Metabolites as Biomarkers of Adverse Reactions Following Vaccination: A Pilot Study using Nuclear Magnetic Resonance Metabolomics

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Public Health Division
Immunization Healthcare

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Disclaimer

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Adverse Events Following Immunizations (AEFI)

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- An Adverse Event Following Immunization (AEFI) is an adverse reaction to a vaccination that goes above and beyond the usual side effects that are known to be associated with vaccinations.
  - Vary in clinical severity from very mild to incapacitating
  - Rarely, an aberrant immune reaction from vaccination results in a potentially serious adverse event
    - A serious adverse event related to the smallpox vaccine is myocarditis and/or pericarditis (myopericarditis).

Metabolomics

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- Metabolomics is the study of the small-molecule metabolite profile of a biological organism.
  - Functional view of the organism as determined by the sum total of DNA, RNA, proteins and environmental factors
  - More accurate picture of the actual phenotype of the organism
- Metabolomics may help identify a particular metabolic signature or “metabotype” in patients who are predisposed to developing AEFI such as a severe systemic reaction or myocarditis, that currently is difficult or impossible to identify prior to the development of the AEFI.

Study Goals

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- **Exploratory Study Goals**
  - Use metabolomics to help determine if metabotypes of baseline samples could be used to predict AEFI
  - Reveal potential biomarkers of AEFI by comparing case and control samples

Study Impact

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- The ability to predict who may experience an adverse event following immunization (AEFI) based on metabolic profiles could significantly impact immunization health outcomes and help lead the way to personalized immunization healthcare.

Study Design

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- Pre- and post-vaccine samples from 100 subjects were processed for metabolomics analysis across the following vaccine response groups:
  - **Group 1** : 5 subjects had an AEFI and developed clinically diagnosed myocarditis (**+MyoC**)
  - **Group 2** : 30 subjects had evidence of elevated troponins, but without symptoms following immunization (**+Trop - Sx**)
  - **Group 3** : 31 subjects had systemic symptoms following immunization (**SSFI**)
  - **Group 4** : 34 subjects had no symptoms and served as AEFI controls (**no AEFI**)

Methods- Sample Preparation

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- 200 frozen serum samples were prepared for metabolomics analysis.
- 22 Quality Control (QC) samples were also generated from smaller aliquots of the source material.
- Methanol (60%) was added to each of the 200 experimental and 22 QC samples for extraction.
- Samples were vortexed, centrifuged and supernatants were transferred to new tubes and lyophilized to dryness.
- Samples were reconstituted in phosphate-buffered D_2O master mix, vortexed and centrifuged.
- A 600 µL aliquot of the supernatants was transferred into NMR tubes for data acquisition on a 700 MHz spectrometer.

Data Acquisition and Analysis

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- ^{1}H NMR spectra of individual and pooled samples were acquired on a Bruker Avance 700 MHz NMR spectrometer using standard data acquisition and processing methods.
- NMR spectra were processed using intelligent binning. The binned data and associated metadata were transferred to Umetrics SIMCA-P-14.1 for multivariate analysis by Principal Components Analysis (PCA) and Orthogonal Partial Least Squares-Discriminant Analysis (OPLS-DA).
- The bins important to the differentiation of phenotypes were determined through inspection of Variable Influence on Projection (VIP) plots (SIMCA), statistical tests, and fold changes (SAS).
- Bins that were found to be important for group separation (VIP $\geq$ 1, p-value $<$ 0.10 and fold change $\geq$ 2) were library-matched to metabolites using Chenomx NMR Suite 8.1 Professional.

