Supplementary Material

Urinary phenotyping of SARS-CoV-2 infection connects clinical diagnostics with metabolomics and uncovers impaired NAD+ pathway and SIRT1 activation

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1. Supplementary results

1.1. Table S1. List of urinary metabolites included in the study.

The table reports the metabolites which met all the criteria described in the Methods section. Metabolites typed in green color were measured using both NMR- and LC-MS-based technologies. In contrast, the following metabolites were excluded due to detection rate lower than 70%: on 1-Methyladenosine, 1-Methylhistidine, 2-Furoylglycine, 3-Aminoisobutyric acid, 4-Ethylphenol, 4-Hydroxyhippuric acid, 4-Hydroxyphenylacetic acid, 4-Hydroxyphenylpyruvic acid, Benzoic acid, Choline, Citraconic acid, D-Gluconic acid, D-Mannose, D-Panthenol, DL-Tyrosine, Glycerol, Paracetamol, Paracetamol-glucuronide.

NMR (Bruker IVDR) - selected after rho-sigma filtering **n=44**	**NMR (manually assigned - Chenomx)** **n=5**	**LC-MS** **n=44 + n=4 ddhNs**
Alanine	3-Indoxylsulfate	1-methylhistidine
1-Methylguanidine	cis-Aconitate	3-hydroxyanthranilic acid
1-Methylhydantoin	Hypoxanthine	3-hydroxykynurenine
1-Methylnicotinamide	Pseudouridine	3-methylhistidine
3-Hydroxyisovaleric acid	Urea	4-hydroxyproline
3-Methylglutaconic acid		5-hydroxyindole-acetic acid
Acetic acid		Alanine
Acetoacetic acid		alpha-aminobutyric acid
Acetone		Aminoadipic acid
Adenine		Arginine
Allantoin		Asparagine

Allopurinol		Aspartate
Betaine		beta-aminoisobutyric acid
Caffeine		Citrulline
Citric acid		Cysteine
Creatine		ddhC
Creatinine		ddhC-5'CA
Glucose		ddhC-5'HC
Dimethylamine		ddhU
Erythrol		dopamine
Formic acid		Ethanolamine
Fumaric acid		Glutamic acid
Glycine		Glutamine
Glycolic acid		Glycine
Hippuric acid		Histidine
Inosine		indole-3-acetic acid
Lactic acid		Isoleucine
Methionine		kynurenic acid
N,N-Dimethylglycine		kynurenine
Orotic acid		Leucine
Oxaloacetic acid		Lysine
Oxypurinol		Methionine
Pantothenic acid		neopterin
Phenylacetic acid		nicotinamide-riboside
Proline betaine		Ornithine
Pyruvic acid		Phenylalanine
Succinic acid		picolinic acid
Syringic acid		Proline
Tartaric acid		quinolinic acid
Taurine		Sarcosine
Theobromine		Serine
Trigonelline		serotonin
Trimethylamine		Taurine
Valine		Threonine
		tryptophan
		Tyrosine
		Valine
		xanthurenic acid

1.2. Aim 1 - Identification of the metabolite changes in AcuteCOV urine samples compared to CTR using 1H-NMR spectroscopy.

1.2.1. Hierarchical cluster analysis to investigate sample-driven clusterization and metabolite distribution

Cluster analysis was performed using dCHIP software (clustering method: average linkage; distance metric: 1–Spearman's rank correlation).

1.2.1.1. Sample clustering

The analysis identified 5 major significant clusters: 1) involved 187 samples and was over-represented by samples belonging to the AcuteCOV group (AcuteCOV vs CTR: 120 vs 67, $p<10^{-6}$); 2) involved 57 samples and was over-represented by samples belonging to the CTR group (AcuteCOV vs CTR: 11 vs 46, p=0.00008); 3) involved 54 samples and was over-represented by samples belonging to the CTR group (AcuteCOV vs CTR: 12 vs 42, p=0.00071); 4) involved 33 samples and was over-represented by samples belonging to the CTR group (AcuteCOV vs CTR: 4 vs 29, p=0.00009); 5) involved 22 samples and was over-represented by samples belonging to the CTR group (AcuteCOV vs CTR: 1 vs 21, p=0.00005). The heatmap was built using log2-transformed metabolite concentrations. Rows refer to as patients, while columns denote the metabolites evaluated in urine samples.

