Siderophore-based differentiation of *Aspergillus fumigatus* colonization and invasion

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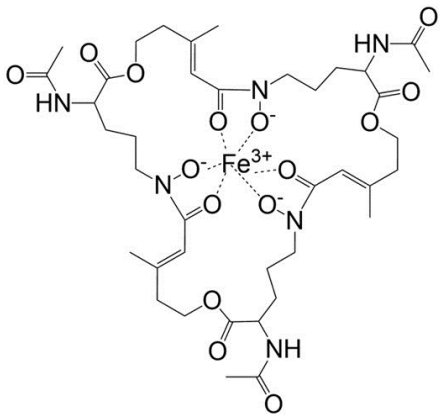
Aspergillus fumigatus

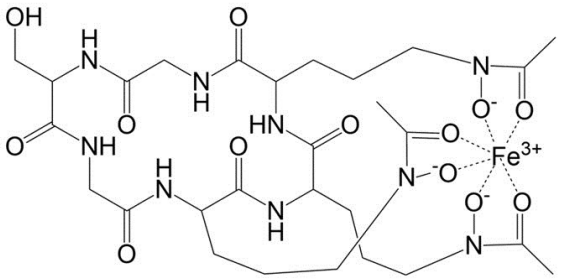
- Omnipresent airborn pathogen
- In iron limited conditions – activation of iron acquisition strategies:
  - Reductive iron assimilation
  - **Siderophore-mediated iron acquisition**