Subject Characteristics

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Characteristic	+MyoC (n= 5)	+Trop – Sx (n= 30)	SSFI (n= 31)	no AEFI (n= 34)
Age, years (Mean, SD)	26.6 (6.9)	21.7 (3.3)	24.4 (7.4)	23.2 (6.5)
Male	4 (80%)	29 (96.7%)	27 (87.1%)	30 (88.24%)
Race				
White	5 (100%)	21 (70%)	23 (74.2%)	23 (67.7%)
Black	0	1 (3.3%)	3 (9.7%)	5 (14.7%)
Hispanic	0	3 (10%)	4 (12.9%)	2 (5.9%)
Asian	0	3 (10%)	0	2 (5.9%)
Other	0	2 (6.7%)	1 (3.2%)	2 (5.9%)
Non-Smoker*	2 (40%)	22 (73.3%)	13 (41.9%)	21 (61.8%)
Excellent Health (pre-vaccine)	3 (60%)	23 (76.7%)	16 (51.6%)	28 (82.4%)
Family History of CAD	1 (20%)	2 (6.9%)	2 (6.5%)	6 (17.7%)

*never smoker, former (>1 year)

How do baseline or post-vaccine only samples separate across the 4 Groups?

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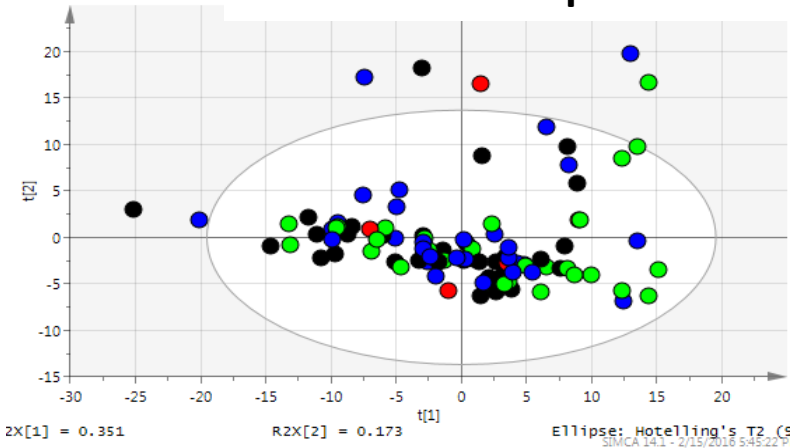
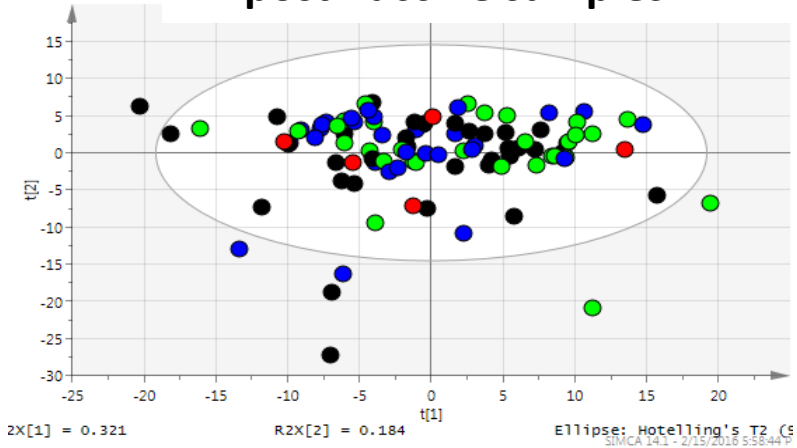
All baseline samples



All post-vaccine samples



- Group 1 (+MyoC)
- Group 2 (+Trop - Sx)
- Group 3 (SSFI)
- Group 4 (no AEFI)

In PCA there were no clear patterns of separation based on Group categorization.

How do samples from subjects that developed MyoC compare at baseline versus post-vaccination?

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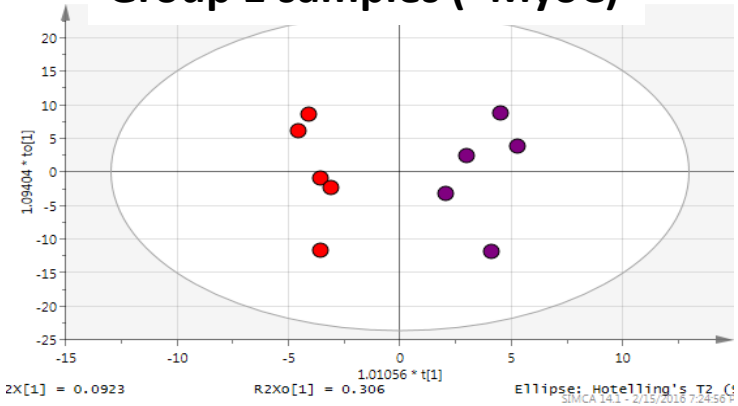
Group 1 samples (+MyoC)



