Björkhem-Bergman et al. (2013) published a study in healthy adults evaluating endogenous 4β-hydroxycholesterol-to-cholesterol ratio to measure CYP3A induction. The authors compare CYP3A induction fold-changes and determined correlations between 4β-hydroxycholesterol-to-cholesterol ratio and midazolam clearance after rifampicin administration. Midazolam clearance is a validated biomarker to evaluate CYP3A activity. We commend the authors for attempting to find alternative, simple, and cost-effective methods to evaluate CYP3A activity. However, we are concerned that the study results may lead to inappropriate use of the 4β-hydroxycholesterol-to-cholesterol ratio for evaluating CYP3A-mediated drug-drug interactions.

A statistically significant, but weak, relationship was reported between 4β-hydroxycholesterol-to-cholesterol ratio and midazolam clearance (coefficient of determination \(r^2 = 0.29\), \(P < 0.01\)). The authors state that the ratio “...might be used as a marker to evaluate CYP3A activity at baseline and not only during induction” (Björkhem-Bergman et al., 2013). Although correlation coefficients \((r)\) and/or \(r^2\) values are commonly reported in the literature and used to assume suitability of a cytochrome P450 (P450) probe (Fuhr et al., 2007), \(r\) and \(r^2\) values provide limited information. Correlation coefficients describe the strength and direction of an association between independent and dependent variables. Coefficients of determination describe the degree of variability between variables. Most importantly, \(r\) and \(r^2\) values are not a measure of predictive performance (Sheiner and Beal, 1981; Bland and Altman, 1986). Additional limitations of \(r^2\) values include the inability to determine if the most appropriate set of independent variables was selected, whether independent variables are causes of changes in the dependent variable, and whether omitted-variable bias exists (Nagelkerke, 1991; Draper and Smith, 1998).

Additionally, in a previously published study, the authors compared 4β-hydroxycholesterol to another validated CYP3A probe, quinine, and reported weak \(r\) values with statistical significance \((r = -0.24\) to \(-0.5, P < 0.05\)) (Diczfalusy et al., 2008). However, statistically significant \(r\) and/or \(r^2\) values do not substantiate that a P450 probe is valid for general use. Correlation coefficients and/or coefficients of determination are often overvalued and result in exaggerated conclusions. A P450 probe should adhere to validation criteria to be considered appropriate for use (Watkins, 1994; Zaiqler et al., 2000; Fuhr et al., 2007). Given the limitations of \(r\) and \(r^2\) values in general, as well as in these studies (Diczfalusy et al., 2008; Björkhem-Bergman et al., 2013), we do not believe that the 4β-hydroxycholesterol-to-cholesterol ratio has been validated as a biomarker for the measurement of CYP3A activity.

We commend the authors for attempting to find alternative, simple, and cost-effective methods to evaluate CYP3A activity. However, we are concerned that the study results may lead to inappropriate use of the 4β-hydroxycholesterol-to-cholesterol ratio for evaluating CYP3A-mediated drug-drug interactions.

**References**


**Authorship Contributions**

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