider successively the three main parts, with the first: what is today's definition of hemochromatosis. It has changed a lot, you will see. Then we will see what is new or important for the diagnosis. Finally, what is new for the treatment.

1:17

So, let's start with the first point, which is what is hemochromatosis? The data that I will present here are the main conclusions of an international workshop that was held last year in Heidelberg during a BioIron meeting. So, it is the conclusions from this working group.

Today we consider that hemochromatosis corresponds to, of course, to iron overload – iron overload of genetic origin and with hepcidin deficiency. So, let's see now this notion of hepcidin deficiency. In order to better understand this notion, I just would like to introduce some focus reminders on iron metabolism.

2:10

If we consider the main actor of iron metabolism, you have the liver, you have the duodenum, digestive tube, you have your bone marrow and the spleen, and also the blood plasma.

The iron in the plasma comes from two main sources. On the one hand, it comes from the digestive tube from the elementary iron. And on the other hand, it comes from the spleen because iron in the spleen, itself comes from the degradation of old red blood cells which reach the spleen, dying within this organ and releasing the iron. And this iron will be recycled and will go into the plasma.

So, a double source of iron. Once iron is inside the plasma it cannot remain as such because metal in the liquid cannot circulate. So, the iron has to be transported, and it is transported by a protein whose name is transferrin. And as you can see here, normally, physiologically, there is an excess of transferrin compared to the amount of iron in the plasma and this correspond to the clinical, practical notion of transferrin saturation.

3:33

If we represent transferrin no more as a boat but as a vase in the plasma, you have this yellow vase – transferrin – and normally iron fills part of this vase that is near partial occupation of this vase. We know that transferrin saturation corresponds to the ratio of iron to transferrin, and that normally it is less than 45%.

4:03

If we continue to see the fate of this iron linked to transferrin, we know that the main fate is to go to the bone marrow where the transferrin will release its iron, and this iron will be used to produce new red blood cells which go into the circulation.

So, to go back now to the notion of hepcidin deficiency: If we consider hemochromatosis, whether due to the main mutation of the HFE protein – the C282Y/C282Y mutation by far the most frequent cause of hemochromatosis – or due to other rare mutations that I have indicated some of them here, hemojuvelin or transferrin receptor 2 – whatever the name, it is not important – the notion is that these various mutations will cause the same type of disease because this mutation shares the same mechanism. What is this mechanism? Those mutations, HFE or non-HFE, will act at the hepatic level, and at the hepatic level, the effect of these different mutations will be the same, that is decreased production by the liver of this protein called hepcidin. Hepcidin is a very important protein; it's the iron regulator of the iron metabolism in the body. And so, the effect of this mutation is to decrease the production by the liver of hepcidin. Since hepcidin is a hormone, that means that hepcidin, once it is produced by the liver, is released into the circulation. So, if you have a decreased production of hepcidin by the liver, this will lead to a decreased concentration of hepcidin in the plasma. You will have hypohepcidinemia, and this hypohepcidinemia will have a double consequence. On the digestive side, the effect of this low hepcidin level will be to stimulate this protein, which is a very important protein, called ferroportin. Ferroportin is the only protein known today to be able to export the iron from the cell into the plasma. So, when you have a lack of hepcidin you have an increased activity of this export protein, which means, at the duodenal level, that you will have an increased entry of iron into the plasma: hyperabsorption of iron. And at the same time, at the spleen level, you will have the same mechanism because ferroportin is also present and very active in the spleen, so low hepcidin level will create an increased activity of export of this ferroportin protein and therefore an increased release of iron into the plasma. So, the consequence of hepcidin deficiency is, through an increased activity of ferroportin, to create a permanent and important amount of iron inside the plasma.

7:20

So, of course if iron increases in the plasma, this means that you will have an increase in transferrin saturation. When you have an increased transferrin saturation, this corresponds often to the appearance in the plasma of a new form of iron, which is no more linked to transferrin, because transferrin starts to be saturated and which is called Non-Transferrin Bound Iron (NTBI). And this Non-Transferrin Bound Iron (NTBI) has a double property. The first property is a kinetic one.

