

Supplemental Material

Supplemental Table 1: List of metabolites measured with the AbsoluteIDQ® p180 Kit GAC, Helmholtz Zentrum München.

Acylcarnitines (40)			
C0	Carnitine	C10:1	Decenoylcarnitine
C2	Acetylcarnitine	C10:2	Decadienylcarnitine
C3	Propionylcarnitine	C12	Dodecanoylcarnitine
C3:1	Propenoylcarnitine	C12:1	Dodecenoylcarnitine
C3-OH	Hydroxypropionylcarnitine	C12-DC	Dodecanedioylcarnitine
C4	Butyrylcarnitine	C14	Tetradecanoylcarnitine
C4:1	Butenoylcarnitine	C14:1	Tetradecenoylcarnitine
C4-OH (C3-DC)	Hydroxybutyrylcarnitine	C14:1-OH	Hydroxytetradecenoylcarnitine
C5	Valerylcarnitine	C14:2	Tetradecadienylcarnitine
C5:1	Tiglylcarnitine	C14:2-OH	Hydroxytetradecadienylcarnitine
C5:1-DC	Glutaconylcarnitine	C16	Hexadecanoylcarnitine
C5-DC (C6-OH)	Glutaryl carnitine (Hydroxyhexanoylcarnitine)	C16:1	Hexadecenoylcarnitine
C5-M-DC	Methylglutaryl carnitine	C16:1-OH	Hydroxyhexadecenoylcarnitine
C5-OH (C3-DC-M)	Hydroxyvalerylcarnitine (Methylmalonylcarnitine)	C16:2	Hexadecadienylcarnitine
C6 (C4:1-DC)	Hexanoylcarnitine (Fumaryl carnitine)	C16:2-OH	Hydroxyhexadecadienylcarnitine
C6:1	Hexenoylcarnitine	C16-OH	Hydroxyhexadecanoylcarnitine
C7-DC	Pimelylcarnitine	C18	Octadecanoylcarnitine
C8	Octanoylcarnitine	C18:1	Octadecenoylcarnitine
C9	Nonanoylcarnitine	C18:1-OH	Hydroxyoctadecenoylcarnitine
C10	Decanoylcarnitine	C18:2	Octadecadienylcarnitine
Amino Acids (21)			
Ala	Alanine	Lys	Lysine
Arg	Arginine	Met	Methionine
Asn	Asparagine	Orn	Ornithine
Asp	Aspartate	Phe	Phenylalanine
Cit	Citrulline	Pro	Proline
Gln	Glutamine	Ser	Serine
Glu	Glutamate	Thr	Threonine
Gly	Glycine	Trp	Tryptophan
His	Histidine	Tyr	Tyrosine
Ile	Isoleucine	Val	Valine
Leu	Leucine		
Monosaccharides (1)			
Sum of Hexoses (including Glucose)			
Glycerophospholipids (90)			
lysoPC a C14:0	PC aa C34:1	PC aa C42:0	PC ae C38:2
lysoPC a C16:0	PC aa C34:2	PC aa C42:1	PC ae C38:3
lysoPC a C16:1	PC aa C34:3	PC aa C42:2	PC ae C38:4
lysoPC a C17:0	PC aa C34:4	PC aa C42:4	PC ae C38:5
lysoPC a C18:0	PC aa C36:0	PC aa C42:5	PC ae C38:6
lysoPC a C18:1	PC aa C36:1	PC aa C42:6	PC ae C40:1
lysoPC a C18:2	PC aa C36:2	PC ae C30:0	PC ae C40:2
lysoPC a C20:3	PC aa C36:3	PC ae C30:1	PC ae C40:3

