Quantitative Simulations Predict Treatment Strategies Against Fungal Infections in Virtual Neutropenic Patients

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Motivation

With over 70 %, neutrophils represent the highest fraction of blood leukocytes. Since they can migrate to sites of infection and clear the organism from pathogens they constitute an important part of the immune system.
However, diseases or medical treatments can result in a reduction in the absolute neutrophil count (ANC) in blood called neutropenia. Neutropenia can be due to a disturbed development of neutrophils in the bone marrow, a disturbed migration to the blood stream or a rapid consumption due to an infection.
The severity and the duration of neutropenia directly correlates with a higher risk for infections. Such infections can be caused by bacteria but also fungal pathogens, such as Candida spp. that reside as human commensals on the skin and mucosae .

Aim:

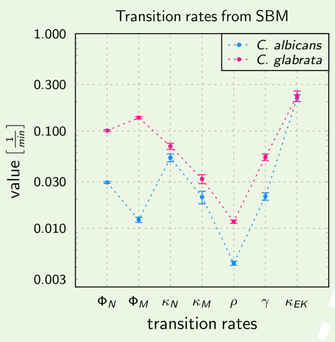
In the current study we use a prevously established bottom-up approach to simulate virtual neutropenic patients. Thereby, we investigate whole-blood infections with the two opportunistic fungal pathogens *C. albicans* and *C. glabrata* and test possible treatment strategies *in silico*.

Legend: Health · Disease · Treatment · Outlook

We performed whole blood infection assays, where blood from healthy donors is infected with *C. albicans* or *C. glabrata* cells. With phagocytosis assays and survival plates we determined pathogen populations of alive, killed, extracellular and phagocytosed cells by monocytes and neutrophils.

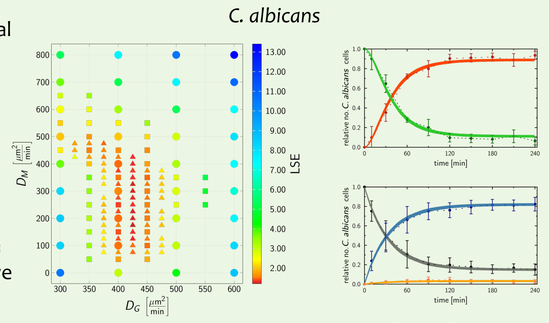
Rate		Description
Φ_N	...	phagocytosis rate of neutrophils
Φ_M	...	phagocytosis rate of monocytes
κ_N	...	killing rate of neutrophils
κ_M	...	killing rate of monocytes
ρ	...	pathogen immune evasion
κ_{EK}	...	secretion of anti-microbial peptides by neutrophils upon first phagocytosis
γ	...	half-life time of anti-microbial peptides

The SBM consists of states and transitions between these states that resemble the biological system. Fitting the SBM to the experimental data allowed quantification of immune reaction rates, such as phagocytosis and killing rates.

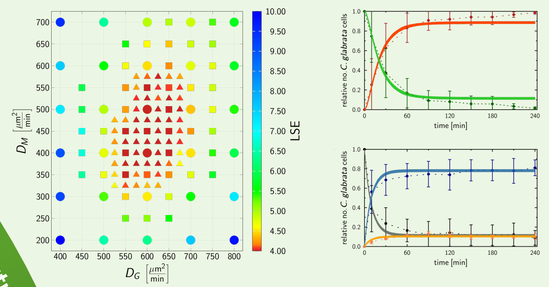


To investigate also spatial aspects of the biological system we build an ABM, where single cells are simulated in a continous three-dimensional environment.

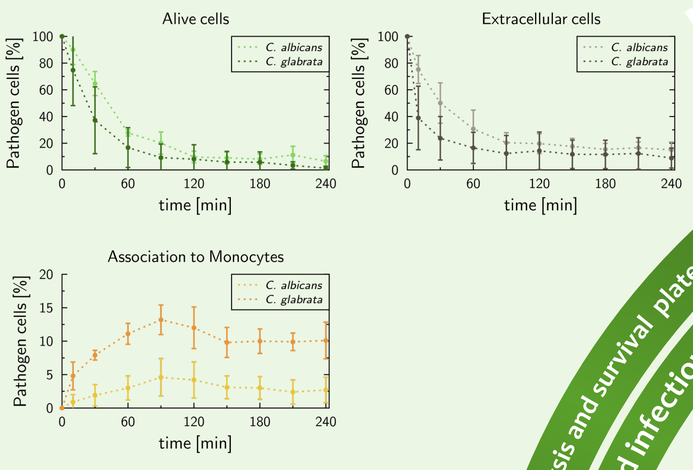
Based on the experimental data and the previously fitted rates we could determine diffusion coefficients of immune cells.