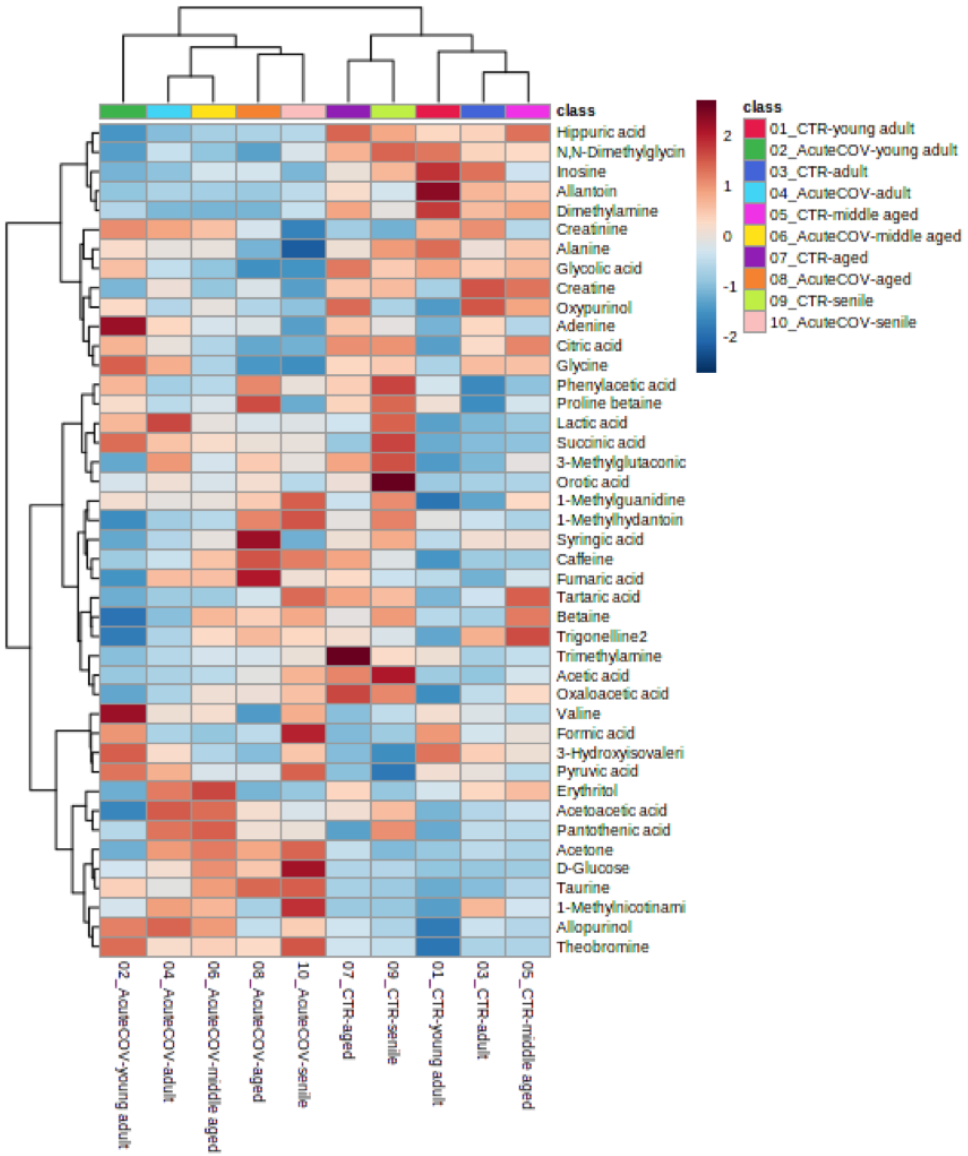
1.2.1.2. Table S2. Metabolite clustering

Cluster	Tot Proportion of Variation Explained	Most Representative metabolite	Number of Members	Other members
1	0.053	Trimethylamine	4	Acetic acid, 3-Methylglutaconic acid, Inosine
2	0.049	Glycine	6	Citric acid, Alanine, N,N-Dimethylglycine, Allantoin, Formic acid
3	0.045	3-Hydroxyisovaleric acid	3	Pyruvic acid
4	0.043	Creatinine	5	Dimethylamine, Syringic acid, 1-Methylguanidine, 1-Methylhydantoin
5	0.042	Allopurinol	5	Oxypurinol, 1-Methylnicotinamide, Adenine, Proline betaine
6	0.038	Hippuric acid	4	Trigonelline, Glycolic acid, Tartaric acid
7	0.037	Caffeine	4	Glucose, Phenylacetic acid, Theobromine
8	0.037	Taurine	4	Betaine, Valine, Choline
9	0.033	Acetoacetic acid	2	Acetone

10	0.03	Fumaric acid	2	Lactic acid
11	0.024	Creatine	2	Erythritol
12	0.023	Oxaloacetic acid	2	Orotic acid
13	0.023	Succinic acid	1	-

