Gut microbiota-derived trimethylamine-N-oxide and indoxyl sulfate in acute PE

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Background

- **Trimethylamine-N-oxide (TMAO)** is formed by the oxidation of trimethylamine (TMA) by hepatic favin-containing monooxygenases.
  - TMA is generated in the colon by the gut microbiome from dietary amines (phosphatidylcholine/choline, L-carnitine) if not fully absorbed during digestion within the small intestine.
  - TMAO may be indegested directly with fish/seafood intake.
- Microorganisms associated with increased TMAO production were difficult and incosistently identified with 16S RNA sequencing but most likely include the phylum *Acinetobacteria, Firmicutes, Proteobacteria*.
- A choline rich diet may induce dysbiosis by leading to an increase in the phyla *Firmicutes* and *Proteobacteria.*

Plasma TMAO levels largely depend on the composition of the intestinal flora and the state of the mucus layer, direct TMAO intake, and its renal excretion.

Plasma TMA levels exceed TMAO 5-7x.

- **Indoxyl sulfate** metabolized in the liver from indole converted from dietary tryptophan by bacterial tryptophanases in the colon is a protein-bound uremic toxin.

TMAO: caustive factor, compensatory metabolite, or marker of disease?

TMAO may modulate platlet and blood cell function: ➢increased platelet activation through enhancing stimulus-dependent $Ca2^{+}$ release from cytosolic stores (Zhu et al., Cell, 2016) ➢increased macrophage foam cell deposition ➢increased leukocyte adhesion to endothelium ➢Induction of inflammatory cytokines	**Studies have shown protective functions of TMAO, including the stabilization of proteins and cells exposed to hydrostatic and osmotic stresses in animal models.**	**Some observational studies report elevated TMAO concentrations (*ie* > 4.6 umol/L) to be an independent predictor of arterial thrombosis** (Wang et al., Nature.; Koeth et al. Nature Med.; Nei et al. Stroke; Heianza et al. JAMA) **VTE: in one cross-sectional study TMAO levels showed an U-shaped association with mortality, with the optiumum level ~ 4 umol/L** (Aujesky et al., Thrombosis Research)

Aim

To characterize TMAO and indoxyl sulfate concentrations in PE patients stratified into subgroups according to ESC early mortality risk.

Methods

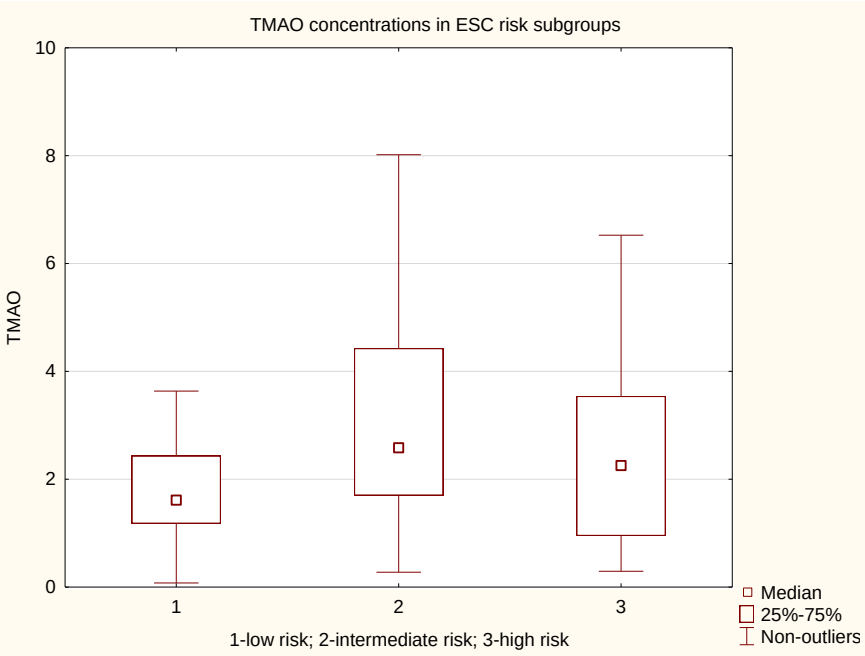
- 181 patients hospitalized due to PE between 2016-2018.
- Blood samples collected at admission, stored in -80 °C until measurement.
- TMAO and indoxyl concentrations were measured in duplicates using high-performance liquid chromatography coupled with mass spectrometry.
- The combined clinical endpoint (CE) was in-hospital mortality and/or in-hospital hemodynamic deterioration.
- Echo RVD defined as RV/LV≥1.0
- Elevated troponin T levels defined as > 14 ng/l.
- Correlations were assessed using Spearman's correlation coefficient. U-Mann Whitey test or Kruskal-Wallis test was used to assess associations between parameters.