7:54

Indeed, if we see the kinetics of the iron which is bound to transferrin, we can say that this iron bound to transferrin circulates rather slowly in the plasma, and that its target is, as we have seen, the bone marrow. It is quite different if you consider non-transferrin bound iron. Non-transferrin bound iron circulates very quickly in the plasma and its target is not at all the bone marrow but, especially at the first step, the liver. So, it will deliver very quickly this NTBI into the liver. And so we can say that NTBI, which appears when transferrin saturation increases, rushes into the liver and will be the main cause for the excess of iron deposition within the liver, and I have just illustrated this here:

8:48

You have a liver biopsy of a patient with hemochromatosis. And you can see these blue iron deposits within the hepatocytes. So, the first property of NTBI is to rush into the liver. The second property relates to its toxicity.

9:10

When transferrin saturation is over 75%, appears in the plasma a special form of NTBI which is called RPI for Reactive Plasma Iron (also LPI for labile plasma iron). And this is important because this Reactive Plasma Iron represents the potentially toxic form of circulating iron. In other words, when you have a lot of transferrin saturation, transferrin is iron saturated, part of the NTBI is RPI and this iron, which is NTBI; rushes into the liver but here it exerts its toxicity because this type of iron, RPI, is characterized by its propensity to produce what we call reactive oxygen species which are elements which are damaging, especially for the membranes of the cell. Hence today, it is accepted, it's admitted that organ damage, that we see in hemochromatosis, is mainly due to the toxic effect of this special form of iron, and this is true for the liver toxicity, this is true for the pancreas damage, and also for the heart damage.

10:27

So, to sum up the definition, we can say that iron overload hemochromatosis corresponds to iron overload of genetic origin. This genetic origin may correspond essentially to HFE mutation, much more rarely to non-HFE mutations, and the common denominator in terms of mechanism is that it is due to hepcidin deficiency which is responsible for this triangle: increased transferrin

saturation, the very earliest marker of hemochromatosis; liver iron overload; and no spleen iron overload (because you have seen that due to low hepcidin and high ferroportin, the spleen releases constantly and massively its iron into circulation).

11:17

So, in the mirror view, what is NOT hemochromatosis? You have two categories of disease.

11:25

First iron overload, which is not of genetic origin, that we can call acquired iron overload. And you have two main type of acquired iron overload. One is due to multiple transfusions, that situation you can see for instance in chronic anemia in children with thalassemia, and also the second situation is when you have chronic anemia of another cause and you supplement with intravenous iron, and excessive intravenous iron supplementation can produce acquired iron overload. So, this is the first category of chronic iron overload which does not correspond to hemochromatosis. They are not genetic, they are acquired.

The second set of diseases which are not hemochromatosis, corresponds to iron overload which are genetic but not related to hepcidin deficiency, and we can call this situation non-hemochromatosis genetic iron overload and I just here will allude to this disease called ferroprotin disease that I will say a few words on it in the diagnostic part that we will now start.

12:46

Now we have seen the definition of hemochromatosis with a common denominator of hepcidin deficiency. Let's now move to the diagnostic aspect of hemochromatosis and we will see successively: HFE hemochromatosis – classical hemochromatosis, probably 98% of hemochromatosis; say a few words on non- HFE hemochromatosis; and very few words on non-hemochromatosis genetic iron overload. So, let's start with the diagnosis of HFE hemochromatosis.

13:23

The main notion here, is that this diagnosis has become totally non-invasive. In other words, you don't need any longer to perform a liver biopsy to reach a diagnosis of hemochromatosis. You just rely on the triangle of data which is the following:

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You have clinical data, biological data and imaging data. Let's say a word about each of them. First what is the clinical expression of the classical HFE- hemochromatosis? The first expression is in fact nothing. You can see nothing during 30, 40 and sometimes 50 years. And it is a big problem because during this "black box", if you don't check systematically for iron parameters you will not suspect that you have an underlying silent disease, a major problem for hemochromatosis.

When the clinical expression appears, you have two main types of syndromes or symptoms or signs. Three signs which impair the quality of life but do not compromise, not jeopardize, life expectancy.

14:35

This is first, chronic unexplained fatigue which can be very important in these patients.

14:48

The second type of signs are rheumatology signs. And you do know that the hand particularly is affected, especially at this level here, and is responsible for the symptoms that we have described as a “painful handshake”.

15:10

And the second location that should suggest hemochromatosis rheumatism is when you have this location at the ankles. There are very few chronic foot arthritis which are located at the ankle.