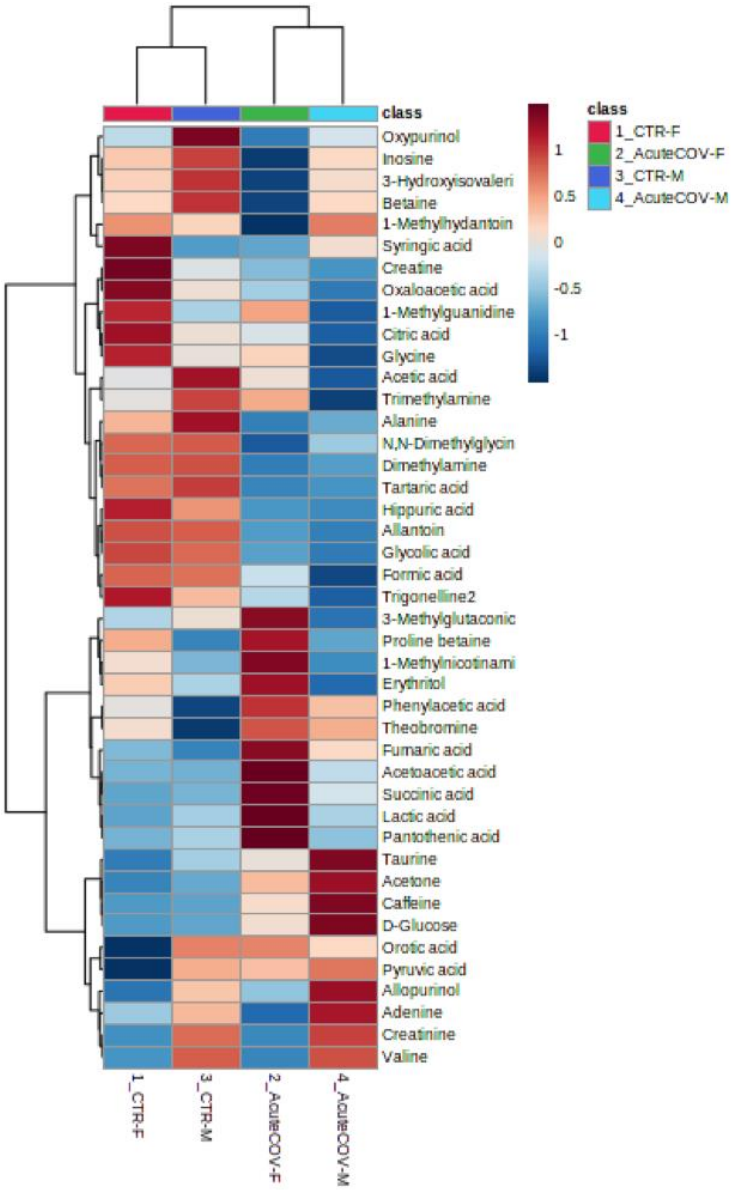
1.2.2. Figure S1. Differences in the urinary metabolite concentration across age groups

Hierarchical clustering, Ward method.



1.2.3. Figure S2. Differences in the urinary metabolite concentration across gender groups

Hierarchical clustering, Ward method.



1.2.4. Table S3. Differences in the metabolite changes induced by SARS-CoV-2 in urine samples across gender and age groups

Log2 fold changes are reported. Two-way ANOVA, Tukey's post hoc test.

		log2FC AcuteCOV vs CTR								
		Sex			Age					
		F	M		young adult	adult	middle aged	aged	senile	
Modulation	**Metabolite**	**n=276**	**n=276**	**p int[a]**	**n=11**	**n=142**	**n=295**	**n=77**	**n=27**	**p int[b]**
↑ in AcuteCOV										
	Glucose	1.2	1.8		1.2	1.3	1.7	1.1	1.9	
	Lactic acid	1.1	0.1	0.004	1.3	1.3	0.5	0.1	-0.7	0.049
	Acetone	0.9	1.1		0.0	0.7	1.1	0.9	1.1	
	Acetoacetic acid	0.8	0.2		-0.4	0.8	0.6	0.1	-0.3	
	Taurine	0.6	0.9	0.042	1.1	0.5	0.8	1.0	1.0	
	Succinic acid	0.7	0.3	0.001	1.3	0.8	0.5	0.5	-0.5	0.048
	Fumaric acid	0.5	0.4		0.0	0.6	0.3	0.6	0.0	
	Orotic acid	0.8	0.1		0.8	1.0	0.6	0.2	-2.4	0.048
	Caffeine	0.1	0.4		0.3	0.0	0.4	0.2	0.2	
	Theobromine	0.1	0.3		0.8	0.1	0.2	0.2	0.3	
	1-Methylguanidine	0.1	0.1		0.5	0.2	0.0	0.2	0.0	
↓ in AcuteCOV										
	Tartaric acid	-1.6	-1.6		-0.3	-0.8	-2.2	-1.1	-0.1	
	Creatine	-1.8	-1.0	0.041	-0.9	-0.9	-2.1	-0.5	-3.5	
	Hippuric acid	-1.1	-0.8		-1.3	-0.9	-1.0	-0.9	-0.9	
	Allantoin	-0.8	-0.9		-1.8	-1.0	-0.9	-0.8	-0.4	
	Citric acid	-0.6	-0.7		1.0	-0.1	-0.8	-1.0	-1.1	0.001
	Trigonelline	-0.6	-0.6		-0.8	-0.9	-0.7	0.2	0.1	0.048
	Oxypurinol	-0.6	-0.7		1.4	-1.2	-0.5	-1.0	-0.3	
	Dimethylamine	-0.5	-0.4		-0.5	-0.5	-0.5	-0.5	-0.2	

	Glycine	-0.3	-0.5		0.6	0.0	-0.5	-0.8	-1.0	0.009
	N,N-Dimethylglycine	-0.6	-0.3		-0.7	-0.2	-0.3	-0.5	-0.4	
	Glycolic acid	-0.3	-0.3		0.0	-0.3	-0.4	-0.5	-0.6	
different response across gender groups										
	Erythritol	0.5	-0.7	0.048	-0.6	0.3	0.3	-0.7	0.0	
	Syringic acid	-0.2	0.2	0.001	-0.2	-0.2	0.0	0.4	-0.5	
	Pantothenic acid	0.3	0	0.033	0.0	0.2	0.2	0.2	-0.1	
different response across age groups										
	Formic acid	-0.2	-0.3		0.0	-0.3	-0.4	0.2	0.6	0.001
	Inosine	-0.4	-0.1		-0.7	-0.5	0.0	0.0	-0.5	0.045