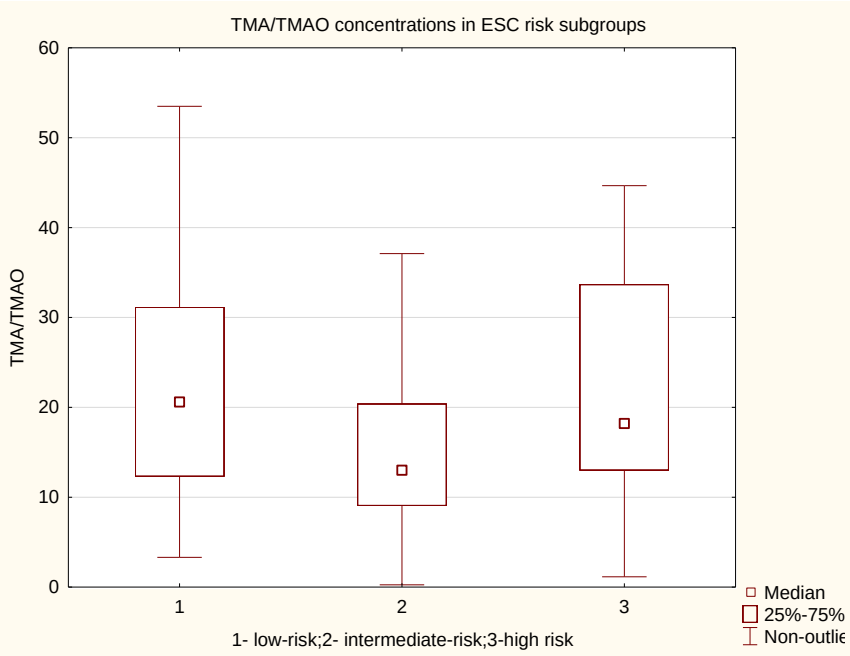
Study group characteristics

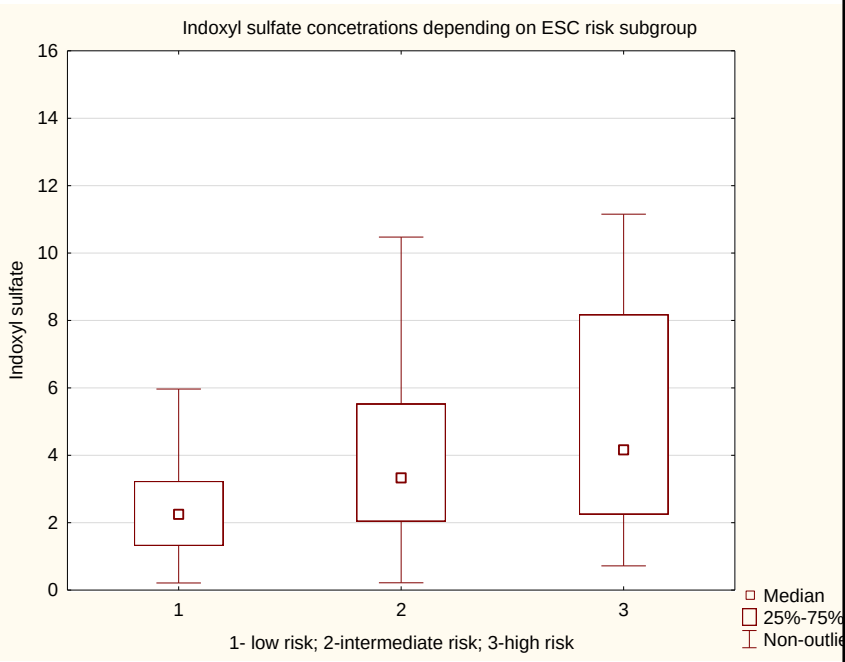
	All (N=181 pts)	low risk (N=50 pts)	intermediate risk (N=119 pts)	high risk (N=12 pts)	p-value
TMAO (umol/L)	2.2 (1.5-3.6)	1.6 (1.2-2.4)	2.6 (1.7-4.4)	2.3 (1.0-3.5)	0.001
Indoxyl (mg/L)	2.9 (1.8-4.7)	2.2 (1.3-3.2)	3.3 (2.0-5.5)	4.2 (2.3-8.2)	0.008
age (years)	64 (46-79)	46 (34-60)	70 (59-83)	71.5 (59.5-84)	<0.001
body weight (kg)	80 (70-95)	80.5 (72.0-97.0)	80.0 (67.0-94.0)	70.0 (66.0-77.0)	0.16
heart rate (bpm)	84 (72-100)	80.0 (70.0-90.0)	85.0 (73.0-100.0)	105.0 (88.5-112.5)	0.004
SBP (mmHg)	130 (120-140)	130.0 (120-140)	130.0 (120-140)	70.0 (55-75)	<0.001
RV/LV 4C	0.9 (0.7-1.14)	0.8 (0.7-0.9)	1.0 (0.8-1.2)	1.3 (1.1-1.4)	<0.001
IVC (mm)	16 (13-19)	13 (12-16)	17.0 (13.0-20.0)	23.0 (15.0-25.0)	0.001
