HIV-Associated Tuberculosis: Does the Iron-Regulatory Hormone Hepcidin Connect Anemia With Poor Prognosis?

Andrew E. Armitage¹ and Ed Moran²

¹MRC Human Immunology Unit, MRC Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, and ²Department of Infectious Diseases and Tropical Medicine, Birmingham Heartlands Hospital, Heart of England NHS Foundation Trust, United Kingdom

(See the major article by Kerkhoff et al on pages 61–70.)

Keywords. HIV-associated tuberculosis; hepcidin; anemia; iron; mortality.

Coinfection with Mycobacterium tuberculosis represents one of the major global health challenges associated with the human immunodeficiency virus type 1 (HIV-1) pandemic. HIV-associated tuberculosis is the leading cause of AIDS-related mortality, predominantly affecting resource-limited settings in sub-Saharan Africa [1]. Anemia is a frequent comorbidity of HIV infection, M. tuberculosis infection, and HIV–M. tuberculosis coinfection; in each case, anemia is predictive of mortality, independently of other well-established risk factors [2, 3]. The etiology of these infection-associated anemias is likely multifactorial, but anemia of inflammation (commonly referred to as anemia of chronic disease), which is associated with perturbations in iron status, may play an important role [4]. Sequestration of iron within macrophages accompanied by impaired iron absorption is commonly observed during inflammation, leading to functional iron deficiency and, if persistent, to iron-restricted erythropoiesis and anemia. Importantly, these phenotypes are also hallmarks of increased activity of the iron-regulatory hormone hepcidin.

Hepcidin is a liver-produced peptide that determines both systemic levels and anatomical compartmentalization of iron [5]. It is expressed in response to iron to maintain homeostasis but also as part of the acute phase inflammatory response, primarly via the interleukin 6/STAT3 pathway. Conversely, iron deficiency and periods of erythropoietic demand result in hepcidin suppression. Hepcidin activity causes degradation of the enterocyte- and macrophage-expressed iron exporter ferroportin, resulting in inhibition of dietary iron uptake, sequestration of recycled erythrocyte iron in macrophages, and reductions in serum iron [6]. Although anemia of inflammation may be caused in part by direct cytokine-mediated suppression of erythropoiesis, iron-restricted erythropoiesis mediated by hepcidin is also becoming well established as a key component of the pathogenic mechanism [7, 8].

Iron is a pathogenic determinant of many infectious conditions, not only because of its relationship with anemia, but also since invading pathogens typically require iron for effective replication [9]. The impact of iron status on infection pathogenesis likely differs according to the specific niches of the invading pathogens—whether they are extracellular, macrophage-tropic, hepatocytic, or erythrocytic [9]. While hepcidin-induced hypoferremia may protect against extracellular infections in mice, hepcidin activity and associated shifts in iron compartmentalization may differentially affect pathogens that use alternative niches, such as Plasmodium or Salmonella species [10–12]. The macrophage-tropic M. tuberculosis uses diverse means of scavenging host cell iron, including direct uptake of iron-loaded transferrin and heme, and by producing siderophores, such as mycobactins [13–15]. Similarly, HIV-1 replication can be enhanced by increased cellular iron [16]. Despite all of this, investigations of hepcidin’s involvement in many human infectious conditions, including HIV–M. tuberculosis coinfection, remain limited.

In this issue of The Journal of Infectious Diseases, Kerkhoff et al present a detailed investigation of the relationships of hepcidin status with anemia, tuberculosis severity, and mortality risk in a well-characterized cohort of 232 HIV-infected adults from South Africa [17]. Participants were unselected, consecutively enrolled inpatients with newly diagnosed active M. tuberculosis coinfection or match-ed antiretroviral therapy-naive ambulatory outpatients with or without M. tuberculosis coinfection. The cohort included patients with pulmonary, extrapulmonary, and disseminated tuberculosis, allowing the most thorough observational evaluation to date of the behavior of hepcidin in this context.