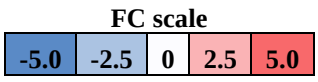
FC scale

-5.0	-2.5	0	2.5	5.0

1.2.5. Table S4. Differences in the metabolite changes induced by SARS-CoV-2 in urine samples across BMI groups.

Log2 Fold changes are reported; Two-way ANOVA, Tukey's post hoc test

log2FC AcuteCOV vs CTR					
		BMI			
		normal weight	overweight	obesity	
Modulation	Metabolite	n=202	n=188	n=122	p int[b]
↑ in AcuteCOV					
	Glucose	0.8	1.5	2.2	
	Lactic acid	0.7	0.1	1.0	
	Acetone	1.3	1.1	0.4	
	Acetoacetic acid	0.8	0.3	0.5	
	Taurine	0.6	0.9	0.6	
	Succinic acid	0.8	0.4	0.3	0.036
	Fumaric acid	0.4	0.0	0.9	
	Orotic acid	0.1	0.4	0.8	
	Caffeine	0.0	0.4	0.1	
	Theobromine	0.2	0.2	0.2	
	1-Methylguanidine	0.1	0.0	0.0	
↓ in AcuteCOV					
	Tartaric acid	-1.6	-1.3	-1.5	
	Creatine	-0.8	-2.5	-1.2	
	Hippuric acid	-0.8	-1.0	-1.1	
	Allantoin	-1.1	-0.8	-1.0	
	Citric acid	-0.3	-0.8	-0.7	
	Trigonelline	-0.5	-0.6	-0.4	
	Oxypurinol	-1.2	-0.5	-0.2	
	Dimethylamine	-0.4	-0.4	-0.8	0.009
	Glycine	0.0	-0.4	-0.6	0.031
	N,N-Dimethylglycine	-0.2	-0.5	-0.4	
	Glycolic acid	-0.4	-0.3	-0.3	0.019



1.3. Aim 2 - Characterization of the AcuteCOV urinary metabolic signature

1.3.1. Table S5. Differences in the urinary metabolite concentration between hospitalized and nonhospitalized patients

Mann-Whitney test, FC >1.2, FDR-adjusted p-values are shown.

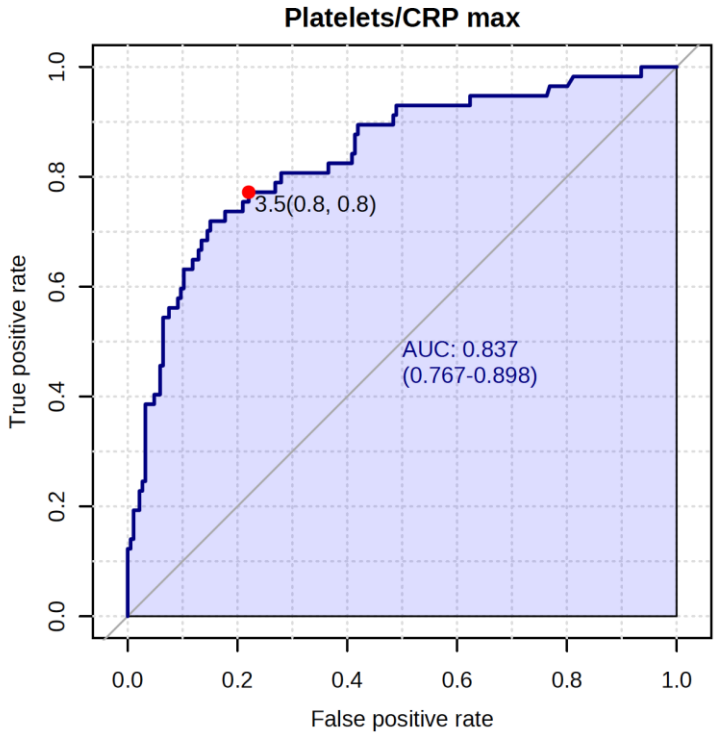
Modulation	Metabolite	log2 FC	FDR-adj p
↑ hospitalized patients			
	3-hydroxykynurenine	2.13	****
	kynurenine	1.29	****
	ddhC-5'HC	1.15	***
	ddhC-5'CA	1.02	***
	ddhC	0.79	***
	ddhU	0.51	**
	neopterin	0.50	**
	3-hydroxyanthranilic acid	0.47	*
	taurine	0.44	**
	quinolinic acid	0.31	*
	phenylalanine	0.27	*
	glucose	0.27	*
↓ in hospitalized patients			
	citric acid	-0.83	***
	trigonelline	-0.82	**
	allantoin	-0.58	*
	glycine	-0.57	**
	formic acid	-0.34	*
	1-methylnicotinamide	-0.32	*
	alanine	-0.31	**
	threonine	-0.29	**

