Characterization of plasma metabolite profile in HIV+ persons with or without IRIS

National Institute of Allergy and Infectious Diseases

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Background

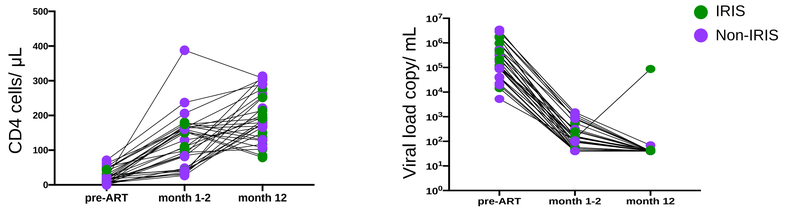
Immune reconstitution inflammatory syndrome (IRIS):

- IRIS is an antigen-driven inflammatory disorder occurring in HIV-infected patients after the initiation of antiretroviral therapy (ART). IRIS can cause significant morbidity and mortality despite successful HIV virologic suppression.
- IRIS pathogenesis is not fully delineated, however, metabolic dysregulation has been frequently associated with inflammatory conditions.
- We hypothesized that evaluation of metabolic pathways could further elucidate IRIS pathogenesis.

Methods

Patient cohort

HIV-infected ART naïve individuals (n=30, IRIS n=13, non-IRIS n=17) with CD4<100 cells/μL were identified in a prospective study (NCT02147405).



Metabolite profiling and plasma cytokine detection

Non-targeted global metabolomics profiling was performed by Metabolon Inc. Frozen plasma samples from each patient at different time points were subjected to methanol extraction then split into aliquots for analysis by ultrahigh performance liquid chromatography/mass spectrometry. Metabolites were identified by comparison to a reference library. Plasma cytokines were measured using Meso Scale multiplex cytokine detection kit.

Data analysis

The differentially expressed metabolites were determined by multiple t-tests selected with p-value≤0.05. Hierarchical cluster analysis using the Ward's method. PCA was performed using JMP Statistical Discovery PRO (V13). Decision trees were employed to identify a minimal set of markers allowing separation between IRIS from non-IRIS and mycobacterial IRIS and other IRIS using J48 algorithm implemented in the WEKA program. To estimate the accuracy of decision tree models, we employed a 10-fold cross validation methodology. Sensibility and specificity were measured from the confusion matrix and the receiver–operating characteristic curve (ROC) were performed using JMP Statistical Discovery PRO (V13). The co-expression analysis was performed using the CEMiTool package, which determines the modules that are altered in a specific sample group.

Results

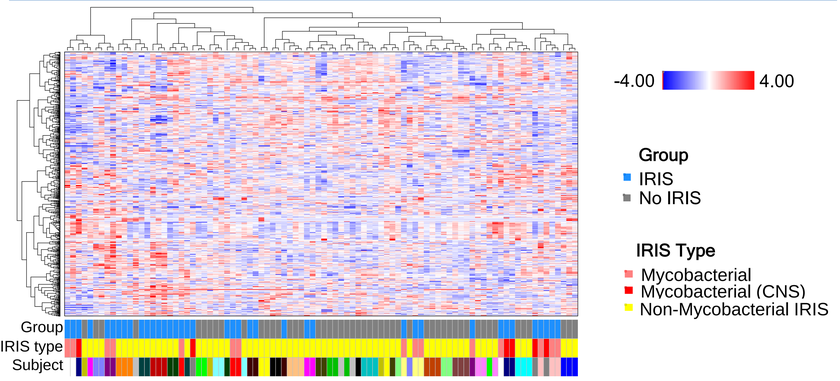


Figure 1: Hierarchical clustering showing overall metabolic profile (832 metabolites) comparisons for each individual samples.

Results

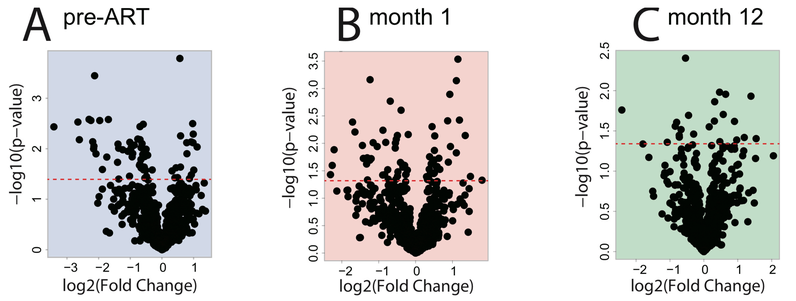


Figure 2: Volcano plots depicting individual metabolites of IRIS vs. non-IRIS patients are shown for pre-ART (A), month 1 (B), and month 12 (C) after initiation of ART. The significance threshold is indicated by the red dashed line.