15:22

Often this arthritis is associated with traumatic aspect due to early osteoporosis. So, we have seen chronic fatigue, we have seen rheumatic disease, arthritis, osteoporosis.

15:38

The third sign is of course hyperpigmentation as illustrated here. So, these types of syndromes impair the quality of life, but if the disease evolves a lot, becomes more severe, you can have three types of syndromes which may really be life-threatening.

16:01

The first one is of course the liver syndrome and you do know that iron excess over a long time can damage the liver, can produce some scarring effects that we call fibrosis, and when fibrosis is very important, you have (16:10) this type of liver which is no more smooth as here, but really (16:21) cirrhosis which is due to iron, not to alcohol, not to hepatitis or steatohepatitis (liver inflammation due to fatty liver) and the particularity of this hemochromatosis cirrhosis is that, despite the fact you have a true cirrhosis and a lot of iron within the liver, there is a preserved liver function. Indeed, liver function tests are almost normal apart from a slight chronic increase in transaminases. As for every type of cirrhosis (whatever its cause), there is a risk of developing hepatic cancer.

16:57

Indeed, every cirrhosis is about to develop cancer whatever its cause, and it’s true for hemochromatosis, as for the other causes of cirrhosis.

17:10

So, first the liver. Second is the pancreas, and you do know that you can develop diabetes, insulin dependent diabetes.

17:18

And the third organ that can be severely affected, but much more rarely than previously thought in HFE hemochromatosis, is the heart, with arrhythmia, eventually possibility of cardiac failure. These are the main known syndromes of classical hemochromatosis.

17:40

Let's now move on to biological signs, and we will see briefly, successively, transferrin saturation, ferritin and genetics. So, a few words on transferrin saturation.

17:56

We have seen that transferrin saturation is the earliest biochemical abnormality that you see in the course of the disease. Increase in transferrin saturation is often over 60 and 50% in men and women respectively, and in fact often 80 to 100%. And it's important to check twice the abnormality before continuing the diagnostic approach because the transferrin saturation may be a rather fluctuating parameter.

18:26

The message is the following: normal transferrin saturation excludes hemochromatosis.

18:34

Let's now say a word about ferritin, and just before, a few words about what ferritin is. We have seen that transferrin is the protein which carries and transports iron into circulation. Ferritin has a different role.

18:49

If you consider the liver and the iron which has reached the liver, it cannot remain as such in the cells because it would be toxic. Thus, it will be incorporated, integrated, within the protein which is called ferritin. So, ferritin is an iron storage protein. And part of this ferritin leaks into the plasma so that the amount of ferritin in the plasma reflects, is correlated, with the amount of iron in the liver. If you have high ferritin it usually means that you have high body iron excess. And so to come back to the clinical aspects, it's clear that if you assay the ferritin in hemochromatosis, you will most often find an increase in ferritin and it's over 300 ng/mL (or µg/L) in men and 200 in females. But it's very important when you have an increased ferritinemia to be aware of the false positive situations for which you have an increase in ferritin but which is not related to iron overload. And it is very frequent. In fact, the most frequent cause of hyperferritinemia is not iron overload. Throughout the world it's what we call

the metabolic syndrome. And the metabolic syndrome corresponds to all these patients who have a little bit “too much of everything”: too much of weight, too much of blood pressure, too much of cholesterol, too much of glycemia, sometimes of uricemia. This is what we call dysmetabolic hyperferritinemia. If you wish, it is not a ferritin of iron but a “ferritin of fat”. The second cause which can produce hyperferritinemia without iron overload is inflammation because ferritin is called an inflammatory protein. The third cause which can produce hyperferritinemia independently of iron excess is alcoholism because alcohol increases the production of ferritin. So, the reflex when somebody has a hyperferritinemia is to rule out these three causes of hyperferritinemia and then you can really deduce that your hyperferritinemia does reflect body iron excess.

21:24

Let's now say a word about the genetic test. Of course, when we suspect hemochromatosis, we check first if there is: high transferrin saturation? Then we are allowed to ask for the C282Y mutation, and the expected result from the lab is the following: We expect C282Y/C282Y homozygosity for this mutation (one mutation received from the father; the other mutation received from the mother). As to the meaning of other genetic profiles: we do know that H63D heterozygosity has no diagnostic value. It's the same for H63D homozygosity: no diagnostic value. And it’s the same, and even worse for this mutation called S65C: no diagnostic value.

