The hepatic peptide hormone hepcidin is the central regulator of iron metabolism and mediator of anemia of inflammation. To date, only one specific immuno-dot assay to measure hepcidin in urine had been documented. Here we report an alternative approach for quantification of hepcidin in urine by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS). Peptide peaks were detected corresponding to the 3 forms of hepcidin normally found in urine. The identity of the peak equivalent to hepcidin-25 was confirmed using synthetic human hepcidin-25. Validation of our MS data on samples with various hepcidin levels showed a strong correlation with previous immuno-dot assay results (Spearman R = 0.9275, P < .001). Most importantly, this hepcidin assay clearly discriminates between relevant clinical iron disorders. In conclusion, this novel MS urine hepcidin assay is easy to perform and available to a wide audience. This enables the implementation of hepcidin measurements in large clinical studies. (Blood. 2005;106:3268-3270)
To investigate the feasibility of a mass spectrometry–based assay for the quantification of urine hepcidin, a pilot SELDI-TOF-MS was conducted where the spectra of a patient with septicemia and a healthy volunteer were generated. Figure 1Ai-ii shows in both spectra a clear peak at 2788 m/z that corresponds with the peak mass of 2789 m/z from the synthetic human hepcidin-25 peptide (Figure 1Aiii). Besides hepcidin-25, the urine spectra also show peaks that correspond with reported masses of the N-terminally truncated hepcidin-20 and -22 (respectively 2192 m/z and 2436 m/z, as measured by MALDI-TOF-MS).2 As expected, the intensities of the hepcidin peaks are strongly increased (about 3-fold) in the case of septicemia (Figure 1Ai-ii). The results indicated hepcidin was detectable and quantifiable in urine samples by SELDI-TOF-MS. As the lack of commercially available peptides hampers the mass confirmation of the 20– and 22–amino-acid hepcidin forms, measurements will be based on the hepcidin-25 peptide until new insights on the 20– and 22–amino-acid peptides will approve a change in the data analysis protocol.

Validation of SELDI-TOF-MS measurements

To validate SELDI-TOF-MS measurements, we performed SELDI-TOF-MS on urine samples from our previous study,14 in which...
hepcidin concentration was determined by the immuno-dot assay. The samples were from 10 volunteers injected with lipopolysaccharide (LPS) from whom we collected urine at 4 time points within a 22-hour time frame.\(^1\) Statistic analysis showed a strong significant correlation between the 2 methods (Spearman \(R = 0.9275, P < .001\)) and no significant differences between methods for each volunteer at each time point (Paired \(t\) test \(P > .05\)). These results prove that the SELDI-TOF-MS approach for urinary hepcidin measurements is comparable to the published immunoassay method. In addition to providing accurate results, the assay is fast, simple, and high-throughput, and therefore suitable for large experimental clinical studies.

**Implementation in clinical practice**

To investigate whether hepcidin quantification by mass spectrometry can distinguish between different clinical iron metabolism disorders, urine from patients with several iron-related diseases were used for SELDI-TOF-MS measurements. Figure 1B shows that patients suffering from septicemia as well as those injected with LPS had significant elevated urinary hepcidin excretion compared with healthy subjects (Mann-Whitney \(U\) test, \(P < .05\)). Patients with iron deficiency anemia and (partly) compensated hereditary hemochromatosis showed significant reduced hepcidin excretion compared with healthy subjects (\(P < .05\)). Patients with MDS with transfusion-induced iron overload, serum transferrin saturation values higher than 77\%, and ferritin levels over 500 \(\mu\)g/L showed relatively increased but greatly varying hepcidin levels. This variety precludes differentiation of patients with secondary iron overload from healthy individuals (\(P = .054\)), while median difference with acute infection patients is still significant (\(P < .05\)). These results are consistent with previous reports on hepcidin levels in physiologic and pathophysiologic states.\(^10\),\(^15\)-\(^17\) In addition, the SELDI-TOF-MS method would be suitable for differentiation between (hepcidin-induced) anemia of inflammation, and iron deficiency anemia where hepcidin excretion is physiologically reduced.

In conclusion, we present a novel mass spectrometry–based assay for the high-throughput measurement of hepcidin levels in urine. We anticipate that this will become an important tool to increase our insight in the role of hepcidin in iron metabolism-related disorders.

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**References**