Invited lecture

Bilirubin as a biomarker of disease risk: addressing the analytical challenge

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Introduction. Bilirubin is a lipophilic molecule found in serum at the concentration of 3.5-20 μM, with < 5 % of that being bilirubin diglucuronide. These values result from the balance between daily production of about 300 mg from heme catabolism and the biliary elimination of bilirubin diglucuronide. The clinical value of bilirubinemia has grown from being just a diagnostic biomarker of haemolysis or liver failure to a predictive one. Mild elevations of bilirubin, as in Gilbert's syndrome, are associated with reduced cardiovascular disease and mortality risk. This has sparked the ambition to find non-genetic factors, such as drugs, diets, or life styles, driving mild hyperbilirubinemia. The protective effect of bilirubin is ascribed to the free radical scavenging activity of the redox couple bilirubin/biliverdin. However, high-throughput methods for its analysis are so far lacking, which prevents a deeper understanding of bilirubin homeostasis. We have addressed this unmet diagnostic need by developing a simple, high-throughput method for the analysis of the full set of bile pigments in human blood (i.e., biliverdin, bilirubin, and bilirubin glucuronide), which requires a tiny volume (10 microL) of capillary blood sampled by finger puncture.

Methods. We produced a bifunctional synthetic protein (HUG), composed of a protein scaffold (HELP) fused with UnaG, which binds bilirubin and emits fluorescence. The fluorimetric assay is performed in microtiter plates, requires a multiplate reader, and produces no waste.

Results. We characterised the kinetics of bilirubin binding by HUG. Due to its very high affinity, HUG enabled the fluorimetric titration of bilirubin in albumin-free, neutral solutions in the range 2-100 nM. When in solution as a complex with albumin, all albumin-bound bilirubin was captured by HUG. The HUG method was validated and compared to the standard method based on the diazo reagent. We have applied it for the direct microanalysis of bilirubin in experimental hepatology, human and animal blood.

Discussion and conclusions. This method opens the opportunity to analyze the full set of blood bile pigments in experimental biology and medicine, as well as in clinical trials and personalized medicine studies. We expect to contribute to an improved scientific understanding of bilirubin metabolism and its regulation.

Keywords: bilirubin, biomarker, disease risk