LV EF (%)	60 (55-60)	64.0 (60-65)	60.0 (55-60)	55.0 (50.0-60.0)	<0.001
D-dimer (ng/ml)	5075 (2472-14267)	4330.0 (1769-6416)	5404.5 (2676-19542)	6861.0 (5949-17705)	0.01
Nt-proBNP (pg/ml)	495 (102-3671)	101.0 (47-136)	1307.0 (253-6160)	1673.0 (139-21094)	<0.001
hsTnT (ng/ml)	0.038 (0.021-0.093)	0.0 (0-0)	0.0 (0.0-0.1)	0.1 (0.0-0.2)	<0.001
sCrea (mg/dl)	0.9 (0.8-1.0)	0.8 (0.6-0.9)	1.0 (0.80-1.2)	1.3 (0.9-1.6)	0.001
eGFR (ml/min/1.73m^2)	77.1 (56.8-99)	89.5 (76.4-118.8)	72.9 (55.6-93.6)	48.6 (35.4-89.7)	0.001

TMAO- trimethylamine-N-oxide; TMA- trimethylamine; HR-heart rate; bpm- beats per minute; SBP-systolic blood pressure; RV/LV- right vetnricle/left ventricle; LV EF- left ventricle ejection fraction; IVC-inferior vena cava; eGFR- estimated glomelural filtration rate; sCrea- serum creatinine

