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Abstract # 748

Lipidomic Profiling in HIV: Implications for Risk Prediction Models

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Abstract

Background: HIV positive patients are at increased risk for coronary artery disease (CAD) with both HIV and antiretroviral associated dyslipidaemia contributing. Targeted analysis of over 400 plasma lipid species by mass spectrometry can sensitively identify changes in the plasma lipidome associated with the development of atherosclerosis.

Methods: In a retrospective case-control study we profiled the plasma lipidome (268 lipid species included) of 113 subjects. Cases (n=23) were HIV positive individuals who had a blood sample available in the 12 months prior to the diagnosis of CAD. They were age and sex matched 1:2 with HIV positive individuals without a diagnosis of CAD (n=45, HIV+ controls) and with healthy HIV negative volunteers (n=45, Healthy controls). Risk prediction models incorporating the plasma lipidome were compared with established cardiovascular risk scores.

Results: 104 (92%) were male with a median age of 52.2 years (IQR 40.9-61.4). HIV patients (combined cases and HIV+ controls) were more likely to smoke (31 [45.5%] v's 3 [6.7%], p <0.001), had higher median hsCRP (2.78 [IQR 1.97 – 6.67] v's 0.64 [IQR 0.36 – 1.48], p<0.001) and triglyceride levels (1.77 [IQR 1.47-2.76] v's 1.00 [0.7-1.76], p <0.001) and lower high density lipoprotein cholesterol (0.95 [IQR 0.78 – 1.13] v's 1.50 [IQR 1.1 – 1.7], p <0.001) than healthy controls. 23 (100%) of cases and 40 (88.9%) of HIV+ controls were currently receiving antiretroviral therapy. 83 lipid species and 7 lipid classes were identified that were significantly associated with HIV infection. A further 74 species and 8 classes where significantly associated with future CAD event. 15 species (predominantly in the triacylglycerol and diacylglycerol classes) were elevated in HIV infection and further elevated in HIV subjects who went on to have a CAD event. Risk prediction models incorporating lipid species outperformed (AUC=0.78 (0.775,0.785)) all other tested risk scores (including the Framingham, Reynolds and the Data Collection on Adverse Events of Anti-HIV drugs [D:A:D] risk equations) in the identification of HIV positive subjects who went on to develop CAD.

Conclusions: Treatment-experienced HIV positive patients demonstrate significant differences in plasma lipidome when compared with healthy HIV negative controls. There is a potential application for improved cardiovascular risk screening of HIV positive patients in the clinical setting by including lipid species in risk prediction models.

Background

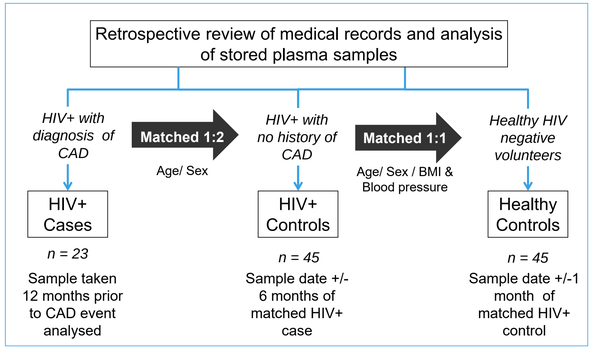
- Currently available cardiovascular risk prediction tools are inaccurate in HIV and recommendations for primary preventative therapy based on their use may lead to inadequate treatment in HIV positive individuals.
- Standard measures of cholesterol and triglycerides do not fully reflect the complex changes in lipid metabolism induced by HIV and ART.
- Plasma lipid profiling, where several hundred lipid species are measured using mass spectrometry has the potential to define underlying changes in lipid metabolism and has shown promise for the enhanced prediction of patients at risk for coronary events, but has not previously been explored in the context of HIV.

Methods

AIMs:

1. To describe the difference in plasma lipid profile between HIV positive patients (with and without CAD) and healthy participants.
2. To develop a predictive model for future cardiovascular events in HIV

Figure 1: Study design



Laboratory Methods:

- Total lipid extraction from a 10ul aliquot of plasma was performed by a single phase chloroform:methanol (2:1) extraction
- Lipid analysis was performed by liquid chromatography, electrospray ionisation-tandem mass spectrometry using a Agilent 1200 liquid chromatography system combined with an Applied Biosystems API 4000 Q/TRAP mass spectrometer with a turbo-ionspray source (350oC) and Analyst 1.5 data system.
- 268 individual lipid species from 24 lipid classes were measured.

Statistical Methods:

- Multiple binary logistic regression was applied to examine the association of plasma lipid species with HIV infection independent of cardiovascular risk (adjusted for family history, smoking, hsCRP) [comparison of HIV control and healthy control group] and for cardiovascular risk in HIV subjects [comparison of HIV case and HIV control].
- Corrected for multiple comparisons using the Benjamin-Hochberg method
- Models were developed to predict cardiovascular events through the application of statistical machine learning approaches (performed in MATLAB 2012b).
- For 3-fold cross-validation, the dataset was split into thirds. The model was trained using two thirds of the data and the performance of the trained model was tested on the remaining third of samples.