lysoPC a C20:4	PC aa C36:4	PC ae C30:2	PC ae C40:4
lysoPC a C24:0	PC aa C36:5	PC ae C32:1	PC ae C40:5
lysoPC a C26:0	PC aa C36:6	PC ae C32:2	PC ae C40:6
lysoPC a C26:1	PC aa C38:0	PC ae C34:0	PC ae C42:0
lysoPC a C28:0	PC aa C38:1	PC ae C34:1	PC ae C42:1
lysoPC a C28:1	PC aa C38:3	PC ae C34:2	PC ae C42:2
PC aa C24:0	PC aa C38:4	PC ae C34:3	PC ae C42:3
PC aa C26:0	PC aa C38:5	PC ae C36:0	PC ae C42:4
PC aa C28:1	PC aa C38:6	PC ae C36:1	PC ae C42:5
PC aa C30:0	PC aa C40:1	PC ae C36:2	PC ae C44:3
PC aa C30:2	PC aa C40:2	PC ae C36:3	PC ae C44:4
PC aa C32:0	PC aa C40:3	PC ae C36:4	PC ae C44:5
PC aa C32:1	PC aa C40:4	PC ae C36:5	PC ae C44:6
PC aa C32:2	PC aa C40:5	PC ae C38:0	
PC aa C32:3	PC aa C40:6	PC ae C38:1	
Sphingolipids (15)			
SM (OH) C14:1	SM C18:0	SM (OH) C22:1	SM (OH) C24:1
SM C16:0	SM C18:1	SM (OH) C22:2	SM C26:0
SM C16:1	SM C20:2	SM C24:0	SM C26:1
SM (OH) C16:1	SM C22:3	SM C24:1	
Biogenic Amines (21)			
Ac-Orn	Acetylornithine	PEA	Phenylethylamine
ADMA	Asymmetric dimethylarginine	*cis-OH-Pro	*cis-4-Hydroxyproline
alpha-AAA	alpha-Aminoadipic acid	trans-OH-Pro	trans-4-Hydroxyproline
*Carnosine	*Carnosine	Putrescine	Putrescine
**Creatinine	Creatinine	SDMA	Symmetric dimethylarginine
*DOPA	*DOPA	Serotonin	Serotonin
*Dopamine	*Dopamine	Spermidine	Spermidine
Histamine	Histamine	Spermine	Spermine
Kynurenine	Kynurenine	Taurine	Taurine
Met-SO	Methionine sulfoxide	total DMA	Total dimethylarginine
*Nitro-Tyr	*Nitrotyrosine		

* Metabolites excluded from the analyses because more than 80% of the samples had values below the detection limit. **Creatinine excluded from the analyses because serum creatinine values from standard baseline clinical chemistry measurements were already available.

Supplemental Table 2. Associated metabolites in unadjusted model after Bonferroni correction in the Gargano Mortality Study 1