While one might expect an acute-phase reactant such as hepcidin to rise in severe HIV–M. tuberculosis coinfection, hepcidin...
transcription may be simultaneously regulated by multiple inputs representing diverse physiological systems, so this should not simply be assumed [5]. For example, although hepcidin is upregulated during uncomplicated malaria, it may be suppressed during severe malarial anemia despite significant inflammation, presumably as a suppressive signal related to high erythropoietic demand dominating during uncomplicated malaria, it may be suppressed during severe malarial anemia of in-ample, although hepcidin is upregulated anemia of in-

Kerkhoff et al generated multivariable Cox regression models, in which hepcidin was found to be an independent predictor of mortality in M. tuberculosis–positive, HIV-infected patients. For each 10-unit increase in hepcidin level (measured by the DRG hepcidin 25 enzyme-linked immunoassay; note that absolute values returned by different hepcidin assays are currently nonequivalent [22]), an 11% increased risk of mortality was found. In contrast, although hemoglobin level was a significant predictor of mortality in univariate analysis, it was not predictive in the adjusted models, indicating that in this set of patients, hepcidin level was more important. A possible explanation is that, whereas there may be multiple causes of low hemoglobin level within this population, individuals with more severe inflammatory anemia, captured best by high hepcidin levels, have the worst prognosis. In other settings, hepcidin performs well as a single index capable of distinguishing inflammatory anemia from iron-deficiency anemia (in which the hepcidin level is low) [23].

So is hepcidin simply another acute-phase marker indicating which patients are sickest and accordingly have the worst prognosis, or could it be more intimately involved with the pathogenic processes occurring during HIV–associated tuberculosis? Are increased hepcidin concentrations a consequence or cause (or both) of worsening disease? The observational data in the present study cannot answer these questions directly, but they add significantly to previous clinical and experimental data in generating testable hypotheses regarding mechanisms.

The authors additionally observed highly significant associations between hepcidin and the acute-phase protein C-reactive protein, strongly linking hepcidin to the acute-phase response. Hepcidin upregulation is, therefore, highly likely a consequence of the inflammatory response to a developing infection. However, although C-reactive protein was predictive of mortality in univariate analysis, like hemoglobin it was not predictive in adjusted models, while hepcidin remained independently associated, consistent with hepcidin being more closely linked to the disease process.

Are there potential mechanisms through which hepcidin could be involved more directly in the pathogenesis of HIV–M. tuberculosis coinfection? On one hand, in vitro studies suggest hepcidin may have direct antimycobacterial properties (noting that the hepcidin concentrations tested were likely supraphysiological) [24, 25], so hepcidin upregulation in infected macrophages might represent a host response aimed at limiting infection. On the other hand, the better-established systemic function of hepcidin may simultaneously contribute to 2 processes relevant to clinical course: first, as described above, hepcidin may promote development of HIV–M. tuberculosis coinfection–associated anemia through serum iron restriction; and second, hepcidin activity enriches iron in the macrophage niche, potentially providing an iron source to favor mycobacterial (and, to an extent, viral) replication [9], while also potentially influencing macrophage immune effector functions [26]. Gene expression profiling suggests that M. tuberculosis experiences the macrophage phagosome as a relatively iron-poor environment, as several iron-acquisition genes, including siderophore genes, are upregulated [27]. Since iron is a crucial factor for M. tuberculosis growth in macrophages [13], increased macrophage iron retention during severe disease may, therefore, provide a source of iron to aid replication and further exacerbate disease in a positive feedback loop. Furthermore, hepcidin-mediated iron retention in lymphocytes may also enhance HIV-1 replication [16]. Additionally, recent studies report that altered iron indices including hepcidin
predict subsequent diagnosis of active *M. tuberculosis* infection in HIV-infected individuals; while the times between iron status assessment and *M. tuberculosis* diagnosis were relatively short (in the order of months), the data suggest that perturbations in iron status related to hepcidin may precede development of active disease [21, 28, 29]. Should further basic science investigations provide mechanistic support for involvement of the hepcidin–iron axis in the pathogenic process of HIV-associated tuberculosis, a potential point of intervention may be revealed. Antagonists of hepcidin production or activity are currently under development, with anemia of inflammation a prime target [7]. Whether these would be effective as means of reducing the pool of accessible macrophage-based iron for *M. tuberculosis* and/or HIV replication while simultaneously alleviating HIV–*M. tuberculosis* coinfection–associated anemia is an interesting question worthy of further examination.

**Notes**

**Financial support.** This work was supported by the UK Medical Research Council.

**Potential conflict of interest.** Both authors: No reported conflicts. Both authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

**References**