

Bob Barrett: This is the podcast from '*Clinical Chemistry*'. I'm Bob Barrett. The past decade has seen an enormous increase in our understanding of iron metabolism, and the recently discovered peptide hormone hepcidin appears to play a central role.

Several human diseases are now associated with alterations in hepcidin concentrations, and its measurement maybe a promising tool in diagnostic medicine.

Our guest in this podcast, Dr. Dorine Swinkels, is a Professor of Clinical Chemistry at the Radboud University in Nijmegen, The Netherlands. Her work focuses on the identification and characterization of novel factors that affect iron homeostasis. She is the author of a recently published review on this topic in the December 2011 issue of '*Clinical Chemistry*'.

So tell us, Dr. Swinkels, what exactly is Hepcidin, and is this something that you always thought might exist and just recently discovered?

Dr. Dorine Swinkels: Hepcidin is predominantly produced by hepatocytes as in 25-amino-acid peptide that is secreted in circulation. Hepcidin is now recognized as the key regulator of systemic iron homeostasis. By its interaction we see cellular iron exporter, ferroportin. And we all know iron is essential for life, but on the other hand, the redox activity of iron can also cause damage primarily by the production of reactive oxygen radicals.

And it was therefore anticipated that iron levels must be tightly regulated not only at the cellular level but also at the systemic level. But until the year 2000, the mechanism for the maintenance of systemic regulation was largely unknown. And with discovery of Hepcidin, different studies, multiple studies contributed insights into its crucial role and the effective communication between cells that absorb iron from the diet, utilize iron, and store iron.

So for instance, Hepcidin has been found in the hormone that's causing increased intestinal iron absorption and iron-deficient subject, but it's causing a decrease in iron uptake in the iron-replete subject.

Bob Barrett: So how exactly does Hepcidin exert this regulatory function?

Dr. Dorine Swinkels: Hepcidin does so by counteracting dysfunctional ferroportin, and the ferroportin, it's the major cellular iron exporter in the membrane of macrophages, hepatocytes,

and the basolateral side of enterocytes. Hepcidin induces the internalization and degradation of ferroportin. And this then results in increased intracellular iron stores, decreased dietary iron absorption, and decreased iron concentrations in the serum. Cell culture in animal and human studies have shown that situations in which demand for iron has increased such as increased red blood cell synthesis elicit a decrease in hepatocellular Hepcidin synthesis. And these conditions that include iron deficiency, hypoxia, anemia, and conditions characterized by increased erythropoietic activity. And this decrease in Hepcidin results in the release of stored iron into circulation and increase in dietary iron absorption.

But on the other hand, infection or inflammation cause an increase in Hepcidin synthesis. And this then leads to a deficiency of iron available for erythropoiesis. It is considered to be the mechanism underlying the macrophage iron sequestration, impairment of intestinal iron absorption, and low serum iron levels. And these are all characteristics of anemia of chronic disease.

Bob Barrett: Okay, doctor. So our understanding of Hepcidin regulation and function has increased substantially since its discovery. But what has been done to translate Hepcidin into a diagnostic tool for iron disorders, and what are the challenges to accomplishing that?

Dr. Dorine Swinkels: First of all, we and others have developed reliable assays to measure Hepcidin in blood and urine by use of mass spectrometry and immunochemical methods. But the availability of reliable assays is a curse. It is because quantification of Hepcidin has been found to be complicated by its tendency to aggregate and to stick to laboratory plastics, is to necessitate implementation of robust laboratory procedures.

Progress in development of conventional immunochemical assay has long been hampered by difficulties to generate specific anti-Hepcidin antibodies in hosts such as the rabbit or the mouse. This is because Hepcidin is small and has a compact structure leaving scars, antigenic epitopes.

Hepcidin also has a high degree of conservation among a wide range of species. This then diminishes the elicitation of an immune response in host animals. And there is also the presence of the smaller Hepcidin-22 and 20-amino acid isoforms. They play no role in the regulation of iron metabolism, but they can interfere with the quantification of Hepcidin amino assays. This is because these assays use antibodies that react with all these Hepcidin isoforms.

(00:05:05)

Bob Barrett: So with several assay procedures available, can you tell us how they agree with each other and what do you think is the preferred assay technique?