Triacetylfusatinine C

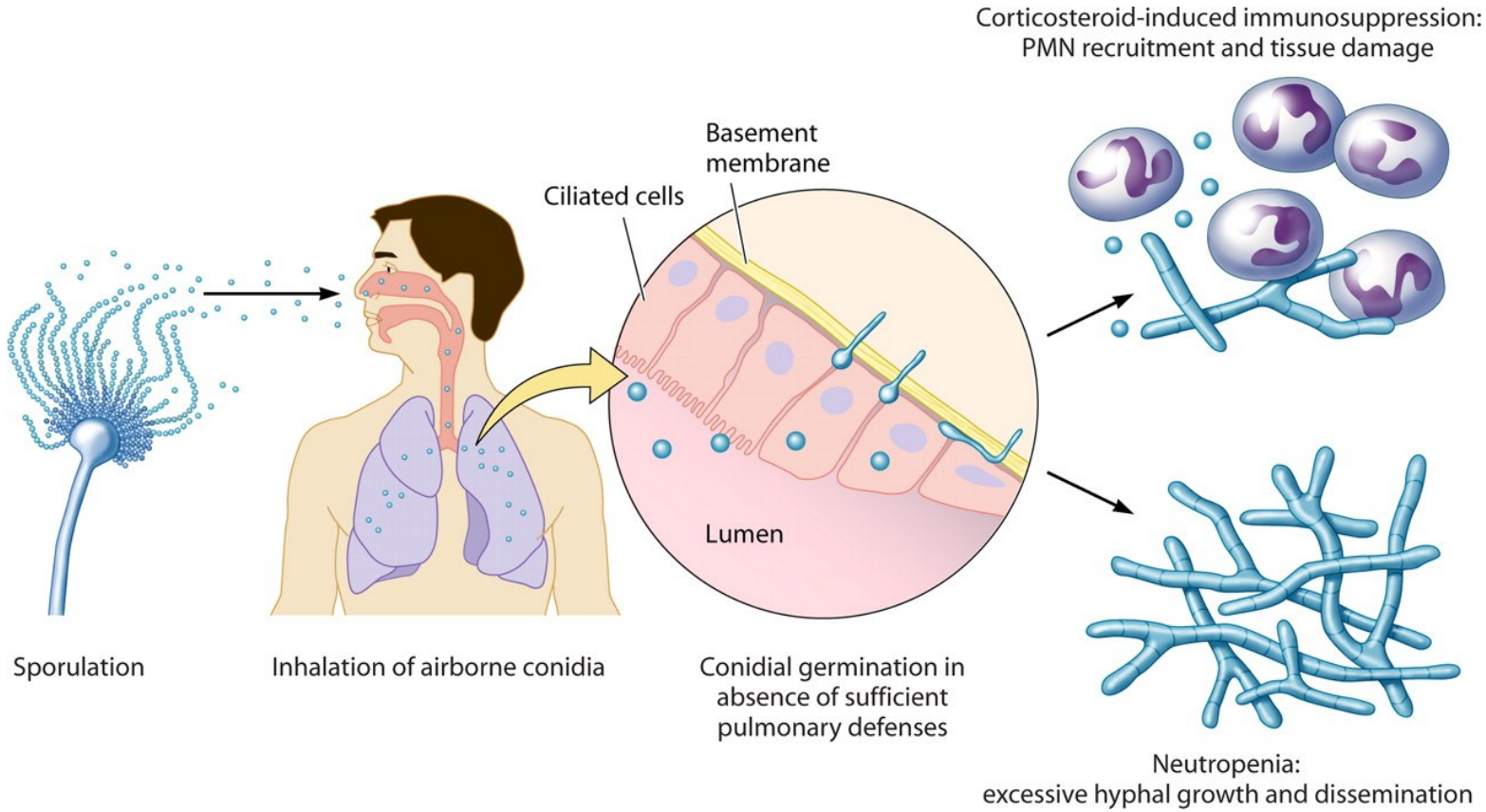


- **Siderophores:**
  - low molecular mass, **high-affinity ferric iron specific chelators** responsible for iron storage and acquisition
  - **Expression is regulated by actual fungal germination stage**

Ferricrocin



Invasive pulmonary aspergillosis in human



Dagenais, T. R. T.; Keller, N. P., *Clinical Microbiology Reviews* **2009,** *22* (3), 447-465

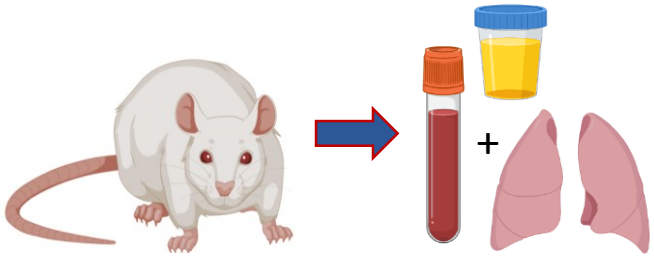
Aims of the study

- Detection of siderophores in invasive pulmonary aspergillosis animal model.
- Determination of *A. fumigatus*-produced siderophores in relation to its germination phase.
- Diagnosis of invasive pulmonary aspergillosis in critically ill patients.

From *in vitro* to *in vivo* study of invasive pulmonary aspergillosis

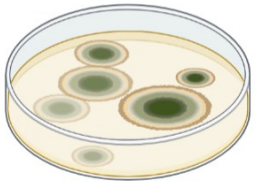
Animal study

Intratracheal application of *A. fumigatus* conidia (10^8 CFU)