22:18

There is more concern about the interpretation – the meaning – of what we call compound heterozygosity C282Y/H63D. What we accept today is the following: this genetic profile is not responsible for true hemochromatosis. It can produce some increased transferrin saturation (50%, 60%, sometime 70%), but by itself it is not supposed to create hyperferritinemia. What we think is that this genetic profile is essentially a booster, has a boosting effect if you want, for increasing ferritin in those situations that I have indicated previously (and especially the dysmetabolic syndrome). So, the overall message is that C282Y/ H63D does not expose to the risk of development of significant iron overload, and this is not a cause of true hemochromatosis. I know that it’s often difficult to be received by the patients that have been diagnosed, sometimes for a long time, as having hemochromatosis, but it’s very important to transmit this message.

23:27

Now we have seen clinical data and biological data. The third set of data which enables us to make the diagnosis of hemochromatosis in a non-invasive way is of course magnetic resonance imaging.

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It has been a revolution for the diagnosis of chronic iron overload. And you have here a typical picture of a hemochromatosis patient. You can see on the left-hand side a “black” liver. It

means that this liver is full of iron. But we should not forget to have also a look at the spleen. And you see here the spleen: it is a "white" spleen because you have no iron excess in the spleen. So, you can see that MRI is very interesting because not only does it permit you to visualize the iron overload – and to quantify it, especially in the liver – but by evaluating the balance between iron in the liver and no iron here in the spleen, it provides you with information on the mechanism of your iron overload.

24:37

We are done with HFE hemochromatosis. A few words on non-HFE hemochromatosis. These are rare situations as I've told you. This is hemochromatosis without homozygosity for C282Y, and you have several types of mutations. I have just indicated two of them here, which are hemochromatosis due to hemojuvelin and TFR2 mutations. There are similarities with HFE, of course, and a few differences. The similarities are due to the fact that we have seen this non-HFE hemochromatosis syndrome, commonly with HFE, involves the "hepcidin deficiency syndrome" which means that you have the triangle: increased transferrin saturation, liver iron overload, no iron in the spleen. And, clinically, those non-HFE hemochromatoses show, as compared to HFE-hemochromatosis very close clinical, biological, and imaging expression. But there are some differences. What are these main differences between non-HFE and HFE hemochromatosis? Non- HFE hemochromatosis is a rare disease. Let me remind you that HFE hemochromatosis is only seen in Caucasians, but non-HFE hemochromatosis can be seen most in Caucasians but also in other ethnicities. So, it's rare, much rarer than HFE but is more diffused throughout the world. The second difference is that usually it affects younger people, less than 30 years old so that the reflex, when you have documented massive important iron overload, especially by MRI, in a person which is less than 30 years old, should be to think of non-HFE hemochromatosis, what we call "juvenile" hemochromatosis. Therefore, usually, non-HFE hemochromatosis, is a more severe disease especially in the liver expression, in the heart expression and of course you need to resort to expert centers for genetic testing because these are rare mutations.

26:58

Let's say a word on non-hemochromatosis genetic iron overload. I remind you that we are here in the situation where iron overload, while of genetic origin, is <u>not</u> due to hepcidin deficiency. This is especially the case of the so-called "ferroportin disease", which is a rare disease but not so rare because it's a dominant disease in contrast to HFE hemochromatosis and non HFE which are recessive diseases (when a disease is "dominant" it's of course more importantly diffused). So, what is, shortly, this ferroportin disease?

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We know that in this disease, the mutation acts at the level essentially of the spleen, and the effects of the mutation of the ferroportin protein is to decrease the property of exporting iron from the cell into the plasma. In other words, when you have a mutation of the ferroportin

disease, you have a decreased activity of Ferroportin (mainly in the spleen), and a decreased release of iron into the plasma which creates a retention of iron in the spleen. And so, this will be reflected by a special biochemical profile in the blood, namely high ferritin (because you have high body iron overload) but normal transferrin saturation (because iron is trapped in the spleen). And this is important because I told you that transferrin saturation, when it is normal, excludes hemochromatosis, but you see here that normal transferrin saturation does not exclude some rare forms of genetic iron overload. This ferroportin disease patient does have a significant iron excess in the body and you can see here a typical profile of a ferroportin disease patient.