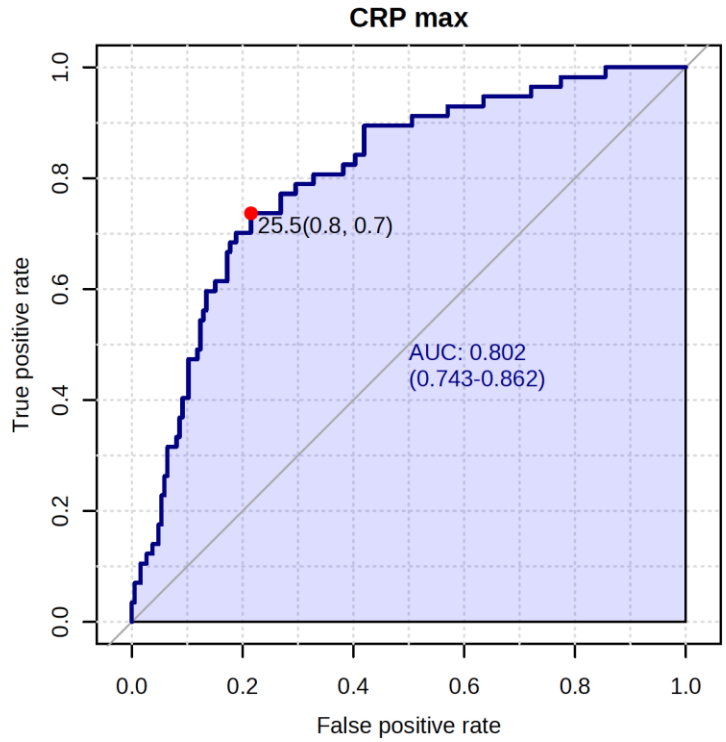
FC scale

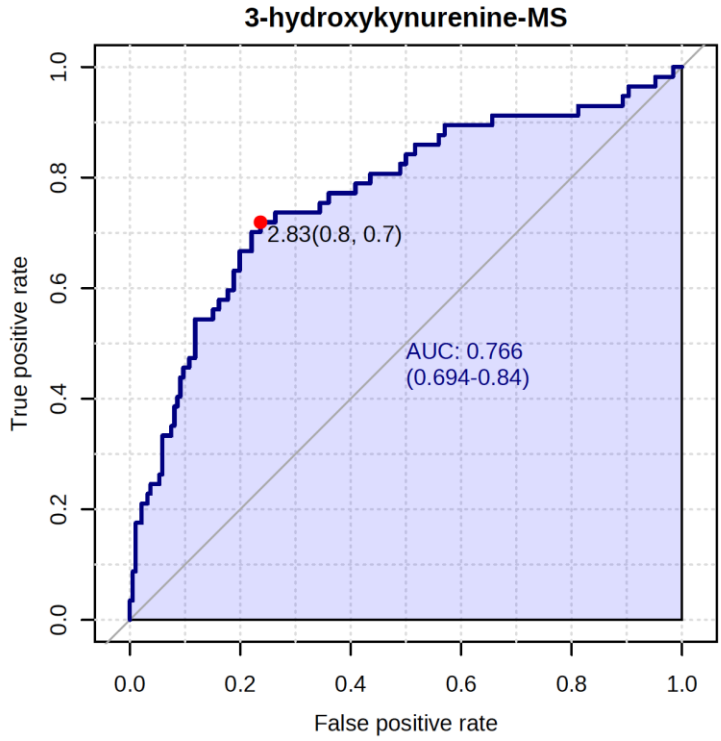
-1.6	-0.8	0	0.8	1.6

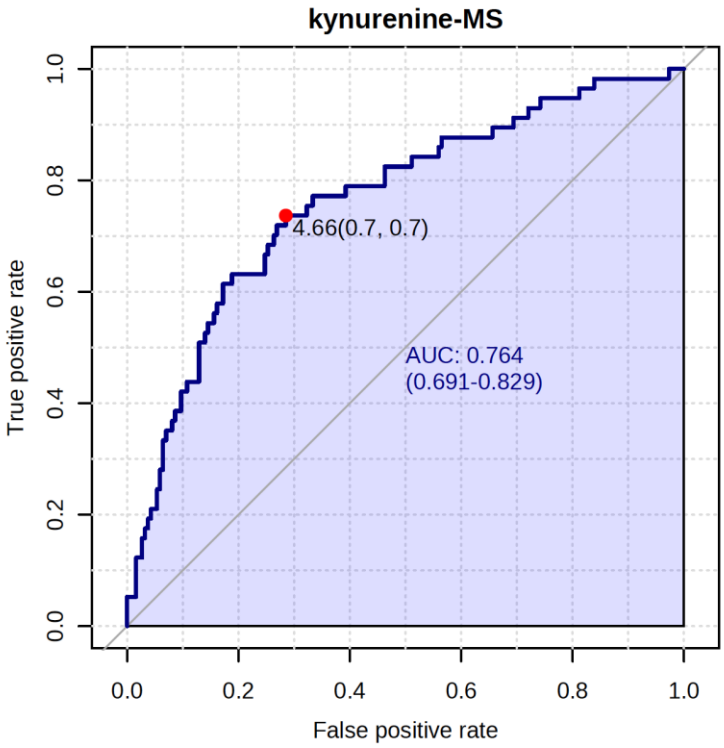
1.3.2. Figure S3. ROC curve analysis to discriminate between hospitalized and nonhospitalized patients