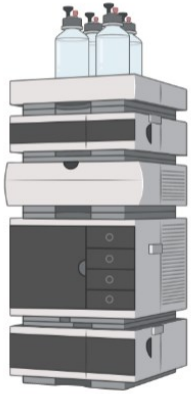
Germination study

Cultivation of *A. fumigatus* conidia in iron-limited mineral medium at pH 7



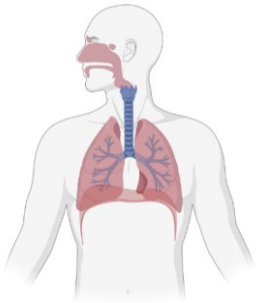
Conidia
Pellet
Supernatant

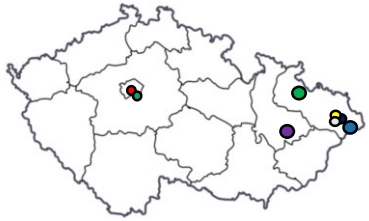
LC-FT-ICR MS
FT-ICR MS Imaging

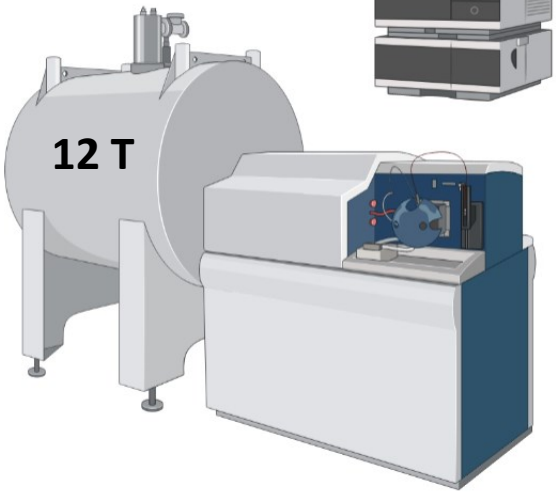


Clinical study

13 patients **diagnosed invasive pulmonary aspergillosis** and **22** patients **having other type of pulmonary disease**