28:48

In the MRI (of Ferroportin disease) you can see here a black spleen – you remember that in hemochromatosis you had a white spleen – a black spleen because iron is essentially located in the spleen – and only "grey" liver because the iron overload is only moderate at the hepatic level. So, you see again the interest in MRI for orientating the mechanisms of chronic iron overload.

29:18

So we can move to the third and final part which is the treatment. What is new and what is important in the treatment?

29:27

Of course, if I show you this slide it is not very new to speak on the phlebotomy today, it's not new but it is so important. Phlebotomies remain and will remain for a very long time the mainstay of the treatment. I just would like here to focus on the blood markers of efficacy when the patient undergoes phlebotomy treatment. As you know, there are two phases, induction phase and the maintenance treatment. What about the markers of efficacy when you are in the induction phase?

30:01

Induction phase is of course to resort to weekly venesections in order to decrease progressively, and to remove all, the established iron excess. And we know that the ferritin – which is, I remind you, the iron storage protein – which leaks into the plasma, the ferritin in the plasma in hemochromatosis is very well correlated with a degree of iron stores. So, the message is that the appropriate marker to follow the efficacy of the treatment during the induction phase, is really ferritin. And the next notion is very important: "Beware transferrin saturation during the induction phase". As you know, if you represent the evolution of the transferrin saturation during the induction phase, we can see that, during most of this phase, despite the fact that you remove effectively iron, and that ferritin progressively decreases, transferrin saturation will remain very high, and totally saturated sometimes. At the very end of the treatment, when ferritin is about 50 micrograms per liter (or nanograms per milliliter),

rather suddenly, transferrin saturation will return to normal values. So, the message is that we should not check the efficacy of venesection in the induction phase by transferrin saturation during most of it.

31:34

What about the maintenance therapy? For maintenance therapy, the main goal is to obtain and to maintain the ferritin level at, let's say, around 50 micrograms per liter. And often this corresponds to transferrin saturation which is also in the normal range, let's say less than 50%. We can say that this fits the ideal profile, "the rule of fifty": 50 for ferritin, less than 50 for transferrin saturation.

However, we have observed over time that there is a subset of patients, which is pretty significant in number, which do not follow this rule. Despite the fact that you have ferritin which is around 50, if you check transferrin saturation, you can see that they are exhibit very high levels of transferrin saturation (over 75%). And the recent work which has been done in our team in Rennes has shown that this type of profile may correspond to a patient who develops more fatigue, more chronic rheumatologic signs. Maybe it's due to the fact that, when transferrin saturation is over 75%, you may have in the plasma this toxic form of iron called reactive plasma iron.

32:55

So, what is, here, the main message? It's that normal ferritin remains the main goal for the treatment. When you have 50 of ferritin you should not be worried because there is no iron overload in your body. But you should not totally forget transferrin saturation. I would recommend checking at least twice a year for transferrin saturation to be sure that you are not in this specific profile of having very high transferrin saturation despite normal ferritin.

33:30

So, what about the other treatments of hemochromatosis? You have two innovative forms of treatment. One is targeted to act at the level of the "consequence", I mean, on iron excess., in other words of treatments that may replace phlebotomy. This is the issue of using oral chelators: the main drug in this field is called deferasirox and its commercial name is Exjade. This oral drug is able to act at two levels. First, it can pick up in the liver the iron and evacuate it through the bile. Moreover, * this chelator is able to pick up the iron, the NTBI at the plasma level. But resorting to oral chelation remains exceedingly rare in hemochromatosis. In most cases, phlebotomies, as you know, are very well tolerated, and you don't need to resort to this type of chelator. The second problem is that it remains today an off-label drug: that means that it can be prescribed, but solely under the responsibility of the GP and with the written consent of the patient because, of course, as all drugs, this chelator may have some side effects. The other innovative strategic way is to act no more at the level of iron excess, but upstream of the

iron excess, I mean to fight hepcidin deficiency, which is responsible for the iron excess. You have here two ways to apply this approach.

35:48

So, what can we do today? It's possible to increase this hepcidin – which is low, I remind you, in hemochromatosis – by synthetic hepcidin which can be produced in the lab. This hepcidin will decrease ferroportin and decrease iron absorption as we have already seen. Indeed, it is possible to synthesize hepcidin and to inject it subcutaneously to increase hepcidin in the body. This is a first approach: hepcidin supplementation. But there is another way for restoring normal hepcidin levels, which is the following.

