4β-hydroxycholesterol-to-cholesterol ratio as an endogenous biomarker in human plasma to determine treatment effects of induction and inhibition of CYP3A4 enzyme activity

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BACKGROUND

- 4β-hydroxycholesterol (4β-OHC) is a metabolite resulting from CYP3A4 (and CYP3A5 to a lesser extent) metabolism of cholesterol.
- The ratio of 4β-OHC/cholesterol has been proposed as an endogenous biomarker of CYP3A4 and CYP3A5 activity.
- A single small volume blood sample can be used to measure 4β-OHC.
- Potential applications as a biomarker of CYP3A enzyme activity include: prediction of the magnitude of inhibition or induction - thus replacing the use of probe drugs for CYP3A activity; and evaluating multi-directional drug-drug interactions. Direct measurement of the metabolite could provide appropriate and accurate means of assessing CYP3A4 activity.

METHODS

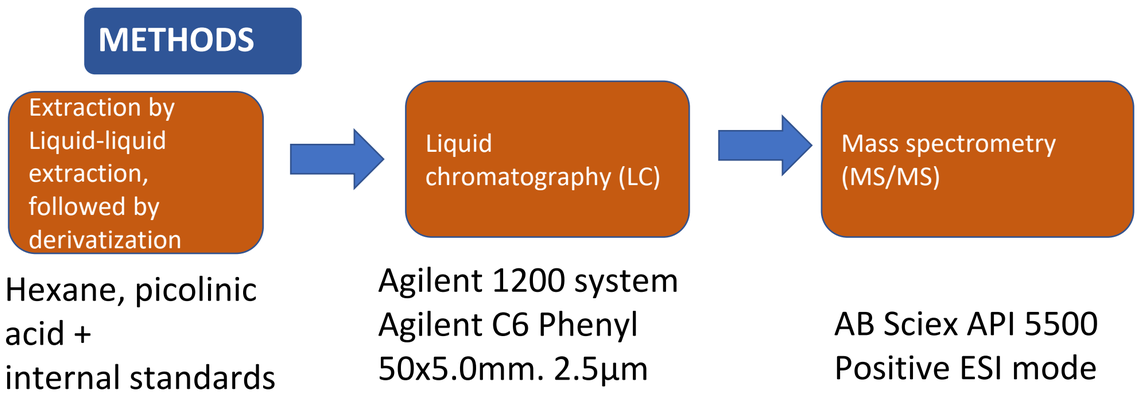

Fig 1: Workflow from sample preparation to analyte detection

- Deuterated internal standards (4β-OHC-d4) for the analyte was used.
- The method provides sufficient accuracy and precision over a calibration range of 2-500 ng/mL
- .Methodology was applied to 2 studies (Darifi and Virtual)
- A multi-level mixed-effects approach was used to create model to investigating 4β-OHC as an endogenous biomarker to describe treatment effects on CYP3A induction and inhibition.

RESULTS

- Within the Darifi study, 4β-OHC/cholesterol changes were in keeping with the observed DRV exposures: baseline 4β-OHC/cholesterol of 3.25 x 10^{-5} on standard darunavir /ritonavir doses of 800/100 mg daily, increased by 56(95% CI: 40, 75) % after 7 days of rifampicin. With adjusted darunavir/ritonavir of 1600/200 mg daily or 800/100 mg twice daily the ratio returned towards baseline values (32 [CI: 19, 61]% and 18 [CI: 0.4, 44]%, respectively).
- A limitation of the ratio however, is that.-Only 4 individuals completed the study which was terminated prematurely due to hepatoxicity.

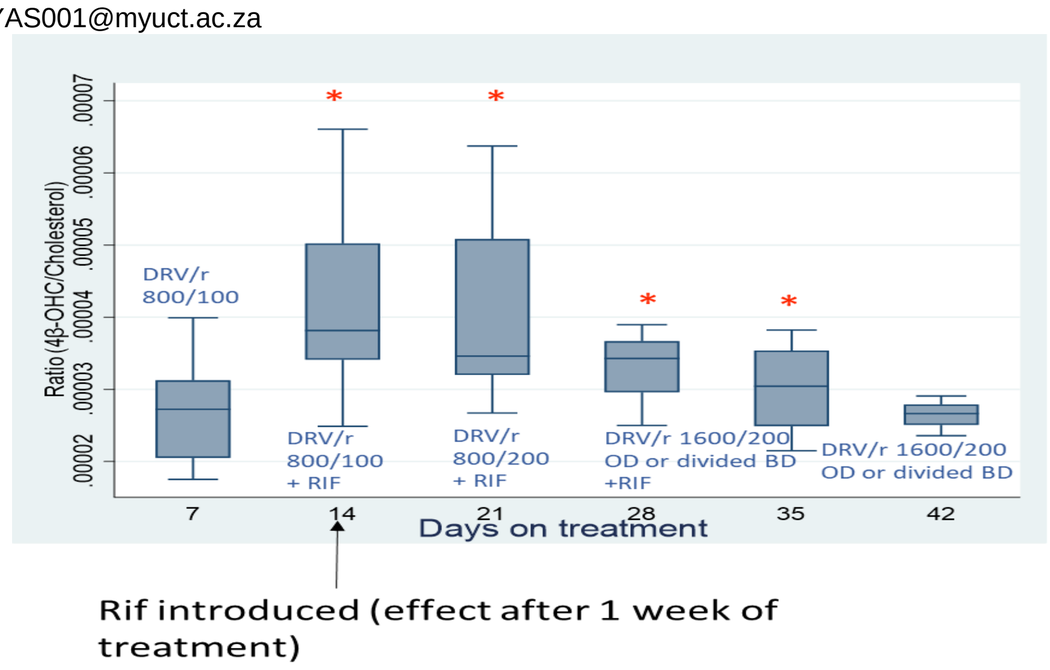

Figure 2: DARIFI Box and Whisker plots of 4β-OHC/Cholesterol ratio vs Visit with mean at each visit for