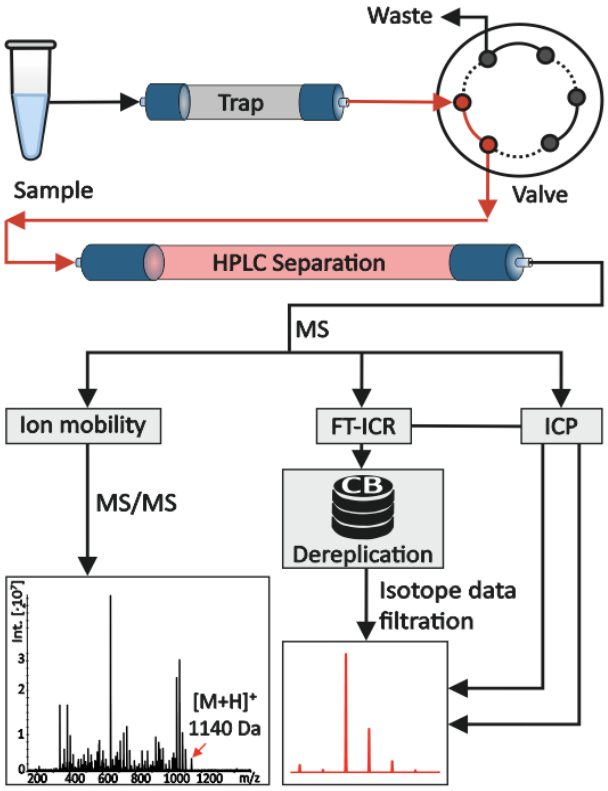


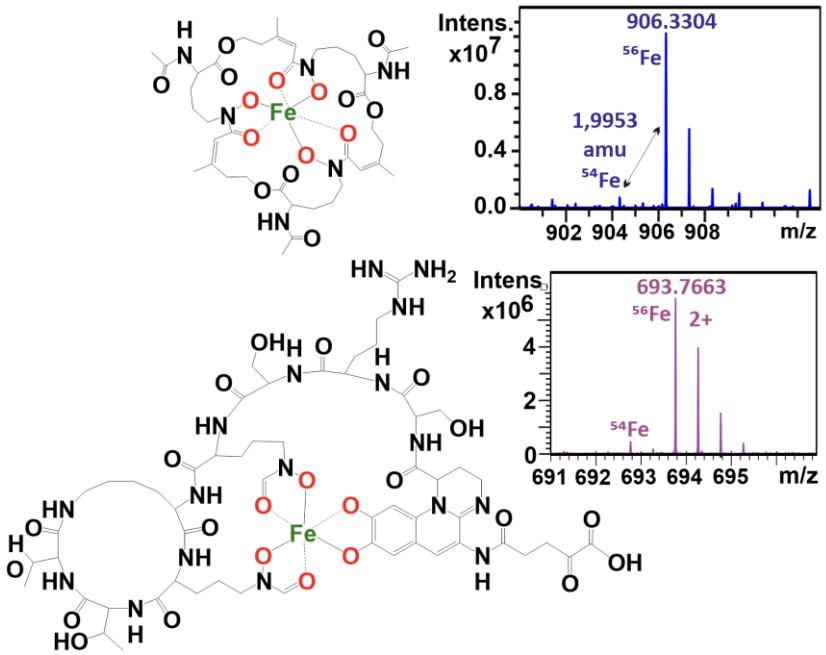




Infection metallomics

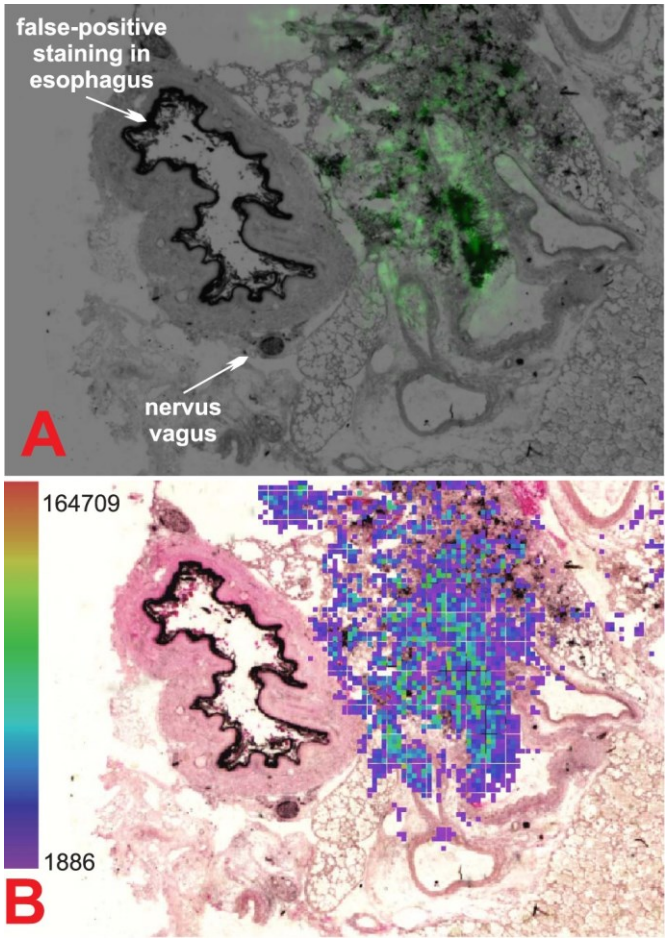
A tool based on the clinical **analysis of metal-containing microbial virulence factors**, specifically, analysis of metal-containing infection biomarkers **using a combination of elemental and molecular MS** that can be potentially applied in a wide range of pathogen-related functional studies



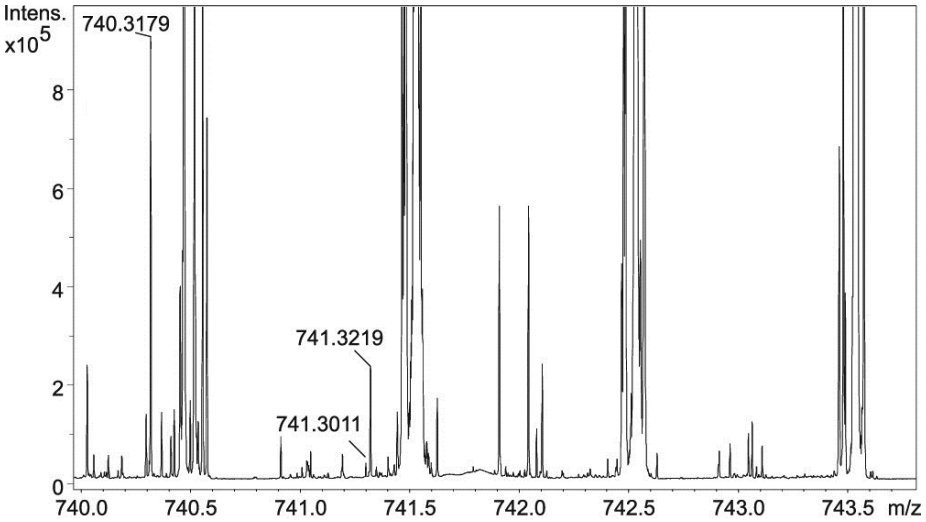


Patil, R.H., et al, 2021 (submitted)