Results

Table 1: Participant Characteristics

	HIV Cases (A) n = 23	HIV Controls (B) n = 45	Healthy Controls (C) n = 45	A v's B	B v's C	A v's C
Male (%)	91.3 (21/23)	91.1 (41/45)	93.3 (42/45)	-	-	-
Age (at sample collection)	51.1 (41.0-61.5)	51.2 (40.7-59.1)	54.0 (48.0-57.3)	0.92	0.82	1
Systolic Blood Pressure (mmHg)	120 (120-140)	120 (110-130)	121 (112-132)	0.13	0.61	0.69
BMI (kg/m²)	22.6 (21.1-27.2)	24.3 (21.8-27.7)	23.6 (22.0-26.5)	0.70	0.97	0.86
Family history CHD (%)	52.2 (12/23)	20.0 (9/45)	0 (0/45)	0.07	**0.004**	**< 0.001**
Current Smoker (%)	52.2 (12/23)	42.2 (19/45)	6.7 (3/45)	0.66	**0.002**	**0.001**
Type 2 Diabetes (%)	17.4 (4/23)	6.7 (3/45)	0 (0/45)	0.24	0.24	**0.02**
hsCRP (mg/L)	2.78 (1.97-6.67)	2.26 (1.15-4.23)	0.64 (0.36-1.48)	0.39	**< 0.001**	**< 0.001**
Current Statin (%)	34.8 (8/23)	6.7 (3/45)	0 (0/45)	**0.02**	0.24	**< 0.001**
Detectable Viral Load (%)	39.1 (9/23)	42.2 (19/45)	-	1	-	-
CD4 Count (cells/µL)	452 (310-700)	320 (174-486)	-	0.08	-	-
Currently on ART (%)	100 (23/23)	88.9 (40/45)	-	0.16	-	-

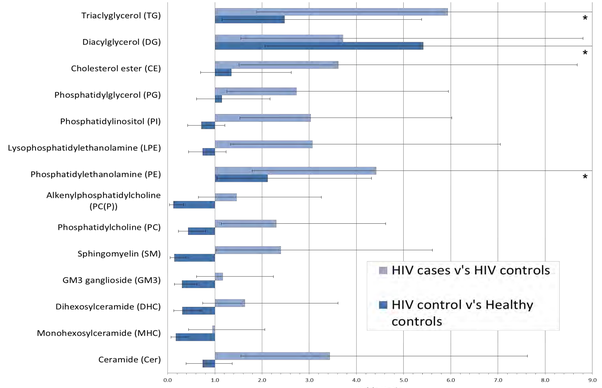


Figure 2: Effect of HIV and future cardiovascular disease on lipid classes* Upper limit 95%CI TG = 18.65; DG = 14.22; PE = 10.91

Odds of HIV infection or future cardiovascular events corresponding to an interquartile range increase in lipid class

Results

- 6 lipid classes were negatively associated with HIV, while diacylglycerols were positively associated (see Figure 2) (Not all species within a class showed the same association)
- 83 individual lipid species were significantly associated with HIV
- Within the HIV+ participants diacylglycerol's showed a further positive association with cardiovascular events along with 7 other lipid classes
- There were 74 individual lipid species which were associated with future cardiovascular events in HIV.

Prediction of Future Cardiovascular Events in HIV

- Area under the receiver operator curve (AUC) for existing risk prediction tools:
  - Framingham 10 year CHD risk = 0.707
  - Reynolds 10 year CHD risk = 0.688
  - D:A:D 10 year CHD risk = 0.686
- Model based on the lipid species performed the best
  - AUC = 0.780 with percentage accuracy of 79%
- Model of combined conventional lipids and lipid species had an AUC of 0.778 & was not statistically significantly different compared to the lipid species model alone.

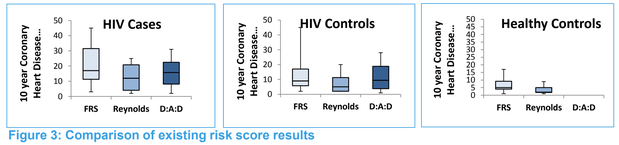


Figure 3: Comparison of existing risk score results

FRS: Framingham risk score. D:A:D: Data Collection on Adverse Events of Anti-HIV drugs equation

Conclusions

- Significant abnormalities in plasma lipid profile were detected in HIV+ individuals compared with healthy volunteers.
- The observed negative association between some lipid species and HIV positive patients may relate to an increase in oxidative stress associated with HIV.
- The strongest markers associated with elevated cardiovascular risk in HIV+ patients were the diacylglycerol [DG] and triacylglycerol [TG] species, where individual species outperform the clinical measure of triglycerides; this observation may have therapeutic implications and warrants further investigation into the effects of treatment of hypertriglyceridemia in HIV+ subjects on DG and TG levels
- Predictive models based on detailed lipid analysis demonstrate the potential to outperform traditional risk equations if confirmed in larger trials this may allow for the accurate, targeted utilisation of primary prevention strategies.

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