N	Family	Metabolite	HR	95% CI		P
				LB	UB	
1	Biogenic Amines	SDMA	Symmetric dimethylarginine	2.01	1.74 2.32	9.1x10 ⁻²²
2	Biogenic Amines	total DMA	Total dimethylarginine	1.91	1.67 2.19	1.3x10 ⁻²⁰
3	Acylcarnitines	C5-DC (C6-OH)	Glutaryl carnitine (Hydroxyhexanoyl carnitine)	1.83	1.61 2.09	1.7x10 ⁻²⁰
4	Acylcarnitines	C5-M-DC	Methylglutaryl carnitine	1.57	1.42 1.73	2.7x10 ⁻¹⁸
5	Amino acids	Cit	Citrulline	1.82	1.57 2.10	4.3x10 ⁻¹⁶
6	Acylcarnitines	C4	Butyryl carnitine	1.68	1.46 1.92	7.0x10 ⁻¹⁴
7	Acylcarnitines	C5-OH (C3-DC-M)	Hydroxyvaleryl carnitine (Methylmalonyl carnitine)	1.64	1.43 1.87	5.7x10 ⁻¹³
8	Acylcarnitines	C6 (C4:1-DC)	Hexanoyl carnitine	1.85	1.56 2.19	1.4x10 ⁻¹²
9	Glycerophospholipids	PC aa C34:4	PC aa C34:4	0.62	0.54 0.72	2.1x10 ⁻¹¹
10	Acylcarnitines	C5:1-DC	Glutaconyl carnitine	1.60	1.39 1.84	2.2x10 ⁻¹¹
11	Biogenic Amines	Kynurenine	Kynurenine	1.56	1.37 1.77	3.1x10 ⁻¹¹
12	Biogenic Amines	Putrescine	Putrescine	1.53	1.35 1.74	3.7x10 ⁻¹¹
13	Acylcarnitines	C5:1	Tiglyl carnitine	1.63	1.40 1.89	1.5x10 ⁻¹⁰
14	Glycerophospholipids	PC aa C32:2	PC aa C32:2	0.66	0.57 0.75	1.9x10 ⁻⁹
15	Biogenic Amines	<i>trans</i> -OH-Pro	<i>trans</i> -4-Hydroxyproline	1.52	1.33 1.75	2.1x10 ⁻⁹
16	Biogenic Amines	Ac-Orn	Acetylornithine	1.57	1.35 1.82	2.8x10 ⁻⁹
17	Sphingolipids	SM (OH) C22:1	SM (OH) C22:1	0.67	0.58 0.77	9.2x10 ⁻⁹
18	Acylcarnitines	C2	Acetyl carnitine	1.55	1.33 1.79	1.0x10 ⁻⁸
19	Acylcarnitines	C5	Valeryl carnitine	1.46	1.28 1.67	1.3x10 ⁻⁸
20	Acylcarnitines	C4-OH (C3-DC)	Hydroxybutyryl carnitine	1.39	1.23 1.57	8.6x10 ⁻⁸
21	Acylcarnitines	C10:2	Decadienyl carnitine	1.32	1.19 1.47	3.9x10 ⁻⁷
22	Acylcarnitines	C16:1	Hexadecenoyl carnitine	1.40	1.22 1.60	1.3x10 ⁻⁶
23	Acylcarnitines	C0	Carnitine	1.38	1.20 1.58	2.9x10 ⁻⁶