Detection of siderophores in lung tissue

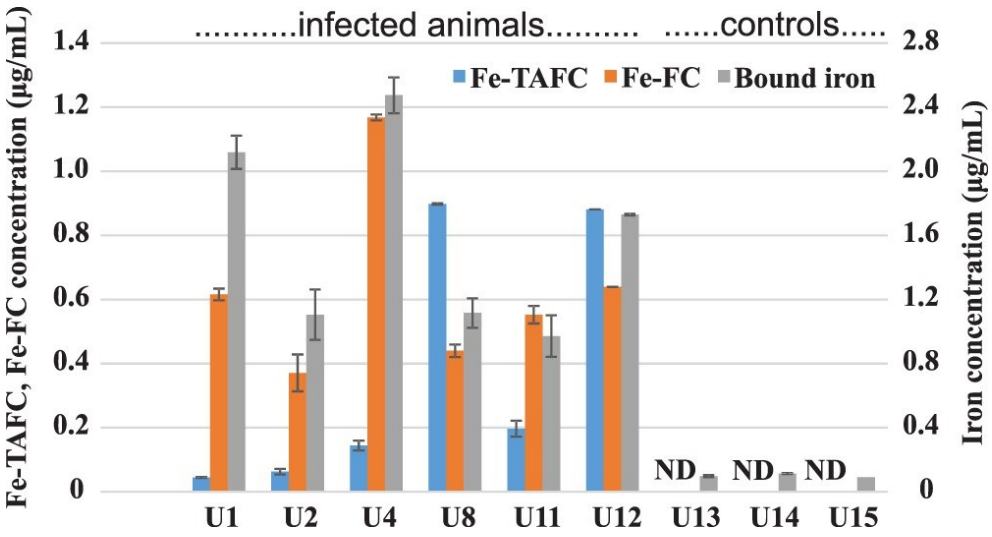


[Ferricrocin+Na]$^+$
m/z 740.3179

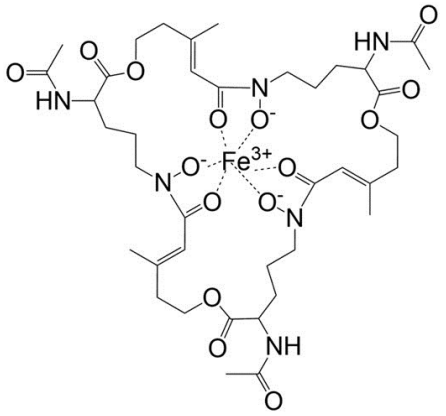


Luptáková, D., *Scientific Reports* **2017,** *7* (1), 16523.