Baseline

Post-vaccine

Significant metabolites identified by group-differentiating bins

Metabolite names	VIP	*p*-value*	Fold Change**
2-oxoisocaproate	0.5	0.063	1.1
3-Methyl-2-oxovalerate	0.6	0.063	-1.1
Acetate | Lysine | Arginine	1.8	0.063	-1.1
Creatine | Creatine Phosphate | Tyrosine	1.3	1	-1.3
Creatinine | Creatine | Creatine phosphate | Lysine	2.7	0.063	-1.3
Fructose | Histidine | Phenylalanine | Serine	1.4	1	-1.2
Glucose (5 bins)	1.3 - 2.5	0.063 - 0.625	-1 - 1
Glucose | Unsaturated lipids	1.2	0.813	1.1
Lactate	1.5	0.625	-1.2
Leucine (2 bins)	1 - 2.1	0.063	-1.1 - -1.1
Lipids (2 bins)	0.7 - 0.9	0.063	-1.1 - -1.1
Lysine | Arginine	0.8	0.063	-1.1
Overlap (4 bins)	0.6 - 1.5	0.063 - 1	1.3 - 7.8
Proline	1	0.063	-1.1
Propylene glycol	1.6	0.063	-1.4
Taurine | Betaine | Glucose | TMAO	2	0.813	-1
Unknown	0.9	0.625	-5.6
VLDL | LDL | HDL | Isocaproate	1.4	0.438	1.1

**Wilcoxon Signed Rank Test*

***A positive fold change indicates median of Post-vaccine > median of Baseline*

How do samples from subjects that had elevated troponins compare at baseline versus post-vaccination?

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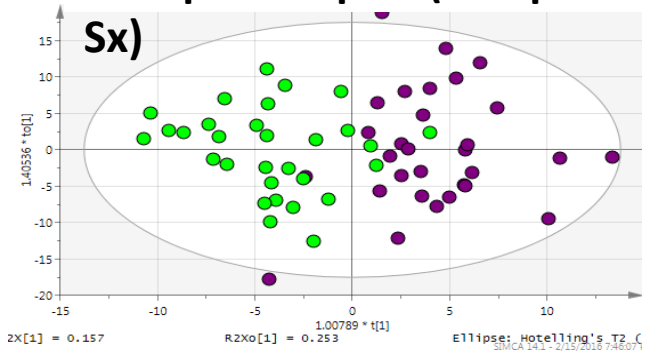
Group 2 samples (+Trop - Sx)



