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Introduction

Heart failure with reduced ejection fraction (HFrEF) is a cardiovascular disease distinguished by low-grade chronic inflammation[1]. Literature suggests that disturbed metabolic pathways within cardiomyocyte mitochondria play a significant role in systemic wide inflammation seen in HFrEF[2]. These mechanisms are linked to the activation of cytokine receptor Interleukin-1 (IL-1). This has led to the testing of repurposed drug therapies such as Kineret (Anakinra) that inhibits the activation of IL-1. To measure the outcomes of Anakinra, investigators have used cardiopulmonary exercise tests (CPET) and high-sensitivity CRP (hs-CRP)[3,4]. Clinical trials using these markers have demonstrated promise for reducing inflammation after 12 weeks of administration[5]. Little is known though as to how Anakinra impacts the heart's metabolic pathways underlying inflammation. Therefore we have taken a hypothesis-driven approach to characterize the HFrEF metabolic pathways affected by Anakinra for different therapeutic durations; for durations of 2 weeks (Ank-2) and for 12 weeks (Ank-12).

Methods

Post-hoc analysis was performed on 49 patients with reduced ejection fraction, mostly African American (79.6%) and male (75.5%) from the VCU REDHART study. Lipids from HFrEF patients' plasma and serum were quantified via a ScieX TripleTOF 6600 mass spectrometry paired to an Agilent 1290 liquid chromatograph (LC). An acquity UPLC CSH C18 column (100 x 2.1 mm; 1.7µm) was used with the LC. Metabolites were acquired by a Leco Pegasus IV TOF mass spectrometer coupled to an Agilent 6890 gas chromatograph (GC) equipped with a Gerstel automatic liner exchange system (ALEX) that included a multipurpose sample (MPS2) dual rail, and a Gerstel CIS cold injection system. Metabolic data were filtered to exclude detected exogenous metabolites identified as medications or products of gut microbiota. Data were normalized, filtered using IQR, log transformed, and pareto scaled. Regularized Linear Discriminant Analysis (rLDA) was used to discover any meaningful group separations based on acquired metabolites and lipids. Metabolic Pathway Analysis selected enriched metabolites that had been annotated on Kyoto Encyclopedia of Genes and Genomes (KEGG). A multivariate analysis generated a biologically valid testable hypothesis on the direction of analytes in response to Anakinra. A final univariate metabolomic analysis revealed that the circulating metabolic profiles of patients treated with Anakinra compared to placebo were significantly different after 12 weeks of treatment (one-tailed t-test, p<0.05). All analyses were performed in Metaboanalyst 4.0 and JMP 14 Pro.

Results

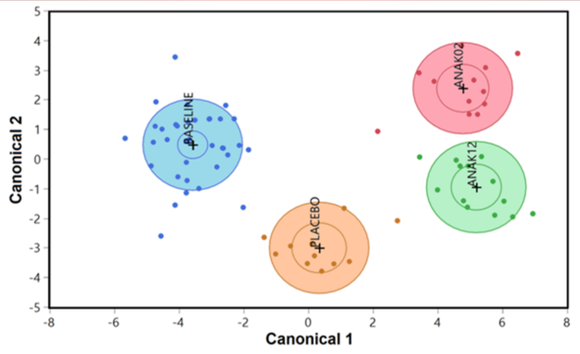

Figure 1: Baseline and treatment groups' separation obtained from analytes. Regularized Linear Discriminant Analysis (r-LDA) demonstrated group separation after stepwise selection of 30 lipids and metabolites. Selected predictors explain the group's differences and spatial separation. Mahalanobis distance from baseline revealed that Anakinra 2 weeks and Anakinra 12 weeks treatment can be distinguished from Placebo (Entropy R^2= 0.99 – 0% misclassification).

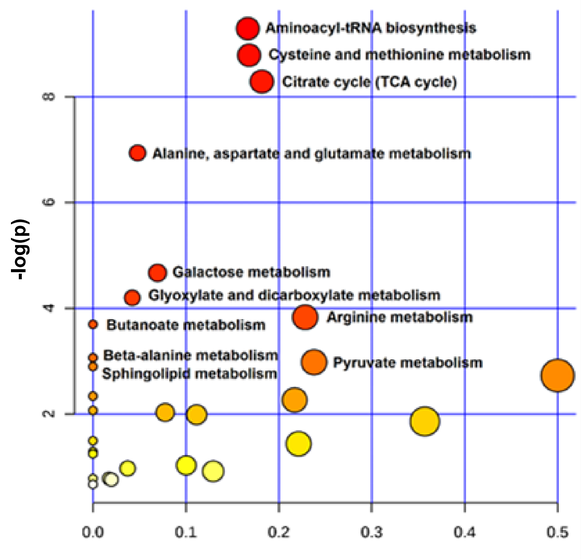

Figure 2: Metabolic pathways associated with Anakinra treatment. Ellipse diameter indicated the magnitude of the impact on the pathway enrichment analysis. All pathways had a statistically significant impact in the enrichment analysis (p<0.05). Ellipse color represent p-value (yellow= low, red=high). Only metabolic pathways with p-value p≤ 0.5 are labeled.