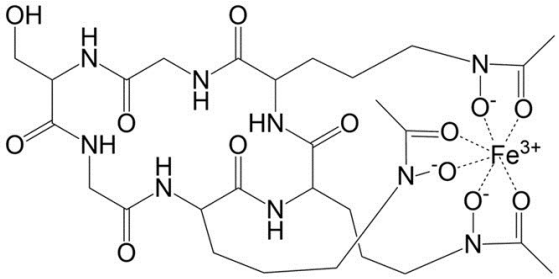
Detection of siderophores in urine



Triacetylfusatinine C

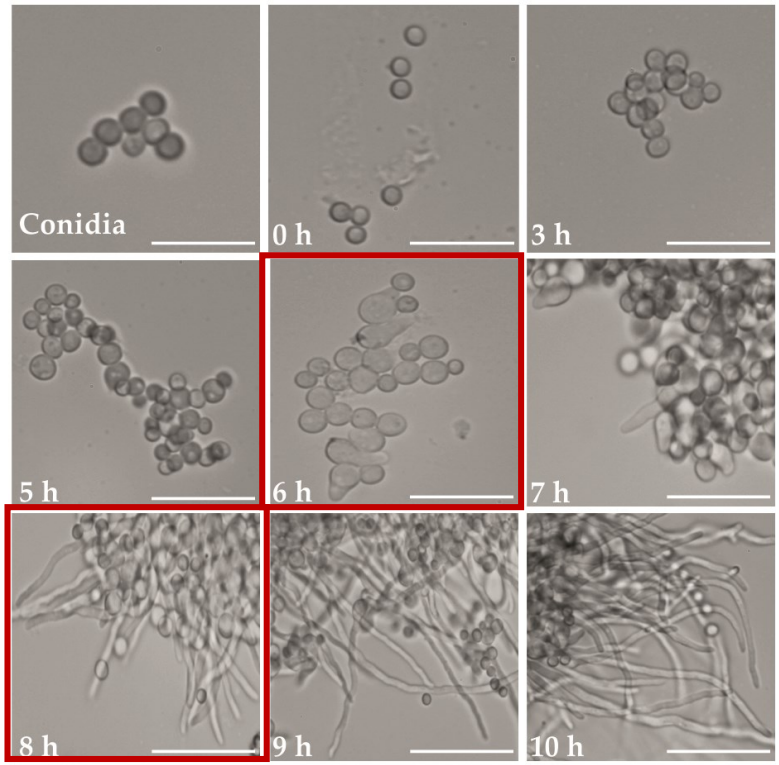


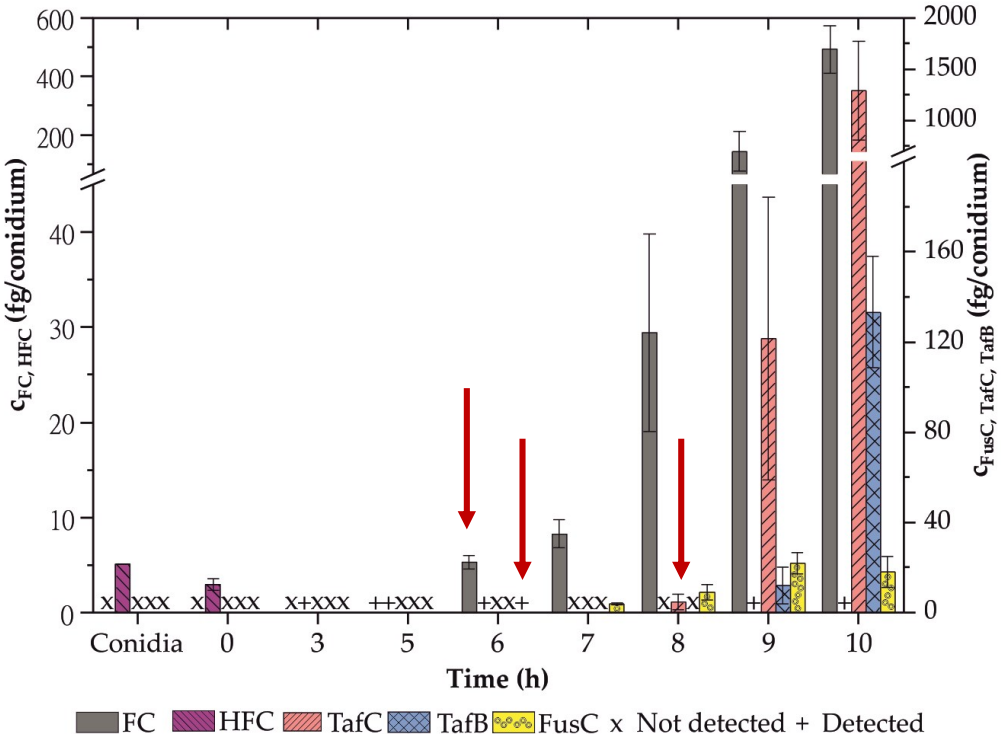
Ferricrocin



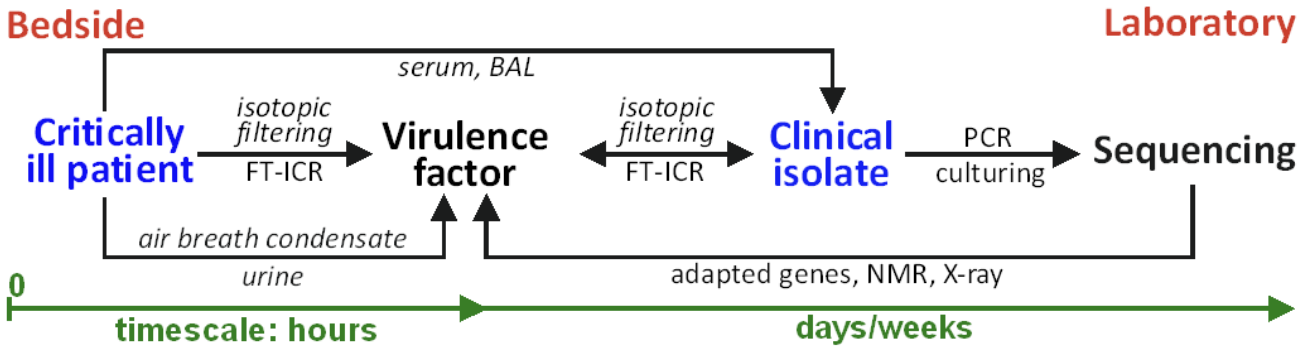
Luptáková, D., *Scientific Reports* **2017,** *7* (1), 16523.

In vitro screening of *A. fumigatus* germination – phase dependent production of siderophores





Monitoring of infection by *infection metallomics*



Non-invasive diagnostics of invasive pulmonary aspergillosis in urine

No.	GD	Age	Underlying disease	Risk factor	*Infection Metallomics*				Conventional clinical approach	
					uGtx/crea index*	uFC/crea index*	uTafC/crea index*	uTafB/crea index*	sGM (ODI)	sBG (pg/mL)
#1	M	50	Multiple myeloma	Neutropenia	15.8±1.0	1.5±0.3	17.2±0.2	0.4±0.0	1.56	501
#2	F	66	Cellular lymphoma	Neutropenia	2.8±0.2	nd	32.1±0.4	det	3.14	115
#3	M	79	Flu (H1N1)	Flu (H1N1)	85.2±3.7	8.3±0.7	82.9±1.5	5.4±0.1	5.9	276
#4	M	71	BB		nd	11.9±2.7	35.7±0.4	11.5±0.4	0.89	>523
#5	M	66	COPD, flu (H1N1)		nd	nd	1.7±0.1	nd	0.1	161
#6	M	50	BB	Diabetes mellitus II	33.8±1.4	4.2±0.5	2.3±0.1	0.4±0.0	0.26	155
#7	M	65	ARDS	Steroids	67.9±6.6	40.6±2.2	3117.4±78.2	59.6±3.0	7.9	>523