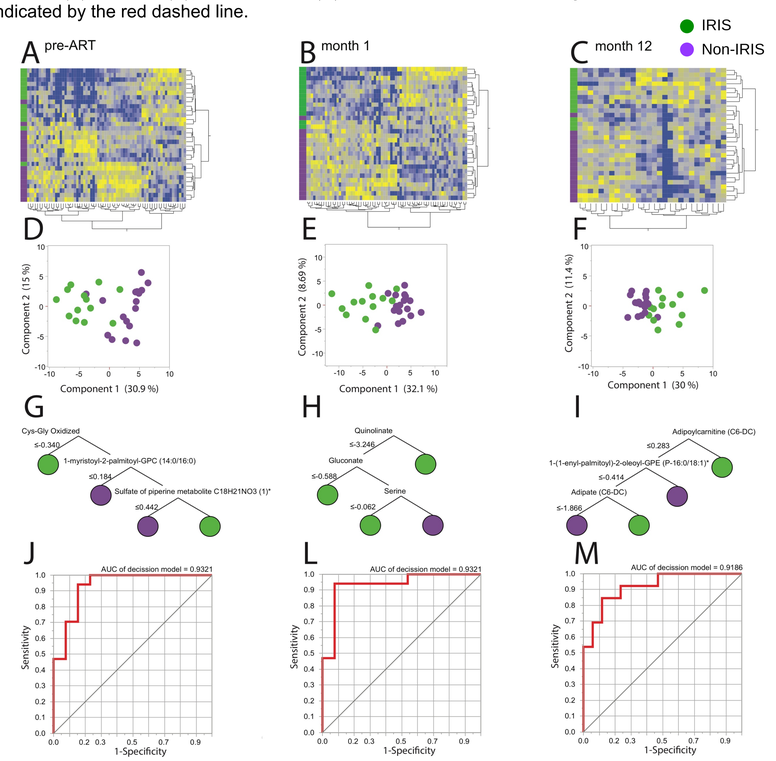


Figure 3: Identified differentially expressed metabolites were used in an unsupervised cluster analysis and PCA for pre-ART (A, D), 1 month (B, E), and 12 months (C, F) post ART. Decision trees of these differentially expressed metabolites are shown in G, H, I, and ROC analysis for decision tree discrimination power are shown in J, L, M for pre-ART, 1 month, and 12 months post ART respectively.

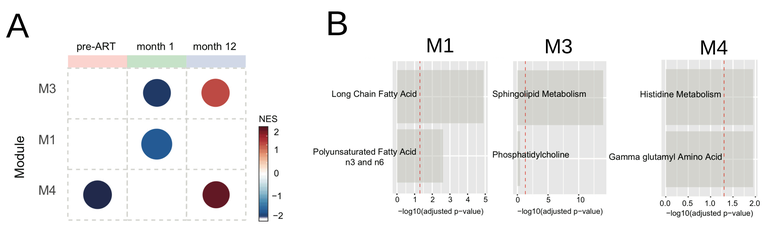


Figure 4: Co-expressed metabolic pathway modules that differentiated IRIS vs. no-IRIS at the three time points are shown in A and B.

Results

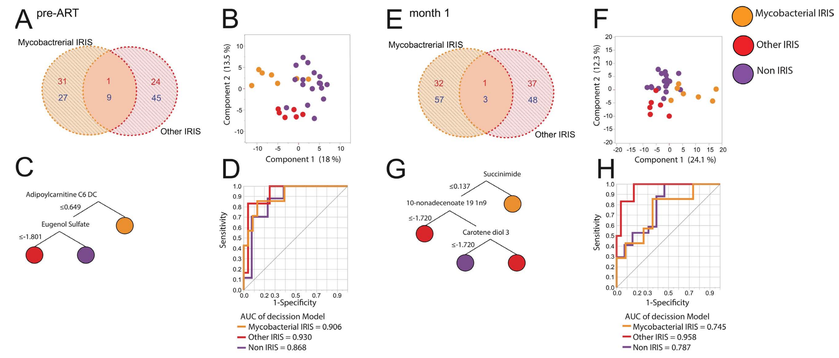


Figure 5. Mycobacterial IRIS compared with other types of IRIS presents with distinct metabolic profile. Venn diagrams are showing the number of differentially expressed metabolites at pre-ART (A), and 1 month post ART (E). Additionally, these differentially expressed metabolites were analyzed by PCA (B, F), decision tree, and ROC analysis for evaluation of decision tree discrimination power (D, H).

Figure 6: Plasma biomarkers are correlated with metabolic pathways. Spearman correlation between values of plasma biomarkers and the normalized enrichment score of co-expressed pathway modules are shown in A and B with p-value <0.05 and correlation coefficient (r) of above 0.5 or below -0.5.

Conclusions

- HIV patients, who develop IRIS, have a distinct metabolite profile. Specific metabolites may also serve as predicting markers.
- Mycobacterial IRIS exhibits unique differentially expressed metabolites compared with other types of IRIS.
- Plasma biomarkers such as IL-2, IL-8 and IL-12p70 correlate with metabolic pathways in the families of lipid and amino acid metabolism.

Acknowledgements

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