Variable	AUC	Specificity	Sensitivity
Platelets/CRP	0.837	0.78(0.712-0.839)	0.772(0.632-0.877)
CRP	0.802	0.785(0.731-0.842)	0.737(0.622-0.834)
3-hydroxykynurenine	0.765	0.763(0.707-0.817)	0.719(0.614-0.816)
kynurenine	0.764	0.715(0.642-0.769)	0.737(0.613-0.851)



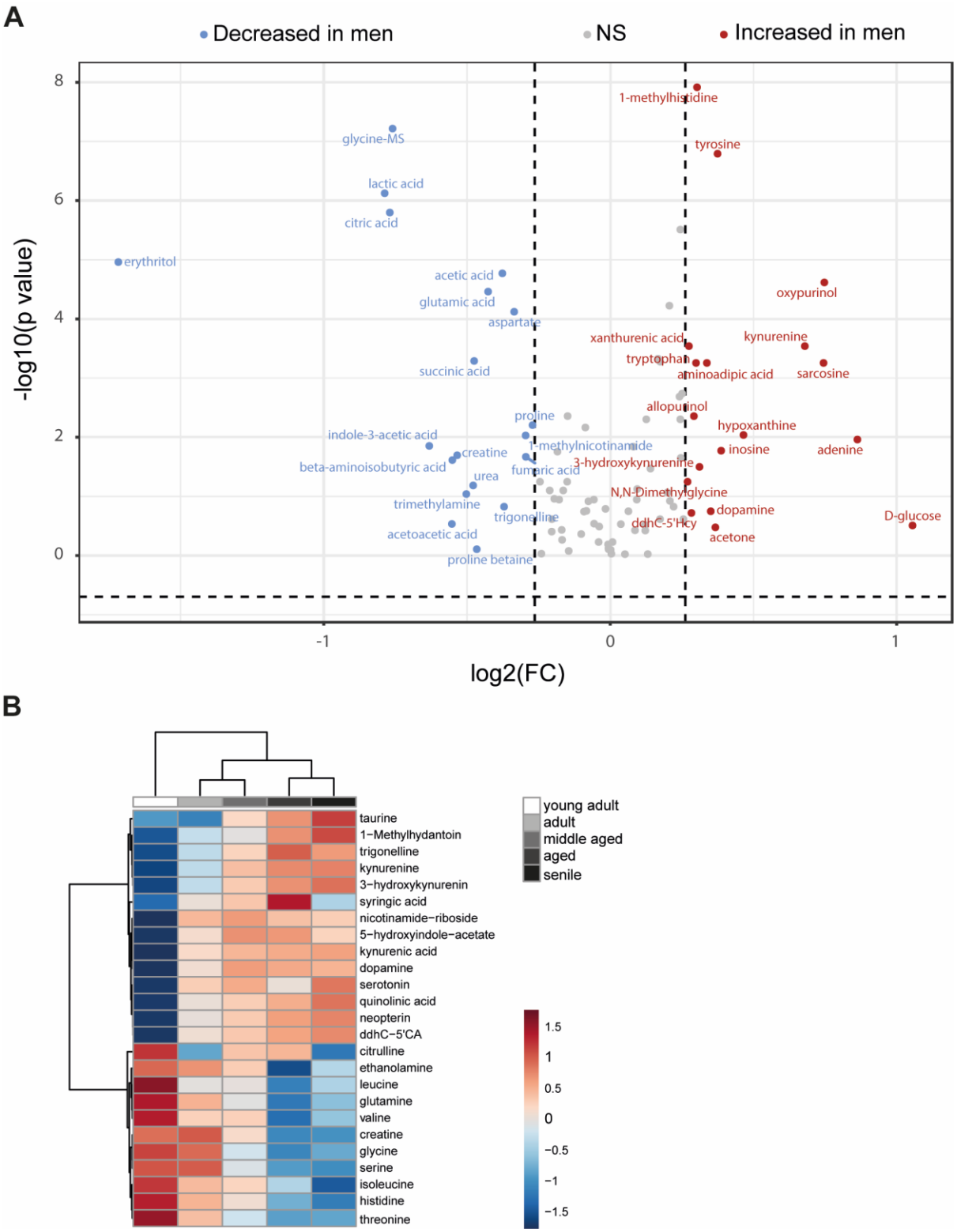

CRP max
True positive rate
False positive rate
25.5(0.8, 0.7)
AUC: 0.802
(0.743-0.862)
0.0
0.2
0.4
0.6
0.8
1.0


3-hydroxykynurenine-MS
True positive rate
False positive rate
2.83(0.8, 0.7)
AUC: 0.766
(0.694-0.84)
0.0
0.2
0.4
0.6
0.8
1.0


kynurenine-MS
True positive rate
False positive rate
0.0
0.2
0.4
0.6
0.8
1.0
4.66(0.7, 0.7)
AUC: 0.764
(0.691-0.829)