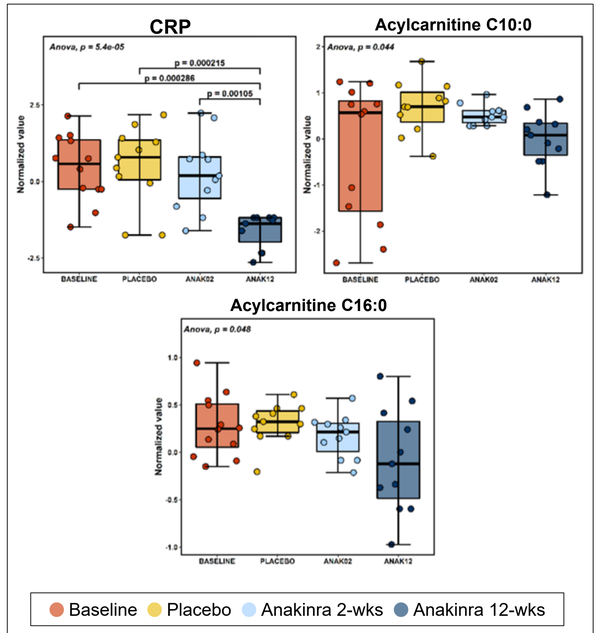

Figure 3: Comparison of significant CRP and acylcarnitine modulations from baseline and treatment groups. Treatment groups were Anakinra 2 weeks, Anakinra 12 weeks and placebo 12 weeks. All values (y-axis) are normalized. Analysis of variance (ANOVA) retrieved p≤0.05 per analyte.

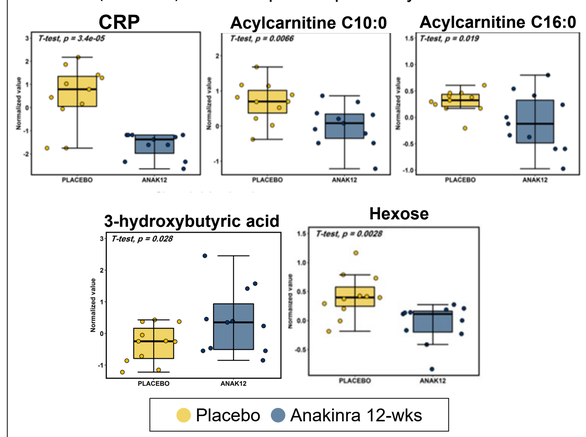

Figure 4: Anakinra 12-weeks yields benefits compared to placebo. The derived hypothesis that Anakinra after 12 weeks would carry a different effect on the metabolome compared to placebo, tested with one-tailed t-test. All values (y-axis) are normalized. Metabolites statistically different (p≤0.05) suggested metabolic changes following 12 weeks of Anakinra treatment.

Function	Metabolites	PL	A12	t-test p-value
Modulation of Inflammation`	CRP		*	0.001
	Acylcarnitine C10:0		*	0.007
	Acylcarnitine C16:0		*	0.023
Energy Consumption	Hexose		*	0.003
	3-hydroxybutyric acid		*	0.030

Table 1: Metabolomic functions affected by Anakinra following 12-weeks treatment. Hypothesis-driven test of Anakinra effect different from placebo after 12 weeks (one-tailed Student's t-test). The hypothesis tested the downregulation of inflammatory markers, and upregulation of metabolites and pathways participating in cardiac energy production. Effects are represented by color (green = low, red= high).

Conclusions

Metabolites and lipids revealed inflammatory and energetic pathways responding to Ank-12. The TCA cycle and butanoate metabolism were among those reported. Downstream IL-1, inflammatory hsCRP was expected to decrease. After Ank-12 hsCRP levels markedly decreased indicating reduced systemic inflammation. Medium and long-chain acylcarnitines (Acyl) C10:0 and C16:0 are elevated due to oxidative stress, an imbalance that leads to inflammation. It was hypothesized concentrations of Acyl would decrease after Ank-12; due to decreasing inflammation and restoring cardiac function re-enabling cardiac mitochondrial FA β-oxidation (FAO). Healthy hearts rely on FAO for ~95% of ATP production. During HFrEF there is a shift towards utilizing glucose and ketones[6]. Overall glucose metabolism also improved with treatment indicated by decrease in hexose in response to treatment. For ketone bodies, 3-hydroxybutyric acid (BHA) is consumed by failing hearts. Higher levels of BHA following Ank-12 implies less consumption of and reliance on BHA. This study provides the metabolomic logic behind Ank-12's response for patients.

Limitations and Future Directions

Small sample size may have skewed the results of Anakinra effect towards representing an African American male subgroup. Baseline metabolic variability highly affected the power of the analysis in detecting metabolites post-treatment. Variability was overcome by a Random Forest outlier detection algorithm to select baseline values that did not significantly differ from the treatment groups. Nonetheless, meaningful changes resultant of IL-1 blockade were detected after 12 weeks of treatment. Future studies applying metabolomic techniques with more power could show Anakinra alterations earlier than 12 weeks.

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