Baseline

Post-vaccine

Metabolite	VIP	p-value*	Fold Change**
2-Aminobutyrate (2 bins)	0.4 - 0.5	0.003 - 0.02	-1.1 - -1.1
2-oxoisocaproate	0.4	0.034	-1.1
3-Hydroxybutyrate | VLDL | LDL	2.1	0.451	-1.1
3-Methyl-2-oxovalerate	0.6	0.006	-1.1
Acetate | Lysine | Arginine	0.9	0.017	-1.1
Alanine	2.8	<.001	-1.2
Arabinose	0.1	0.282	2.4
Asparagine (3 bins)	0.4 - 0.9	0.005 - 0.027	-1.2 - -1.1
Asparagine | N,N-Dimethylglycine	0.6	0.013	-1.1
Choline | O-Phosphocholine | sn-Glycero-3-phosphocholine	1.8	0.008	-1.1
Citrate (2 bins)	0.7 - 0.9	<.001 - 0.002	-1.2 - -1.1
Citrate | Glutamine	0.5	0.095	-1.1
Creatine | Creatine Phosphate | Tyrosine	1.1	0.476	-1.1
Creatinine | Creatine | Creatine phosphate | Lysine	1.3	0.006	-1.1
Fructose | Histidine | Phenylalanine | Serine	1.3	0.315	-1.1
Glucose (8 bins)	1.6 - 3.9	<.001 - 0.017	1 - 1.2
Glucose | Glycerol	2	0.001	1.1
Glucose | Unsaturated lipids	2	<.001	1.1
Glutamate | Proline	0.8	0.001	-1.1
Glutamine (3 bins)	0.8 - 1.6	<.001 - 0.051	-1.1 - -1.1
Isoleucine | 3-Methyl-2-isovalerate	0.6	0.012	-1.1
Isoleucine | Leucine	1.3	0.629	-1
Isoleucine | Valine	1.1	0.372	-1
Lactate	2.3	0.025	-1.2
Lactate | Threonine	4.7	0.014	-1.2
Leucine (2 bins)	0.4 - 1	0.068 - 0.125	-1.1 - -1.1
Lipids (3 bins)	0.5 - 1.1	<.001 - 0.002	-1.2 - -1.1
Lysine (2 bins)	0.5 - 0.5	0.02 - 0.069	-1.1 - -1.1
Lysine | Arginine	0.6	0.002	-1.1
Methionine | Citrate	0.6	<.001	-1.2
Overlap (9 bins)	0.2 - 1.3	0.004 - 0.476	-1.2 - 3.3
Proline (2 bins)	0.7 - 1.3	<.001 - 0.006	-1.1 - -1.1
Propylene glycol	0.9	0.003	-1.2
Taurine | Betaine | Glucose | TMAO	1.9	0.507	1
Trimethylamine	0.4	0.033	-1.1
Unknown (2 bins)	0.3 - 0.6	0.001 - 0.103	-1.1 - 4.8
Unsaturated lipids	1	0.212	-1.1
VLDL | LDL	1.3	0.934	1
VLDL | LDL | HDL | Isocaproate	1.4	0.11	-1.1
sn-Glycero-3-phosphocholine	0.9	0.094	-1.3

**Paired t-test*

***A positive fold change indicates mean of Post-vaccine > mean of Baseline*

How do SSFI subjects baseline & post-vaccination samples compare?

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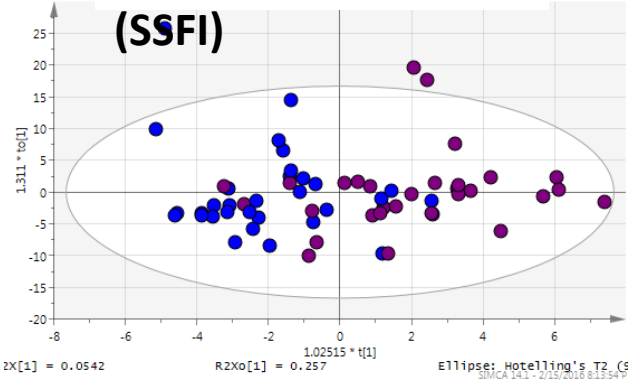
Group 3 samples (SSFI)



Baseline

Post-vaccine

Significant metabolites identified by group-differentiating bins

Metabolite	VIP	p-value*	Fold Change**
3-Hydroxybutyrate | VLDL | LDL	2.3	0.916	-1
Alanine	1.8	0.009	-1.1
Arabinose	0.5	0.003	10.7
Asparagine | N,N-Dimethylglycine	0.7	0.069	-1.1
Choline | O-Phosphocholine | sn-Glycero-3-phosphocholine	1.6	0.13	-1.1
Creatine | Creatine Phosphate | Tyrosine	2.2	0.082	1.2
Creatinine | Creatine | Creatine phosphate | Lysine	1.5	0.11	-1.1
Fructose | Histidine | Phenylalanine | Serine	1.7	0.208	1.1
Glucose (8 bins)	1.6 - 2.6	0.014 - 0.762	1 – 1
Glucose | Glycerol	2.3	0.058	1
Glucose | Unsaturated lipids	1.2	0.576	-1
Glycerol | Isoleucine	2.3	0.004	1.1
Histidine	1.2	0.178	-1.1
Lactate	2.1	0.294	-1.1
Lactate | Threonine	6.1	0.02	-1.1
Methionine | Citrate	0.6	0.006	-1.1
N-Acetylamino acids | Proline	2.4	0.018	1.1
Overlap (10 bins)	0.6 - 1.7	0.003 - 0.178	-1.2 - 4
Oxypurinol	0.3	0.205	-2.1
Proline	1	0.007	1.1
Taurine | Betaine | Glucose | TMAO	2.5	0.147	-1
Threonine (2 bins)	1.1 - 1.2	0.065 - 0.121	1.1 - 1.2
Unknown	0.1	0.221	-2
Unsaturated lipids	1.1	0.754	1
VLDL | LDL	1.5	0.605	-1
VLDL | LDL | HDL | Isocaproate	1.3	0.814	1
Valine	1.3	0.009	1.1
sn-Glycero-3-phosphocholine	1.1	0.125	1.3