#8	M	59	Liver transplant	IST steroids	nd	nd	27.6±0.3	4.2±0.3	0.06	221
#9	M	61	BB	Sepsis**	nd	nd	12.9±0.3	nd	0.27	>523
#10	M	51	Polytrauma	Sepsis**	nd	nd	1.6±0.2	nd	0.24	75
#11	M	68	COPD, TBMA	Chronic ethylism	569.3±13.1	505.1±8.0	1071.1±5.6	251.3±2.2	0.126	>523
#12	M	63	Hepatopathy		nd	nd	det	nd	0.121	439
#13	M	75	Burns	Sepsis**	nd	nd	1.3±0.1	0.5±0.0	0.18	0
			Sensitivity (%)		**46.2**	**53.8**	**100**	**69.2**	**38.4**	**84.6**
			95% CI		17.6 - 71	25 - 81	66.1 – 99.8	39-91	12.7 - 65	57.2 - 98
			Specificity (%)		**100**	**100**	**100**	**100**	**73**	
			95% CI		76.8 - 100	76.8 - 100	76.8 - 100	76.8 - 100	39 - 94	

Summary

- Visualization of *A. fumigatus* infection in rat lung tissue.
- Detection of siderophores – ferricrocin and triacetylfusarinine C in rat plasma and urine.
- Activation of *A. fumigatus* excretome defines a borderline between colonization and invasion.
- **Triacetylfusarinine C**, triacetylfusarinine B, **ferricrocin**, and gliotoxin were markers of enhanced *A. fumigatus* proliferation and were detected non-invasively in urine of 13 patients with invasive pulmonary aspergillosis.

Thank you for your attention

EU FT-ICR MS

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