36:40

It's no more to act at the at the hepcidin production level, but to produce ferroportin inhibition (you act here, downstream compared to hepcidin production). In other words, you will not act at the hepcidin level here, but you at the ferroportin level. There is an interesting candidate drug which can be taken orally and is able to decrease ferroportin and so to decrease iron absorption.

So, what is the situation today for these two approaches? We have three main conclusions. The first notion is the following: that in mouse, it works. We have, what is called, the proof of the concept. Briefly, is if one produces experimental hemochromatosis in mice, it has been shown that these two approaches are able to counteract the development of hemochromatosis. The second conclusion today is that if you consider the effect of these two approaches in normal humans (healthy volunteers), for what we call "phase one" clinical trials, it works also. So, what about hemochromatosis? Here it is much more uncertain and the only trial which has been done in hemochromatosis patients using subcutaneous hepcidin has concerned about 70 patients, and unfortunately the study has been stopped during the interim report because of very mixed results. So, there will still be a long time before we can resort to these new approaches, but I do think that they are irreversible approaches because they are logical in terms of their goals, that is to be effective before iron overload develops or to prevent iron overload reconstitution once iron overload has been removed by induction therapy. If you have a way to restore normal hepcidin levels, and therefore normal iron absorption, you can conceive that it will no longer be necessary to perform the maintenance therapy.

39:09

So, I have finished. I just would like to conclude by the 5 "H". The first H is like Hemochromatosis, the second is like HFE versus non- HFE mutations. The third H is like Hepcidin deficiency and the fourth H is like Homogeneity because we have seen that the major interest of the new terminology is to help in clarifying things in this area, due to the fact that you have the common pathophysiological denominator of hepcidin deficiency underlying the common approach in terms both of diagnosis and treatment. The fifth, and final H is H like

Hope, because the hope is, really, to increase awareness of this disease and we all know that hemochromatosis remains too often misdiagnosed or even ignored. Here I would like to pay tribute to the role of patient associations which is outstanding. I would just like to show you the organization in France: we have a French Federation of Association of Patients with Hemochromatosis (which will become very soon an Association called “France, Fer, Hémochromatose” (FFH). We have created almost 15 years ago the EFAPH, which is the European Federation and you see here the successive presidents. In 2013 in London we also created Haemochromatosis International (HI), and you see its successive presidents; now it is Dianne Prince from Australia; and I am of course happy to quote here the Canadian Hemochromatosis Society. I joined Ray Fynes (from the Canadian Hemochromatosis Society) at the Haemochromatosis International meetings and I would like to thank especially the president, Paul Johnston. I would not like to end this presentation without evoking the memory of Marie Warder. We all know all that she provided for the hemochromatosis patients not only in Canada, but throughout the world. So, I thank you for your attention and I would be happy to answer your questions. Thank you.

Question and Answer Period

41:43

Q: Is joint pain in the hands to be expected?

A: You have seen it, yes indeed.

41:51

Q: Bronzing skin, particularly on the arms? With reddish blotches?

A: Yes, you can see bronzing skin. I don’t know about reddish blotches in hemochromatosis.

42:09

Q: Someone with hemochromatosis who has had knee/ hip replacement asks if it is normal that they continue to have worsening symptoms despite normal iron levels?

A: Unfortunately, it is one of the big problems in hemochromatosis. The arthropathy does not respond well, and often does not respond at all and may even worsen despite removing iron. There is not a direct link between iron excess and arthropathy, and today this is one of the major goals of the research on hemochromatosis, to explain, to understand the particularity of this arthropathy which may be very disabling, especially in manual workers. I just tell you that there has been created what we call a HARI (Haemochromatosis Arthropathy Research Initiative) group, which is devoted to try to understand the pathophysiology of this arthropathy.

43:17

Q: Is there a relationship between pulmonary fibrosis and hemochromatosis?

A: No relationship between pulmonary fibrosis and hemochromatosis.

43:28

Q: Are there other conditions that can cause an elevated transferrin saturation besides iron overload?