**Paired t-test*

***A positive fold change indicates mean of Post-vaccine > mean of Baseline*

How do No AEFI subjects baseline & post-vaccination samples compare?

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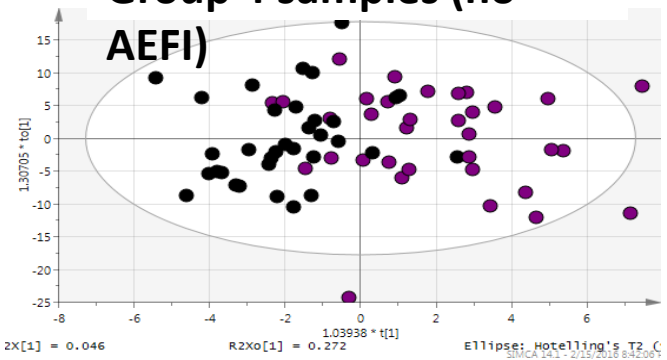

Significant metabolites identified by group-differentiating bins

Metabolite	VIP	p-value*	Fold Change**
3-Hydroxybutyrate | VLDL | LDL	1.8	0.754	-1
3-Hydroxyisobutyrate | Valine | Isobutyrate	1.2	0.063	1.1
Acetate | Lysine | Arginine	3.1	0.006	1.2
Choline | O-Phosphocholine | sn-Glycero-3-phosphocholine	1.2	0.758	-1
Creatine | Creatine Phosphate | Tyrosine	1.2	0.91	-1
Formate	0.4	0.209	2.4
Glucose (4 bins)	1.3 - 2.1	0.589 - 0.993	1 - 1
Glucose | Glycerol	1.5	0.624	1
Glucose | Unsaturated lipids	1.3	0.847	1
Glutamate | Proline	0.7	0.055	1
Glycerol | Isoleucine	2.3	0.041	1.1
Isoleucine | Leucine	2.4	0.006	1.1
Isoleucine | Valine	1.7	0.035	1.1
Lactate	3.4	0.031	-1.2
Lactate | Threonine	6.4	0.05	-1.1
Leucine (2 bins)	0.9 - 2	0.008 - 0.042	1.1 - 1.1
Methionine | Citrate	0.5	0.082	-1.1
N-Acetylamino acids | Proline	2.5	0.065	1.1
Overlap (2 bins)	0.6 - 1	0.098 - 0.269	-1.2 - 1.1
Oxypurinol	0.5	0.357	2.3
Phenylalanine	0.6	0.061	1.1
Proline	0.9	0.068	1.1
Taurine | Betaine | Glucose | TMAO	1.8	0.665	1
Threonine	1	0.436	-1.1
Unknown	0.2	0.471	-2.7
VLDL | LDL | HDL | Isocaproate	1.1	0.941	-1
Valine	1	0.234	1.1
sn-Glycero-3-phosphocholine	1	0.183	-1.2

**Paired t-test*

***A positive fold change indicates mean of Post-vaccine > mean of Baseline*

Metabolites common and unique between Group 1 (+MyoC) and Group 2 (+Trop–Sx).

