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PERSPECTIVE

Utility of endogenous 4β-hydroxycholesterol as a biomarker to assess cytochrome P 450 3A (CYP3A) activity: not quite ready for prime time

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Kvitne et al. published an open-label, three-arm study evaluating hepatic and intestinal cytochrome P450 (CYP) 3A4 activity utilizing the 4β-hydroxycholesterol concentrations as an endogenous biomarker to evaluate CYP3A4 activity in patients with a wide body weight range ($n=78$, BMI 18.5 to greater than 40 kg/m^2) [1]. The authors are to be commended for evaluating organ-specific quantitative CYP3A4 protein expression and microsomal ex vivo activity. The subject participants are also to be commended for providing liver and small intestinal biopsies. 4β-hydroxycholesterol concentrations correlated with hepatic CYP3A4 concentrations (Spearman $r=0.3$, $p=0.027$) and with hepatic microsomal CYP3A4 activity (Spearman $r=0.53$, $p<0.001$). Intestinal CYP3A4 concentrations and microsomal CYP3A4 activity did not correlate with 4β-hydroxycholesterol concentrations. The authors concluded this study "…provides evidence that 4β-hydroxycholesterol concentrations is a suitable marker for hepatic CYP3A4 phenotyp[ing]" [1].

Correlation coefficients (r) and r^2 values are commonly reported in the literature, provide evidence of an association, and are interpreted to assume suitability of a CYP phenotyping probe drug [2, 3]. However, r values are often overvalued, misinterpreted, provide limited information, and are not suitable in validating a phenotyping probe drug and/or endogenous biomarker for general, widespread use. The limitations of r and r^2 values are discussed elsewhere in detail, but include the inability to measure predictive performance, whether independent (effector) variables are causes of changes in the dependent (outcome) variable, and whether omitted-variable bias exists [4–6].

Validating a phenotyping probe drug and/or endogenous biomarker requires an evaluation of predictive performance by way of assessing bias and precision. Bias represents systematic error and can be observed by over- or under-estimates of the parameter of interest (e.g., exposure or clearance). Precision is random error and represents the "effect size" of variation in a prediction [7]. Appropriate methods to determine bias and precision include visual inspection via Bland–Altman analysis or determining mean prediction error (as a measure of bias) and mean absolute error or root mean square error (as measures of precision) [4, 6]. Based on the current study, predictive performance via assessment of bias and precision needed to be evaluated between 4β-hydroxycholesterol concentrations and systemic midazolam clearance. It is interesting to note that 4β-hydroxycholesterol concentrations did not correlate with systemic midazolam clearance (Spearman $r=-0.03$, $p=0.81$) [1].

We acknowledge the minimal invasiveness, ease of measurement, and the ability to discriminate the strength of CYP3A induction as advantages in using 4β-hydroxycholesterol concentrations as a biomarker. However, given the limitations of r and r^2 values, the need to address previous concerns regarding utility [8, 9], and until proper validation steps have been performed, 4β-hydroxycholesterol concentrations are not a valid biomarker for measuring in vivo, real-time CYP3A activity. Validation criteria for CYP phenotyping probe drugs have been proposed and need to be evaluated in the content of 4β-hydroxycholesterol concentrations [2, 10, 11]. Consequently, we are concerned that the study findings may result in the inappropriate use of 4β-hydroxycholesterol concentrations in future studies evaluating CYP3A-mediated drug-drug interactions.

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Declarations

Competing interests The authors declare no competing interests.

Conflict of interest The authors declare no competing interests.

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