A: Yes. I introduced that normal transferrin saturation excludes hemochromatosis. This is true. But increased transferrin saturation may be due to other forms of iron overload and hemochromatosis. For instance, in transfusional iron overload you will also have an increase in transferrin saturation, so it is not specific. You can also have an increase in transferrin saturation which is not due to iron overload. I remind you that transferrin saturation is a ratio of iron to transferrin. So, you have two mechanisms that can increase this ratio: either you have an increase in iron (the numerator) which is the main mechanism in hemochromatosis, or you have a decrease of transferrin (the denominator). And one situation for instance where you have a decreased transferrin is hepatic failure. If you have a patient with very severe cirrhosis and you check transferrin saturation, it may be 100% which is not due to iron overload but due to the fact that hepatic failure leads to absence of production by the liver of transferrin. So, you have almost no more transferrin (meaning that the denominator is very low) and so you have an increase transferrin saturation.

44:50

Q: Can you rule out a hemochromatosis diagnosis if serum ferritin is within the reference range or lower?

A: No, you cannot rule out because you have seen that in the course of hemochromatosis, you have first an increase in transferrin saturation but not yet an increase in ferritin, because transferrin saturation just reflects the iron transport in blood whereas ferritin reflects iron deposition in the organs. So, in hemochromatosis, you have a long phase with only an increasing transferrin saturation but not yet an increase in ferritin. It's what we call "stage two" hemochromatosis. Stage one is only transferrin saturation with normal ferritin. Stage two is increased transferrin saturation with increase in ferritin; stage 3 is when the two markers (transferrin saturation and ferritin) are increased with clinical symptoms which impair the quality of life (fatigue, arthropathy), and 4 is all the previous biochemical and clinical symptoms but associated with signs impairing life expectancy (cirrhosis, heart disease). So, the answer is yes, ferritin can be normal despite hemochromatosis.

46:38

Q: There can be a discrepancy during maintenance therapy between normal ferritin and, despite normal ferritin, high transferrin saturation. What can we do on the therapeutic side to try to correct this?

A: First, I would like to say that what I showed you is based on theoretical view and also on some clinical data that needs yet to be confirmed. So, it's not still widely established but my feeling is that the goal is to restore not only ferritin but also transferrin saturation to go back to normal iron parameters. And so it's normal to try to normalize not only ferritin but also transferrin saturation levels, but it's true that, in practice, it's difficult. What we can do, if you have, for instance, 50 of ferritin and 80% transferrin saturation is to shorten the interval between two successive venesections or to increase a little bit the volume of the venesections. But you should be careful not to make the patient anemic, of course. You can also try to decrease the absorption of iron by other ways. For instance, it may be one indication for restricting iron in the diet, which is not the rule, otherwise. It may be also advised to drink a lot of tea, which decreases the absorption of iron. I mostly think that, in the future, it would be one of the main indications for hepcidin supplementation because when we will be able to correct the hepcidin levels, we will correct the iron hyperabsorption, and those patients with normal ferritin but markedly increased transferrin saturation will benefit from this type of innovative approach.

48:40

Q: Curious about any clinical issues or concerns with someone who is heterozygous.

A: When you are heterozygous for the HFE gene (e.g. you are heterozygous for the C282Y mutation), you have not the disease because it is a recessive disease. You need to have to have two mutations to develop, possibly, the disease. And so, when you are heterozygous (it is 10% of the population) you are not sick and will not become sick. The usual picture in the family of a homozygous patient, C282Y/C282Y, is the following: his/her parents, father or mother, they are not sick – they have no disease because they have only one gene mutation, and it's the fact that there are two mutations that incidentally joined which makes one of the children a homozygote. So, heterozygosity by itself does not cause hemochromatosis.

49:42

Q: Could hemochromatosis affect a person's ability to accept bone grafts or tooth implants?

A: I should say no. I think you have to check maybe the bone density to be sure that there is no major osteoporosis. I don't think that you need to be especially cautious with that. Just check the bone density. Sometimes you have periodontitis in hemochromatosis patients, but for the implants there is no contraindication. Just check that the bone is ok, I think.

50:29

Q: Is it normal to have symptoms despite being stage 1 or stage 2?

A: I think we related here to what we said for the management therapy, stage 1 is when you have increased transferrin saturation with normal ferritin. Stage 2 is when you have increase transferrin saturation plus increase ferritin. The question is to know whether people who have high transferrin saturation despite normal ferritin may develop symptoms. As I told you there is a clinical study which concludes that it may be so, but we need confirmation as always by other clinical studies to be sure of that, So, I would say that it's possible but not yet proven.