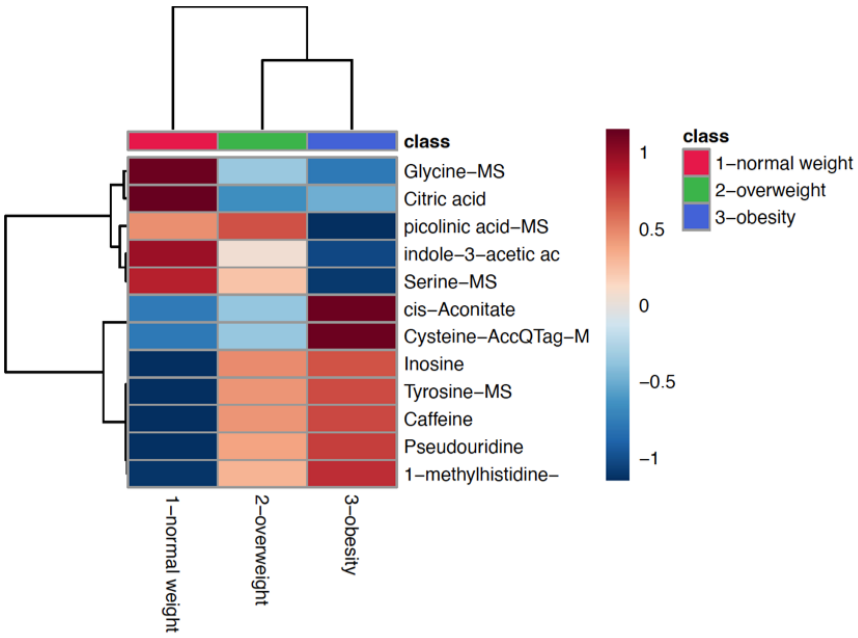
1.3.3. Figure S4. Influence of sex and age factors on the AcuteCOV metabolomic profile.

A) Volcano plot showing the differentially expressed metabolites in the comparison “males vs females” patients. Only metabolites with FC>1.2 were considered, Wilcoxon-Mann-Whitney test. B) Hierarchical clustering of the top 30 metabolites which resulted significantly different across patient age groups. One-way ANOVA, Tukey’s post hoc test, $p<0.05$. Columns denote averaged metabolite urinary concentrations within each age subclass. A two-color scale is used to illustrate the metabolite modulation pattern: deep red denotes +1.5, deep blue denotes -1.5. Abbreviations: F, females; M, males.


A
Decreased in men
NS
Increased in men
-log10(p value)
log2(FC)
1-methylhistidine
tyrosine
glycine-MS
lactic acid
citric acid
erythritol
acetic acid
glutamic acid
aspartate
oxypurinol
xanthurenic acid
kynurenine
tryptophan
succinic acid
aminoadipic acid
sarcosine
allopurinol
proline
hypoxanthine
indole-3-acetic acid
1-methylnicotinamide
inosine
adenine
creatine
3-hydroxykynurenine
beta-aminoisobutyric acid
fumaric acid
urea
N,N-Dimethylglycine
trimethylamine
dopamine
trigonelline
D-glucose
ddhC-5'Hcy
acetoacetic acid
acetone
proline betaine
B
young adult
adult
middle aged
aged
senile
taurine
1−Methylhydantoin
trigonelline
kynurenine
3−hydroxykynurenin
syringic acid
nicotinamide−riboside
5−hydroxyindole−acetate
kynurenic acid
dopamine
serotonin
quinolinic acid
neopterin
ddhC−5'CA
citrulline
ethanolamine
leucine
glutamine
valine
creatine
glycine
serine
isoleucine
histidine
threonine

1.3.4. Figure S5. Influences of BMI on the AcuteCOV metabolomic profile

Hierarchical clustering of the top 12 metabolites which resulted significantly different across BMI groups. One-way ANOVA, Tukey's post hoc test, $p<0.05$. Columns denote averaged metabolite urinary concentrations within each age subclass. A two-color scale is used to illustrate the metabolite modulation pattern: deep red denotes +1.5, deep blue denotes -1.5.



1.4. Aim 3 - Analysis of the antiviral ddhNs and their associations with metabolite profiles and clinical variables

1.4.1. Table S6. Differences in the metabolite concentration across quartiles of "Total ddhNs".

Data are expressed as mean±standard deviation; one-way ANOVA, FDR-adjusted p-values.

