LC-MS analysis of metabolites differentiating IRIS from Non-IRIS in ACTG A5221

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Abstract

Background: Immune reconstitution inflammatory syndrome (IRIS) occurs with potent treatment of HIV in the setting of co-infections such as *M. tuberculosis* (Mtb). IRIS is associated with substantial morbidity and mortality. We sought to better understand this syndrome and to develop a metabolic biosignature in plasma from subjects that developed IRIS. Samples were obtained from subjects enrolled in the immediate treatment arm of ACTG study A5221.

Methods: Subjects from the IRIS and Non-IRIS were matched for age, gender, and CD4 cells by the ACTG Data Center. Plasma samples from as many as 3 time points per subject (entry, IRIS window and follow-up) were analyzed. Plasma was extracted in triplicate with methanol and analyzed by liquid chromatography-mass spectrometry (LC-MS). LC-MS data were analyzed with Mass Profiler Professional (MPP) software (Agilent, Inc.) and XCMS online (Scripps Institute) to select molecular features (MFs) that distinguished IRIS and non-IRIS samples based on statistical significance.

Results: Each triplicate revealed 2,000-5,000 MFs and these were down selected based on a FC of 1.5 between IRIS and non-IRIS at each time point with a significance of p<0.05. The greatest difference between the IRIS and non-IRIS samples was observed with the IRIS window time point and included 59 MFs for all replicates analyzed. Four metabolomics databases (METLIN, HMDB, MTB LIPID MAPS and LIPID MAPS) were searched to provide a putative identification of the MFs. MF presumed to be small peptides and lipids (triglycerides, glycosphingolipids, vitamin D metabolites) dominated the signature. Some of the putative lipids are in well-known inflammatory pathways. Each significant MF was manually crossed checked against our in-laboratory Mtb lipid database and no matches were found, leading us to believe that the lipid molecules identified are of host origin. Targeted LC-MS analyses are currently underway to confirm identities and further assess changes in lipid metabolism of IRIS subjects.

Conclusion: These studies provide evidence that a biosignature based on metabolites can separate IRIS from non-IRIS early in the course of disease and may be able to predict those at risk for IRIS. Lipid species appear in our discriminatory signature. Efforts are ongoing to formally identify each of the significant MF we discovered. Ultimately we aim to confirm and validate our findings using samples collected in prospective trials or sites studying HIV/Mtb co-infected individuals who develop IRIS.

Objectives

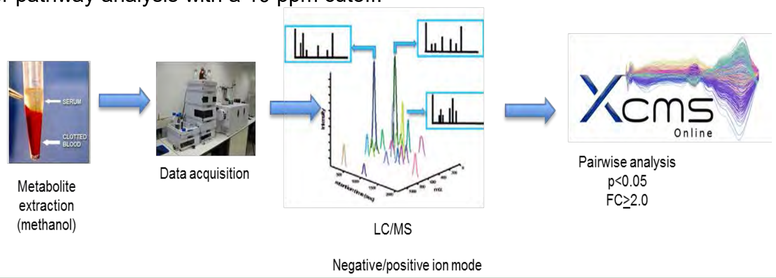
Our aim was to characterize small molecule metabolites that could be diagnostic of IRIS and/or might be able to predict IRIS development.

Methods

We utilized positive and negative ion mode LC-MS methodology to generate large datasets of features for each subject at three time points (Tp) including study entry, a predetermined IRIS window and a late follow-up Tp (Tp 1, 2 and 3 respectively). We sought dysregulated features between the two conditions.

Methods

EDTA-plasma samples were obtained from sites in S. Africa, Malawi, Brazil, Haiti and Peru (4). IRB approvals were via the ACTG and in country approval process. CD4 cell counts ranged from 5 to 212. All subjects were in the immediate ARV (within 2 wks of TB treatment). TB was diagnosed as: confirmed TB or probable TB by study definition. IRIS was defined by published clinical criteria. All patients received efavirenz and truvada. Samples were processed as previously described (1). LC-MS data acquisition occurred using positive and negative ion modes, and a *m/z* range of 100-1700 m/z. Negative and positive mode data were collected from products separated on a C18 and C8 columns, respectively. Pathos (3) was used for pathway analysis with a 10 ppm cutoff.