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Common Library-matched Metabolites

2-oxoisocaproate
Acetate
Betaine
Creatine Phosphate
Fructose
HDL
Isocaproate
LDL
Lipids
Phenylalanine
Propylene glycol
Taurine
Tyrosine
VLDL
3-Methyl-2-oxovalerate
Arginine
Creatine
Creatinine
Glucose
Histidine
Lactate
Leucine
Lysine
Proline
Serine
TMAO
Unsaturated lipids

Unique Group 2 Metabolites

2-Aminobutyrate
Alanine
Asparagine
Citrate
Glutamine
Isoleucine
N,N-Dimethylglycine
Trimethylamine
O-Phosphocholinesn-Glycero-3phosphocholine
3-Methyl-2-isovalerate
3-Hydroxybutyrate
Arabinose
Choline
Glutamate
Glycerol
Methionine
Threonine
Valine

Venn diagram of common and unique metabolites for all AEFI Groups

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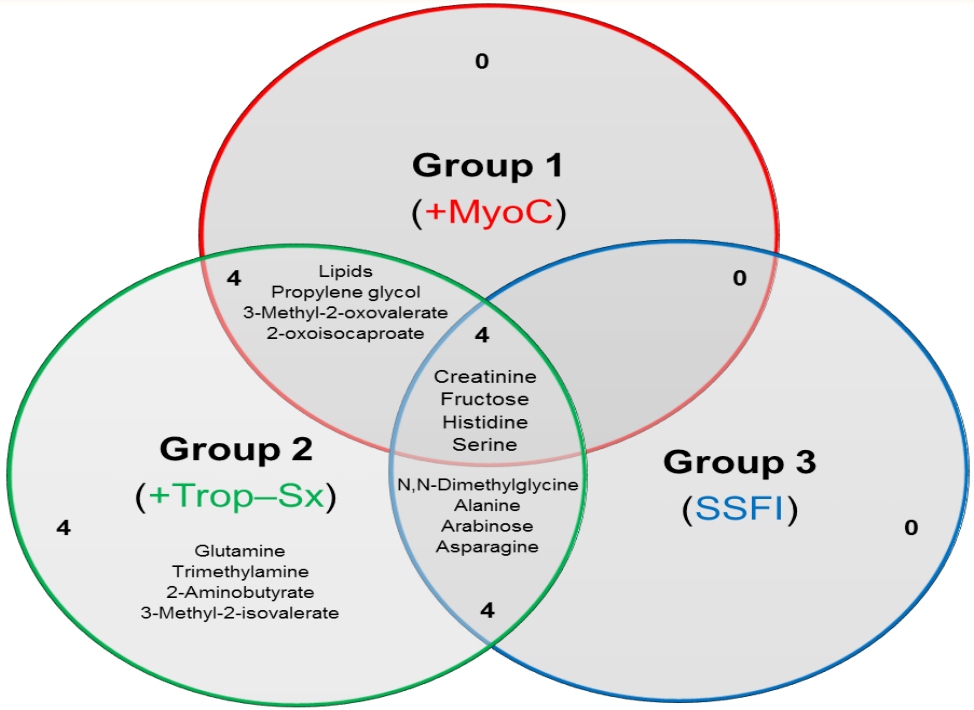

Summary

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- We describe for the first time metabolic profiling of pre- and post-vaccination to include subjects with known AEFI
- We did not identify any unique metabolic signatures pre-vaccination that was able to predict which individuals developed an AEFI
- Broad spectrum metabolomics differentiated pre- and post-vaccination samples for those individuals with serious AEFIs
- A few metabolites unique to the three clinical groups with AEFI. (creatinine, fructose, histidine and serine)

Current Conclusions

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- Individuals may have differential responses to smallpox vaccination that can be detected by metabolic profiling.
- Baseline and post-vaccination metabolic profiles from subjects who experienced AEFI with subclinical (elevated troponins) or clinically verified myocarditis were more clearly differentiated in the multivariate analysis compared to subjects who experienced no AEFI or only systemic symptoms (SSFI).
- Additional metabolomics analysis in the field of vaccinology is warranted.

Contributions

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Questions