24	Acylcarnitines	C12:1	Dodecenoyl carnitine	1.36	1.19 1.55	3.5x10 ⁻⁶
25	Acylcarnitines	C18:1	Octadecenoyl carnitine	1.33	1.18 1.51	4.6x10 ⁻⁶
26	Acylcarnitines	C3	Propionyl carnitine	1.35	1.19 1.54	4.6x10 ⁻⁶
27	Biogenic Amines	ADMA	Asymmetric dimethylarginine	1.37	1.20 1.57	5.4x10 ⁻⁶
28	Acylcarnitines	C14:1-OH	Hydroxytetradecenoyl carnitine	1.33	1.17 1.51	7.7x10 ⁻⁶
29	Acylcarnitines	C14:1	Tetradecenoyl carnitine	1.35	1.18 1.54	8.3x10 ⁻⁶
30	Acylcarnitines	C7-DC	Pimelyl carnitine	1.37	1.19 1.57	8.3x10 ⁻⁶
31	Amino acids	Asp	Aspartate	1.38	1.20 1.60	9.2x10 ⁻⁶
32	Glycerophospholipids	PC aa C36:4	PC aa C36:4	0.72	0.62 0.83	9.8x10 ⁻⁶
33	Sphingolipids	SM C24:0	SM C24:0	0.72	0.62 0.83	1.0x10 ⁻⁵
34	Glycerophospholipids	PC aa C36:6	PC aa C36:6	0.72	0.62 0.84	1.3x10 ⁻⁵
35	Acylcarnitines	C9	Nonanoyl carnitine	1.30	1.15 1.47	2.1x10 ⁻⁵
36	Glycerophospholipids	PC aa C36:5	PC aa C36:5	0.72	0.62 0.84	2.4x10 ⁻⁵
37	Biogenic Amines	Met-SO	Methionine sulfoxide	1.45	1.22 1.73	2.4x10 ⁻⁵
38	Amino acids	Trp	Tryptophan	0.73	0.63 0.85	4.1x10 ⁻⁵
39	Glycerophospholipids	lysoPC a C18:2	lysoPC a C18:2	0.76	0.66 0.87	4.5x10 ⁻⁵
40	Sphingolipids	SM (OH) C24:1	SM (OH) C24:1	0.77	0.67 0.87	5.0x10 ⁻⁵
41	Glycerophospholipids	PC aa C38:5	PC aa C38:5	0.75	0.65 0.86	6.0x10 ⁻⁵
42	Glycerophospholipids	PC aa C34:3	PC aa C34:3	0.76	0.66 0.87	6.8x10 ⁻⁵
43	Glycerophospholipids	lysoPC a C14:0	lysoPC a C14:0	0.75	0.65 0.87	9.5x10 ⁻⁵
44	Biogenic Amines	Spermidine	Spermidine	1.28	1.13 1.45	1.1x10 ⁻⁴
45	Acylcarnitines	C14	Tetradecanoyl carnitine	1.27	1.12 1.44	1.3x10 ⁻⁴
46	Biogenic Amines	Spermine	Spermine	1.25	1.12 1.41	1.3x10 ⁻⁴
47	Acylcarnitines	C12	Dodecanoyl carnitine	1.28	1.13 1.45	1.3x10 ⁻⁴
48	Amino acids	Glu	Glutamate	1.32	1.14 1.52	1.6x10 ⁻⁴
49	Acylcarnitines	C14:2	Tetradecadienyl carnitine	1.30	1.13 1.48	1.6x10 ⁻⁴