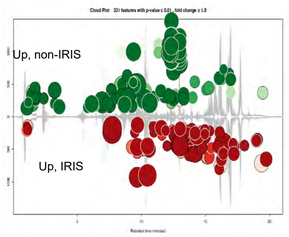


Results

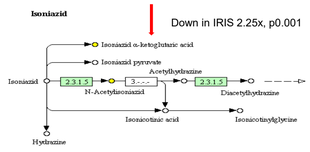
Subject Characteristics

% or medians	Non IRIS (n=28)	IRIS (n=30)
%F	46	36
Wt (kg)	53.5	57.8
Age	35	31
Hgb	10.4	9.3
Alb	3	3
CD4	34	30
VL	5.63	5.63
Countries	RSA, Haiti,Malawi, Brazil	RSA, Haiti,Malawi, Uganda,Botswana
% probable TB*	14	56
Rx arm	100% Immediate	100% Immediate

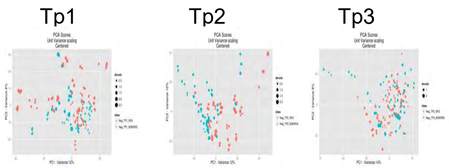
Cloud Plot



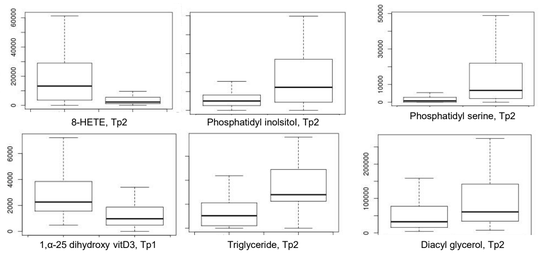
Kegg pathway of drug metabolites tp2



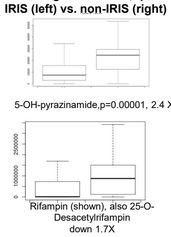
Best separation by PCA occurred at earlier time points



Examples of putative metabolites differentiating IRIS (left) vs. non-IRIS (right), p<0.0;1 fc>1.5



TB drug metabolites, tp2 IRIS (left) vs. non-IRIS (right)

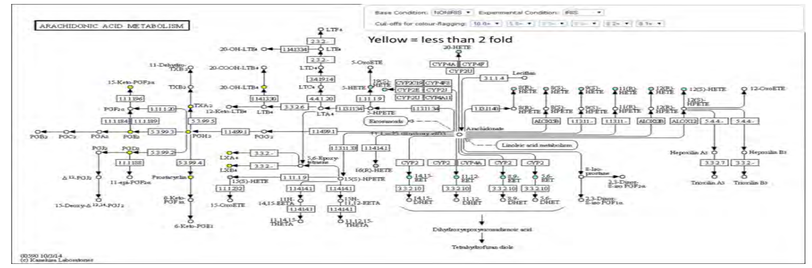


Pathways identified

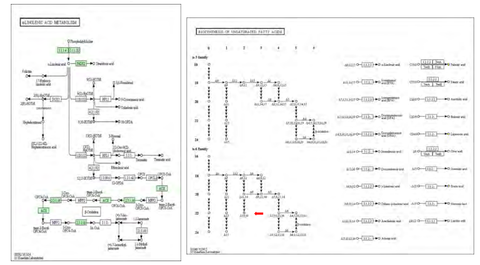
Arachidonic acid metabolism: 19 metabolites out of 74
Linoleic acid metabolism: 17 metabolites out of 25
alpha-Linolenic acid metabolism: 9 metabolites out of 39
Primary bile acid biosynthesis: 6 metabolites out of 47
Ubiquinone/ other terpenoid-quinone biosynthesis: 6 metabolites out of 35
Biosynthesis of unsaturated fatty acids: 5 metabolites out of 49
Phenylalanine metabolism: 5 metabolites out of 64
Steroid hormone biosynthesis: 5 metabolites out of 99
Steroid biosynthesis: 4 metabolites out of 45
Tyrosine metabolism: 4 metabolites out of 74
Drug metabolism - cytochrome P450: 3 metabolites out of 74
Porphyrin and chlorophyll metabolism: 3 metabolites out of 93
Butanoate metabolism: 2 metabolites out of 38
Drug metabolism - other enzymes: 2 metabolites out of 29
Fatty acid biosynthesis: 2 metabolites out of 10

Results

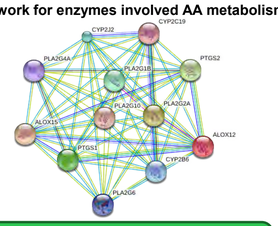
KEGG pathway for Arachidonic acid metabolites



KEGG pathways for linoleic and α-linoleic metabolites



String network for enzymes involved AA metabolism



Conclusions

- The IRIS timepoint (Tp2) gives the best separation in the two groups.
- Most features are of unknown identity, the remaining are putatively identified
- Putative vitD metabolites are higher at Tp1
- Fatty acids are among the most differentially seen metabolites in IRIS. Malnourishment or differences in diet may explain this
- Arachidonic acid, linoleic and α-linoleic were the primary pathways that differed between IRIS and non-IRIS, but no change in prostaglandins or leukotrienes were seen
- Certain TB drug metabolites were lower in IRIS patients, possibly suggesting more Mtb antigens might be driving the inflammation
- Further work to identify key compounds using known synthetic standards is underway.

References

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3. Leader, DP, et al Rapid Commun Mass Spectrom. 2011 Nov 30;25(22):3422-6

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