Table 1: DARIFI 4β-OHC/Cholesterol-Multi-level mixed effects model

Visit 2 (Ref) 4β-OHC/CHO		
REF D7 GM: 3.25 x 10^{-5} ng/ml	% change	P-value
D14	+56 (+40,+75)	<0.001
D21	+53(+33,+75)	<0.001
D28	+46(+20,+78)	<0.001
D35	+32(+19,+61)	0.008
D42	+18(-0.4,+44)	0.117
Age, change/year	-0.2(-0.3,0)	0.052

sex, weight, BMI and CD4 count did not influence the ratio.

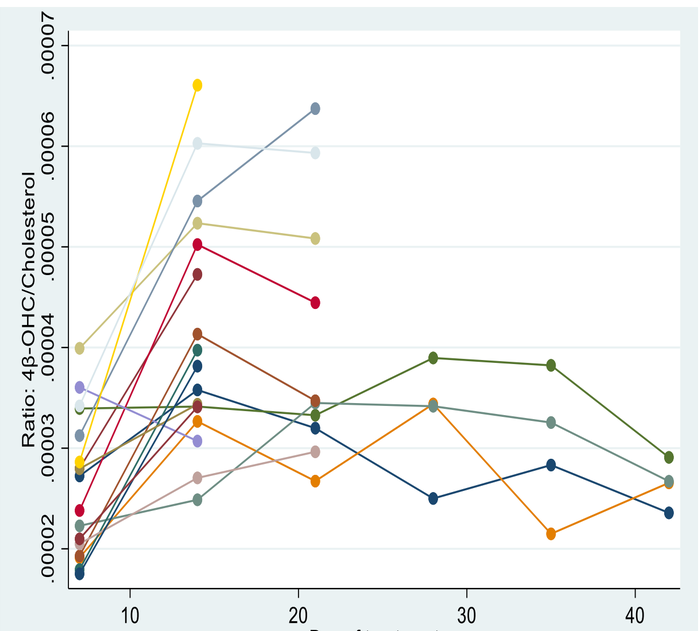

Figure 3: DARIFI Spaghetti plot of 4β-OHC/Cholesterol ratio vs day of treatment

Table 2: Virtual: 4β-OHC/Cholesteroll multi-level mixed effects model

Visit 2 (Ref) 4β-OHC/CHO		
REF D7 GM: 1.36 x 10^{-5} ng/ml	% change	P-value
D14	+76.4 (+56.3,+99)	<0.001
D21	+46.8(+30.1,+65.6)	<0.001
D35	+64(+45.4,+85.1)	<0.001
Age, change/year	-0.2(-0.3,-0.1)	0.011

sex, weight and height count did not influence the ratio.

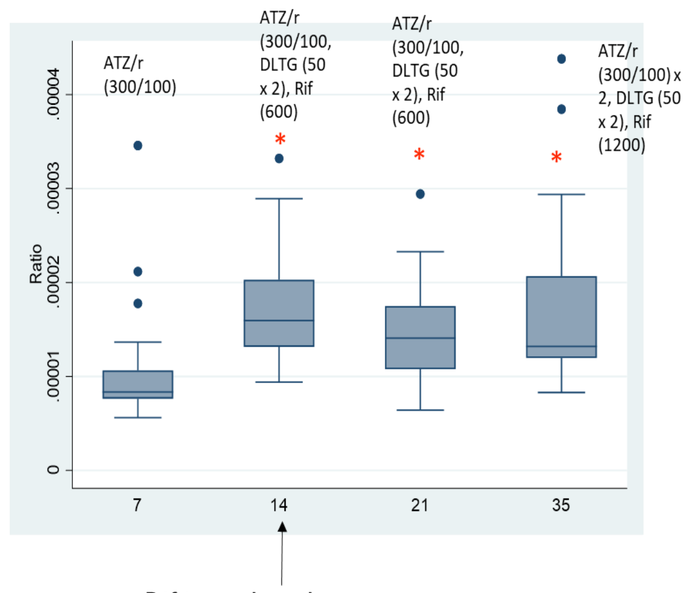

Figure 3: Virtual Box plot of 4β-OHC/Cholesterol ratio vs Days of treatment for all pts

CONCLUSION

- While we had limited sample sizes, our exploratory data suggest that the ratio of 4β-OHC-to-cholesterol may contribute to prediction and understanding of drug-drug interactions.
- Further studies are needed to understand the differences between populations and other factors affecting the 4β-OHC/cholesterol ratio.
- This ratio is potentially useful in a non-study setting as baseline 4β-OHC/CHO ratio could influence drug-drug interactions. Finally, the ratio of 4β-OHC/CHO better correlated with CYP3A activity than when compared to 4β-OHC alone, as the CYP3A pathway is a minor pathway of Cholesterol metabolism.

References

Mao J, Martin I, McLoed J, Nolan G, van Hor R, Vourvahis M, *et al*. Perspective: 4β-Hydroxycholesterol as an emerging endogenous biomarker of hepatic CYP3A drug metabolism review. 2016 Sep; 49(1): 18-34.

Acknowledgements

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