51:46

Q: In areas of Canada with large prevalence of people of Celtic origin, should physicians be screening patients with serum ferritin and transferrin saturation annually to help identify patients early?

A: I should say yes. We should systematically check for transferrin saturation and ferritin in every young Caucasian adult of Celtic, but maybe not on an annual basis. Because if a 30-year-old man has normal transferrin saturation, it is highly unlikely that he will develop hemochromatosis, because transferrin saturation increases very early. So, I think that we should between 20 and 30 check systematically transferrin saturation and ferritin. As you know, it is used to check systematically several times during life, for cholesterol, and glycemia but never transferrin saturation and never ferritin! And if we check ferritin, GPs should also check for transferrin saturation, it's quite important. Maybe for the women it should also be necessary to recheck after menopause, because the metabolism becomes a little bit like males at this time, and we see sometimes females who develop real hemochromatosis at 65 and 70 years old. So, in males probably once between 20 and 30 would be good, and in women once between 20 and 30 and, let's say, at or around 55 years old.

53:39

Q: If ferritin is low, under 20, but transferrin saturation is high, should they have a phlebotomy?

A: I should say we have first to check if this elevation of transferrin saturation is permanent. So, no phlebotomy after a single check, there is no emergency in this situation. As long as ferritin is normal, everything is okay. You should not become obsessed by the transferrin saturation. I just wanted to say, along my talk, that we should not totally ignore it because it may have some meaning in certain circumstances, but we need to confirm both that there is a permanent increase over several months and that this increase is very pronounced. It's only when transferrin saturation is permanently over 75- 80% that you can try the method I proposed. But again, it should not be an obsession. My personal feeling is that when we want to treat somebody, we want to really restore the normal iron parameters and I don't think it's good to have normal ferritin and to have permanently very high increase of transferrin saturation but

again it is not 47, 52, 60, or even 70%! It's when we have permanently very high 80%, 90% transferrin saturation, that it may be damaging although not yet totally established.

55:20

Q: Did I understand correctly that homozygous for H63D will not get iron overload?

A: Yes, we cannot exclude that it can increase slightly transferrin saturation, but there is no evidence in the literature, that such individuals ever developed hemochromatosis. And for some patients who have been reported as developing hemochromatosis, it was before the discovery of other genes, and it turned out that most of these H63D homozygotes with iron overload had associated mutations causing non-HFE hemochromatosis…. So, if you document really massive iron overload in H63D/H63D patient you should seek for non-HFE mutations because H63D homozygosity is not responsible for clinically significant iron overload.

56:23

Q: Do all C282Y/C282Y get iron overload or is it possible they would not?

A: Good question. In fact it's admitted today that if you take the population who is homozygous for C282Y, in women, probably more than 90% of women will never develop any problems of iron overload, and in males it's about only 25% who will develop significant clinically expressed iron overload. So, there are other factors that explain iron excess: if you want, C282Y homozygosity is necessary but not sufficient to develop hemochromatosis. But globally, we can say that 50% of persons who are homozygous for C282Y would justify venesections due to biochemical abnormalities (increased transferrin saturation together with increased ferritin).

57:40

Q: Is there a role for neonatal genetic testing?

A: Not at all because this is a genetic disease which is very silent and not damaging during probably 20-30 years, and so, if you make a genetic test in the neonate, you will have neonates who will turn out to be homozygous and will never develop hemochromatosis. As long as we have no predictive markers for the development of iron overload in C282Y homozygotes, we will not be able to say that this homozygous subject will or will not develop hemochromatosis. This may be very annoying in terms of discrimination to label this neonate as a potential hemochromatosis patient. So, the answer is not. Moreover, there are some data who suggest that to be a homozygous for C282Y could provide some kind of advantage during infancy and adolescence because you need then a lot of iron, so it is pretty good to have this hyperabsorption of iron until probably puberty and the end of growth. There is also an interesting work from a French team showing that there are more HFE mutations in athletes who were very highly distinguished during the Olympic Games and World Games …. It is possible that, in the course of hemochromatosis, there is a transient period where it may be somewhat "beneficial"; but we should also say that, in our experience, those C2828/C282Y

athletes may become sick around 30 years old, maybe because the iron overload has become too important and starts damaging especially the joints. So, everything is not black or white in terms of the effect of C282Y.