Results- ESC risk subgroups



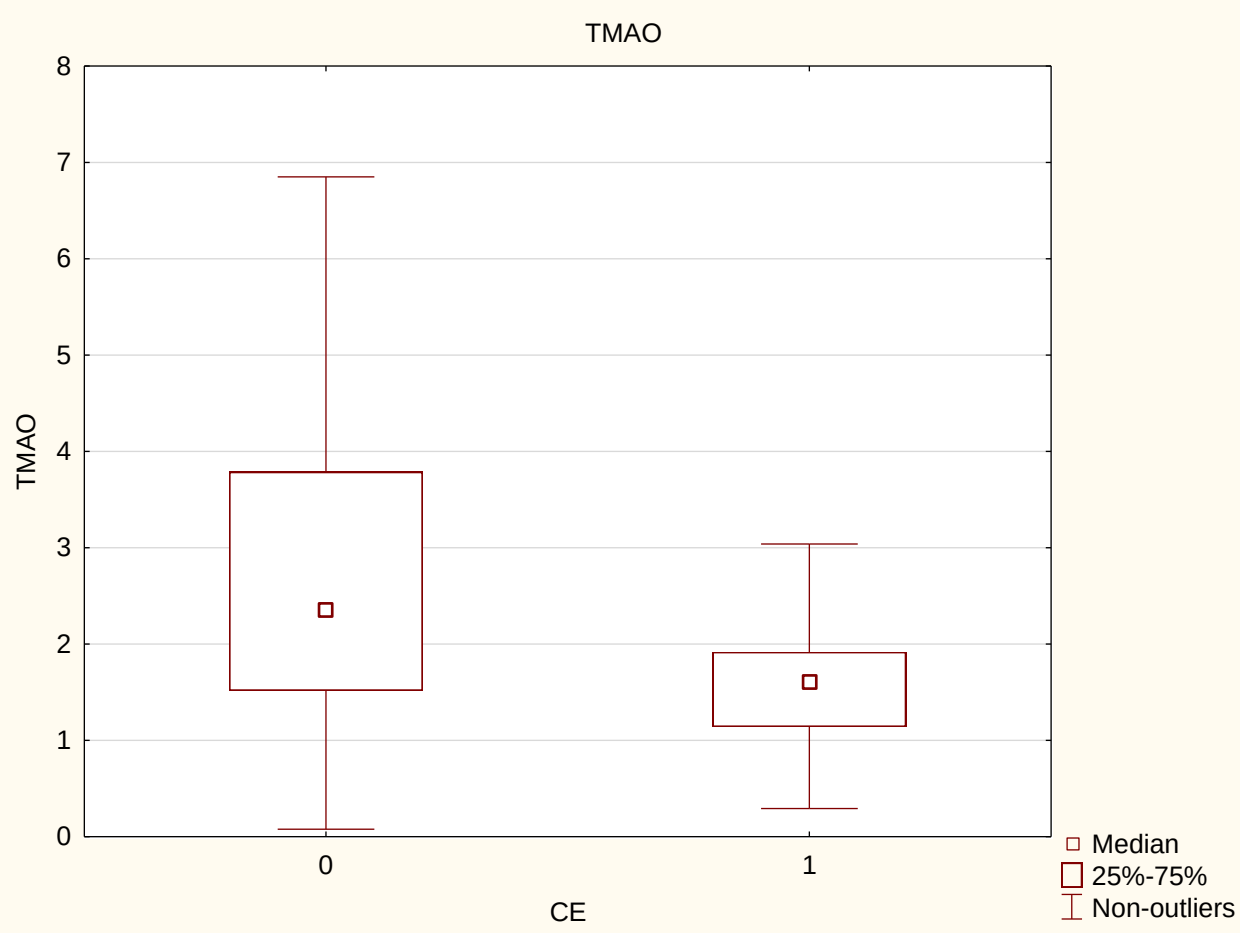


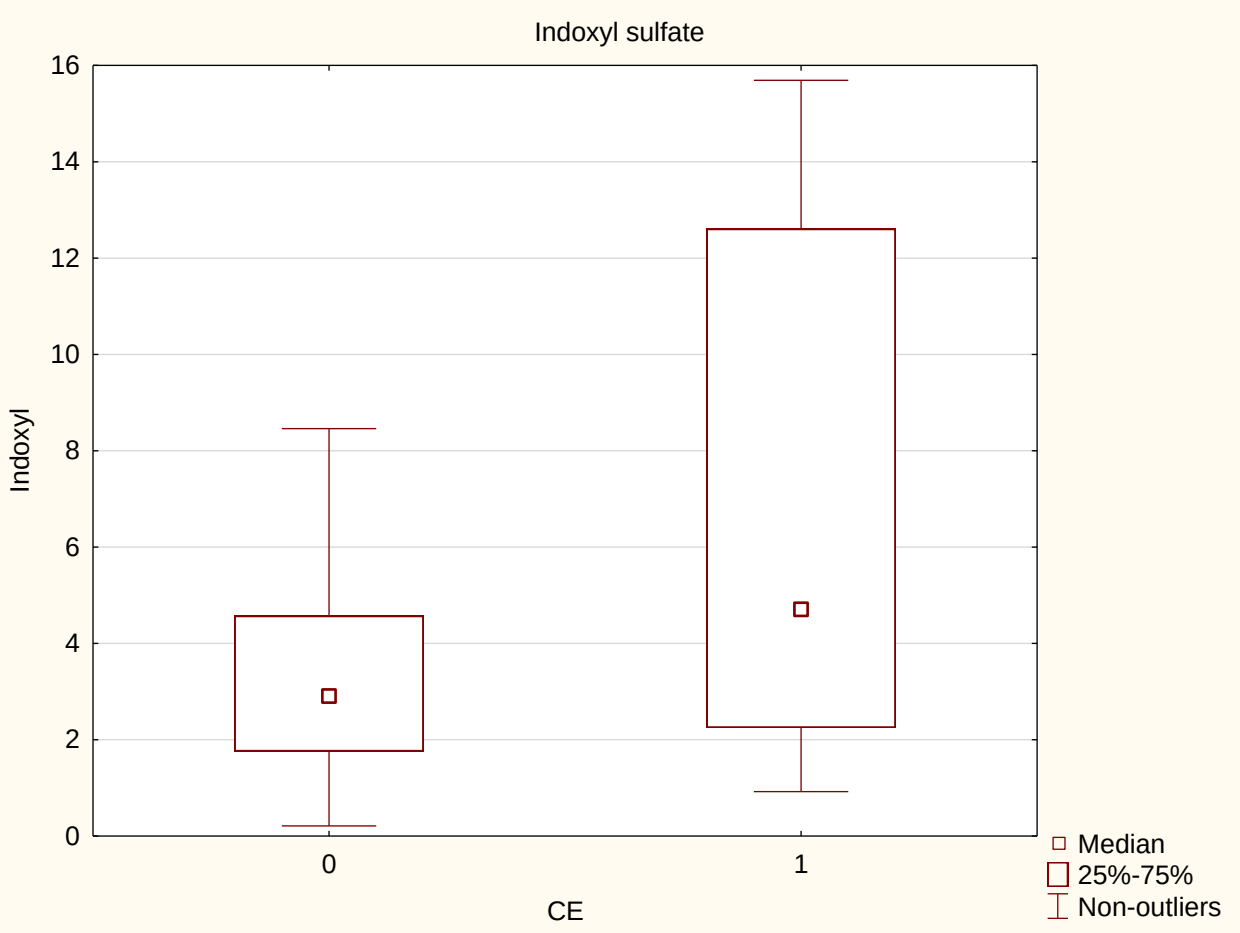


	Echo RVD(+)	Echo RVD (-)	p	hsTnT>ULN	hsTnT<ULN	p
TMAO, median concentration, umol/L	2.6	1.7	0.020	2.6	1.6	0.003
Indoxyl sulfate, median concentration, mg/L	3.3	2.5	0.024	3.7	2.4	0.012

TMAO- trimethylamin-N-oxide, RVD-right ventricle dysfunction; hs-TnT- high-sensitivity troponin T; ULN- upper limit of normal

Results- CE (14 pts)





2.3 vs. 1.6 umol/L; p=0.02

2.9 vs. 4.7 mg/L; p=0.06

Results- TMAO, indoxyl and eGFR

	Spearman's rank correlation coefficent	
	TMAO	Indoxyl
TMAO	1.00	0.02
TMA	0.27	0.02
Indoxyl suflate	0.02	1.00
age	0.13	0.34
body weight	-0.01	0.03
HR	0.16	0.03
SBP	0.01	-0.04
RV/LV 4C	0.03	0.08
LV EF %	-0.11	-0.30
IVC diameter	0.18	0.12
Nt-proBNP	0.09	0.26
D-dimer	-0.05	-0.05
eGFR (MDRD)	-0.01	-0.40
sCrea	-0.02	0.40

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- moderate monotonic correlations between indoxyl sulfate and eGFR and sCrea

Limitations

Relatively small number of enrolled patients, especially from the high-risk subgroup.

No data on dietary patterns.

Conclusions

Higher TMAO levels were associated with features of PE which characterize RVD (RV/LV>1.0, elevated troponin T levels).

Indoxyl sulfate concentrations were higher in higher risk subgroups, which may be explained by decreased renal clearance.