Supplemental Table 3: KTR and cytokines values in the 2 study cohorts

	GMS1 (n=489)	GMS2 (n=321)
KTR	0.04 (0.03–0.05)	0.04 (0.03–0.06)
IL-1 β (pg/mL)	6.6 (4.4–10.9)	8.4 (7.2–9.8)
IL-2 (pg/mL)	26.1 (22.6–32.9)	21.5 (17.8–26.0)
IL-4 (pg/mL)	7.0 (5.2–10.9)	12.5 (9.7–16.7)
IL-6 (pg/mL)	10.3 (7.1–17.6)	12.6 (9.9–17.0)
IL-13 (pg/mL)	7.5 (11.0–29.8)	4.1 (3.3–5.6)
IFN γ (pg/mL)	10.0 (7.3–15.5)	14.6 (11.6–16.8)
TNF- α (pg/mL)	22 (17.9–28.3)	37.1 (17.0–55.0)

Variables are presented as median (interquartile range). GMS: Gargano Mortality Study; KTR: kynurenine/tryptophan ratio; IL-: interleukin; IFN: interferon; TNF-: tumor necrosis factor.

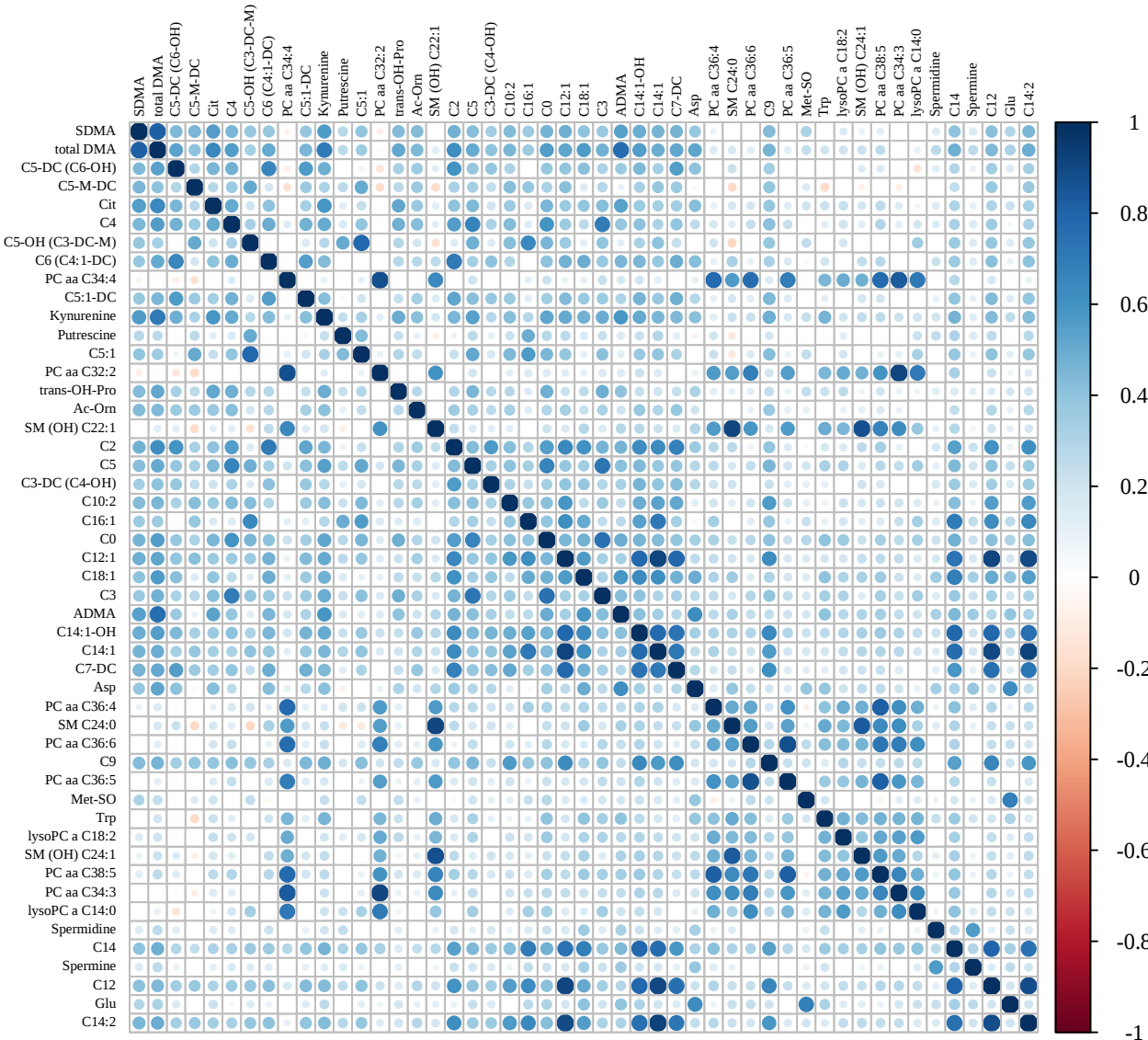
Supplemental Table 4. Correlation between cytokines and KTR in the pooled sample (n = 810; 256 events)

Cytokines	β (SE)	P
IL-1 β	0.06 (0.03)	0.08
IL-2	0.04 (0.03)	0.22
IL-4	-0.13 (0.04)	0.0003
IL-6	0.14 (0.03)	<0.0001
IL-13	-0.25 (0.05)	<0.0001
IFN- γ	0.17 (0.05)	0.001
TNF- α	0.31 (0.06)	<0.0001

All analyses are adjusted for study cohort. Correlations are given as β (SE).

KTR: kynurenine/tryptophan ratio; IL: interleukin; IFN- : interferon; TNF-: tumour necrosis factor.

Supplemental Figure 1. Spearman correlation plot between the 49 associated-metabolites in the discovery sample (Gargano Mortality Study 1)



The areas of circles show the absolute value of corresponding correlation coefficients. The stronger the correlation, the more intense the color of the circle. Insignificant correlations are plotted as blank squares.