Dr. Dorine Swinkels: Mass spectrometry assays require relatively expensive equipment, but they have the advantage of distinguishing between Hepcidin-25, 22, and 20. And as I said before, ELISA assays will measure total Hepcidin levels with, of course depending on the specificity of the antibody, different contributions from each of these three isoforms.

At the same time, we do not know yet the relevance for specifically measuring Hepcidin-25 instead of total Hepcidin for clinical decision-making, and this has not been systemically investigated. But I will send out the samples in two so-called round robins show that Hepcidin levels generally correlate between assays, but that absolute Hepcidin levels vary greatly between assays.

Variation is not surprising giving the absence of a reference method and a validated commutable calibrator or other materials for assay harmonization. It implies that until harmonization is achieved, reference ranges as well as clinical decision limits for certain patient populations specific through techniques should be used.

Bob Barrett: Doctor, is anything known on the reference intervals for serum Hepcidin? And how can healthcare providers interpret serum Hepcidin concentrations in a particular patient?

Dr. Dorine Swinkels: Small and large studies of healthy controls revealed considerable intra-individual variation Hepcidin levels. And this then results in wide reference ranges. Those reference ranges may have limitations when used for interpretation of individual Hepcidin concentrations.

It appears that Hepcidin values like other hormones should be interpreted in the context of other indices of iron metabolism. For instance, it's possible that normal levels of iron in iron-deficiency anemia are inappropriately high and perpetrate iron restriction.

Bob Barrett: Well, let's get to the bottom-line then. What is the potential added value of Hepcidin diagnostics?

Dr. Dorine Swinkels: The development and validation of Hepcidin assays paved the way for a large number of human studies. These studies considerably increased our understanding of the physiologic and pathophysiologic states of iron homeostasis. These studies also demonstrated some

promising applications of Hepcidin for currently unmet needs in diagnostic medicine.

And these include diagnosis, monitoring and prognosis of patients with a variety of iron disorders such as HFE-related hemochromatosis, iron-loading anemia such as thalassemia, and iron-refractory iron-deficiency anemia due to mutations in the Tmprss6 gene.

There's also a few recent studies suggesting that relatively low urine Hepcidin levels are associated with a greater risk of acute kidney injury after coronary artery bypass graft surgery in patients with stable renal function.

Promising applications also include detection of iron deficiency among anemic patients with concurrent inflammation that occurs in several diseases such as anemia of rheumatic diseases and anemia of chronic kidney diseases. This also occurs in developing world. Here Hepcidin had been out to be the long thought guide for iron supplementation.

It would also be great if Hepcidin analysis fulfills the need as a companion diagnostic for EPO treatments. But until now, patients with renal efficiency are a rather complex population in which consistent results have been difficult to obtain.

Bob Barrett: Will there be any role for measurements of Hepcidin in Hepcidin-targeted therapies?

Dr. Dorine Swinkels: Oh, yes. Hepcidin-targeted therapies may improve treatment options for patients suffering from iron disorders. These Hepcidin therapies are not available yet but many compounds on development, either it's Hepcidin agonist or antagonist, it may be combined with EPO treatment or the IV iron chelation and phlebotomy or even replace these treatments.

Therefore, Hepcidin measurements are essential for the preclinical and clinical studies adjusting safety and efficacy of these compounds. And it's registered for the monitoring and assessment indications for these treatments.

Bob Barrett: Finally, doctor, what do you think needs to be done before Hepcidin assays can be fully established as a routine marker in clinical practice?

Dr. Dorine Swinkels: First of all, large and well-designed studies exploiting harmonized assays are required to firmly establish the position of Hepcidin in diagnostic medicine. In this respect, Hepcidin introduction as well as other biomarkers in the clinic would greatly profit from initiatives for prospective

diagnostic accuracy studies, which has been successfully implemented for clinical trials. And also reliable, harmonized, and preferably automated assays should become available for clinical laboratories before Hepcidin can be used in routine clinical practice.

Bob Barrett:

Dr. Dorine Swinkels is a Professor of Clinical Chemistry at the Radboud University in Nijmegen, The Netherlands. She has been our guest in this podcast from '*Clinical Chemistry*'.

I'm Bob Barrett. Thanks for listening!

Total Duration: 10 Minutes