		Total ddhN quartiles				
		Q1	Q2	Q3	Q4	
Modulation	Metabolite	n=59	n=58	n=59	n=60	FDR-adj p
↑ along with increased concentration of antiviral nucleosides						
	1-Methylhistidine	275.91±117.68	309.06±95.85	344.2±111	401.47±103.46	<0.0001
	3-Hydroxyanthranilic acid	4.45±3.66	5.3±4.21	5.69±4.49	7.36±6.43	0.019
	3-Hydroxykynurenine	3.91±16.7	5.42±16.86	8.54±22.58	11.19±13.94	<0.0001
	5-Hydroxyindole-acetic acid	30.56±22.39	55.02±91.33	43.32±42.33	55.67±39.29	<0.0001
	Allopurinol	0.07±0.04	0.07±0.03	0.07±0.04	0.1±0.06	0.002
	alpha-Aminobutyric acid	11.48±6.5	11.32±6.77	12.57±6.73	18.69±11.73	<0.0001
	Aspartate	9.5±10.36	11.97±13.25	14.31±18.91	14.51±13.91	0.004
	Creatinine	10.06±3.99	11.34±3.81	13.58±4.44	17.93±5.78	<0.0001
	Cysteine	199.28±110.48	224.92±143.01	247.92±132.94	311.99±119.47	<0.0001
	Glucose	0.45±0.24	1.53±6.51	0.66±0.68	1.07±1.15	<0.0001
	Dimethylamine	0.28±0.11	0.33±0.11	0.37±0.15	0.4±0.09	<0.0001
	Hypoxanthine	0.08±0.06	0.09±0.08	0.11±0.08	0.17±0.11	<0.0001
	Isoleucine	17.49±8.37	16.08±6.14	18.29±7.7	25.36±10.62	<0.0001
	Kynurenic acid	8.56±4.17	11.22±4.64	12.66±6.08	14.9±6.63	<0.0001
	Kynurenine	3.41±4.45	6.27±8.64	7.03±8.19	16.78±14.72	<0.0001
	Leucine	40.23±17.96	40.3±15.27	45.13±16.92	61.91±21.58	<0.0001
	Methionine	10.41±6.04	9.82±5.18	11.41±5.19	17.2±5.65	<0.0001
	Neopterin	3.66±2.83	9.48±5.54	13.39±7.86	17.25±11.12	<0.0001
	Oxypurinol	0.1±0.08	0.13±0.13	0.17±0.17	0.24±0.2	<0.0001
	Phenylalanine	73.56±39.87	75.53±23.63	94.16±31.58	131.4±47.34	<0.0001
	Pseudouridine	0.15±0.06	0.15±0.06	0.19±0.05	0.24±0.07	<0.0001
	Quinolinic acid	48.53±24.25	78.41±34.04	100.93±48.68	123.23±59.53	<0.0001
	Taurine	999.4±821.2	1395.11±904.86	1595.56±1029.31	1952.64±806.05	<0.0001
	Tryptophan	53.71±30.37	58.54±33.1	62.72±31.89	82.33±40.06	<0.0001
	Tyrosine	119.46±65.39	115.91±50.57	131.14±59.04	179.14±82.36	<0.0001
	Valine	53.96±18.2	55.25±17	59.15±19.29	78.96±27.25	<0.0001
	Xanthurenic acid	4.26±2.78	4.31±3.89	5.88±3.8	6±3.17	<0.0001

↓ along with increased concentration of antiviral nucleosides						
	Acetic acid	0.191±0.394	0.133±0.121	0.154±0.267	0.087±0.091	<0.0001
	Alanine	383.265±116.723	354.959±152.255	302.715±131.027	266.641±105.371	<0.0001
	Allantoin	0.1±0.08	0.08±0.06	0.08±0.06	0.06±0.05	0.010
	Citric acid	2.88±1.43	2.21±1.39	1.85±1.28	1.29±1.01	<0.0001
	Formic acid	0.213±0.156	0.199±0.13	0.147±0.082	0.125±0.075	<0.0001
	Fumaric acid	0.04±0.03	0.04±0.03	0.03±0.02	0.01±0	<0.0001
	Glutamine	733.873±179.468	706.011±242.277	634.086±210.42	602.473±214.399	<0.0001
	Glycine	1473.4±835.48	1290.05±935.76	1084.22±706.07	924.44±401.94	0.001
	Glycolic acid	0.712±0.201	0.715±0.313	0.674±0.251	0.575±0.187	<0.0001
	Guanidinoacetic acid	1.34±4.56	0.71±0.86	1.02±4.13	0.46±0.63	<0.0001
	Hippuric acid	1.934±1.23	2.269±1.743	2.191±1.765	1.442±1.274	0.003
	Indole-3-acetic acid	16.98±15.36	13.54±11.79	10.05±9.87	7.26±6.68	<0.0001
	Lactic acid	0.322±0.197	0.305±0.267	0.235±0.169	0.221±0.083	0.016
	N,N-Dimethylglycine	0.079±0.033	0.081±0.023	0.077±0.067	0.058±0.02	<0.0001
	Orotic acid	0.04±0.03	0.04±0.03	0.03±0.01	0.01±0	<0.0001
	Phenylacetic acid	0.06±0.05	0.06±0.06	0.06±0.06	0.05±0.06	0.005
	Serine	452.118±116.248	432.875±128.963	375.538±109.998	384.368±98.067	0.002
	Threonine	214.741±103.85	176.849±68.047	154.132±71.506	143.658±51.76	<0.0001
	Trigonelline	0.325±0.266	0.388±0.349	0.263±0.233	0.11±0.11	<0.0001
	Urea	8.99±19.21	5.69±3.17	5.86±1.9	4.24±2.01	<0.0001
Other						
	Adenine	0.04±0.03	0.05±0.03	0.04±0.04	0.05±0.06	0.010
	Dopamine	3.02±10.66	2.43±1.26	2.38±1.02	3.1±1.41	<0.0001
	Succinic acid	0.119±0.08	0.113±0.085	0.084±0.049	0.059±0.029	0.004