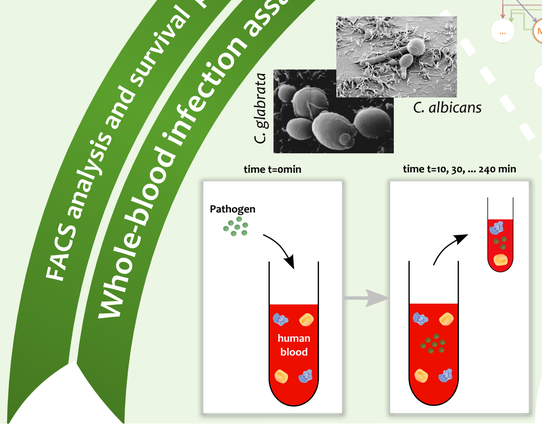


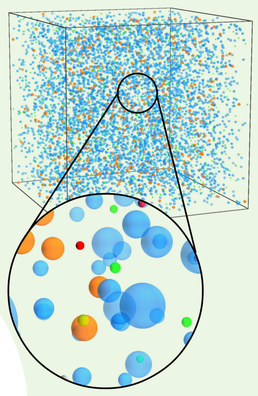
- Minimum: $(D_G^{min}, D_M^{min}) = (425\frac{\mu m^2}{min}, 175\frac{\mu m^2}{min})$
- Infection outcome:
  - neutrophils play major role in the immune response
  - sensitive to variations in the diffusion of neutrophils
  - insensitive in the diffusion of monocytes



- Minimum: $(D_G^{min}, D_M^{min}) = (600\frac{\mu m^2}{min}, 425\frac{\mu m^2}{min})$
- Infection outcome:
  - monoctes are more important than for C. albicans
  - higher diffuision coefficients than for C. albicans







Immune reaction rates
State-based model
Migration parameters of neutrophils and monocytes
Agent-based model
FACS analysis and survival plates
Whole-blood infection assays

Legend

P_K experimental data / P_K ABM data
P_A experimental data / P_A ABM data
P_E experimental data / P_E ABM data
P_G experimental data / P_G ABM data
P_M experimental data / P_M ABM data

Summary:

- experimental data can be fitted with the SBM and ABM
- immune reaction rates and migration parameters can be estimated

- VNP can be simulated:
  - strong decrease of killed C. albicans cells in severe neutropenia
  - for C. *glabrata* monocytes can partially compensate the low number of neutrophils
  - VNP can be 'treated' by increasing phagocytosis rata and/or diffusion coefficients

- 4×10^4 simulations

- all VNP do reach the infection outcome of non-neutropenic patients by increasing neutrophil activation

- required increase in neutrophil activation depends on the severity degree of neutropenia in VNP

Outlook:

- *in silico* supplementation of donor neutrophils
- clarify the mechanism for pathogen immune evasion

Summary
Outlook

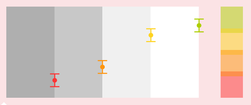
Systems Biology

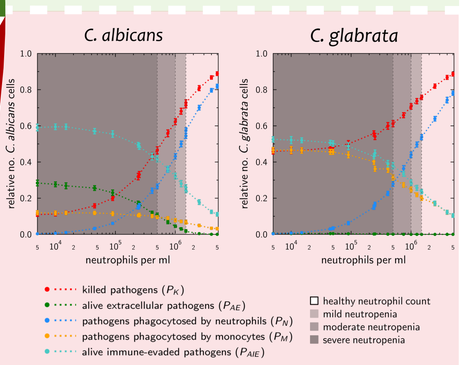
Neutropenia
Infection outcome and pattern

Degrees of Severity of Neutropenia:

	Severe	Moderate	Mild	Healthy
Neutrophils/μl	< 500	500 - 1000	1000 - 1500	> 1500

Pattern at transitions between the different degrees of severity:
considers killed cells and alive immune evaded cells





Pattern for Classification:

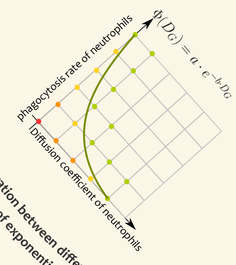
		mild	moderate	severe
C. albicans	P_K	0.713 ± 0.014	0.623 ± 0.017	0.464 ± 0.02
	P_{AIE}	0.26 ± 0.015	0.322 ± 0.016	0.417 ± 0.016
C. glabrata	P_K	0.757 ± 0.012	0.707 ± 0.015	0.612 ± 0.018
	P_{AIE}	0.236 ± 0.012	0.286 ± 0.015	0.38 ± 0.017

5 Virtual Neutropenic Patients (VNP):

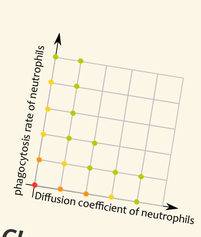
	Severe	Moderate	Mild	Healthy
Neutr./μl	< 500	500 - 1000	1000 - 1500	> 1500
	VNP 5	VNP 4	VNP 3	VNP 2	VNP 1

In silico Treatment

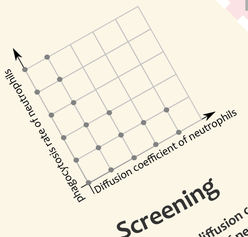
Find separation between different classes by fitting of exponential function



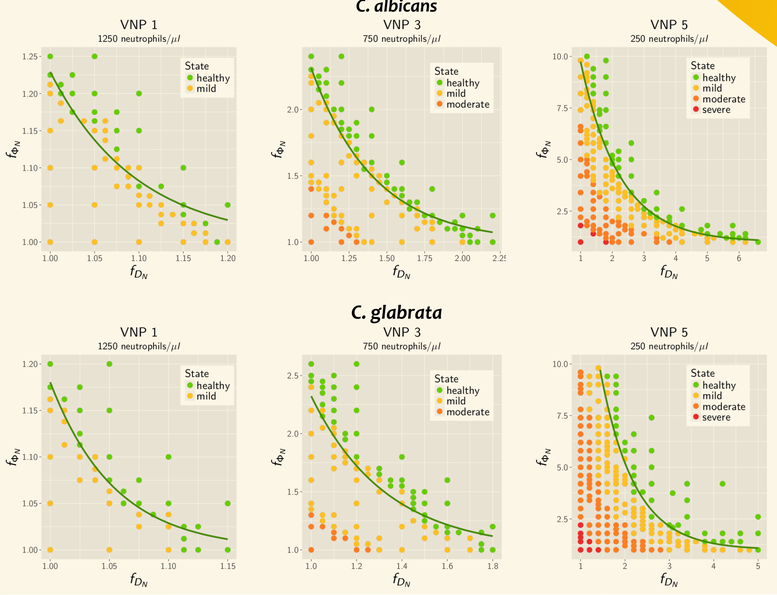
Classification of all simulations according to pattern



Screening
stepwise increase of the diffusion coefficient and the phagocytosis rate of neutrophils



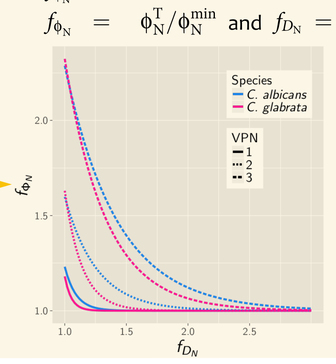
C. albicans

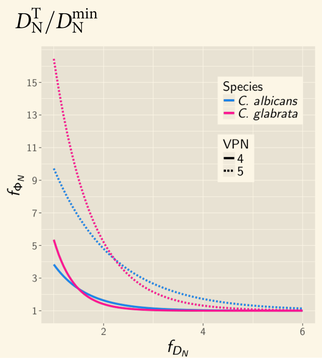


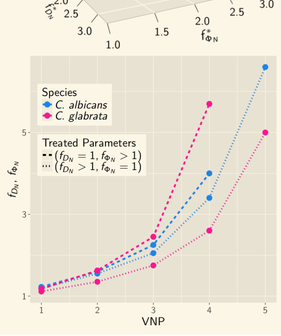
C. glabrata

Curve fitting at transitions:

$$f_{\phi_N} = 1 + a \cdot e^{-b \cdot f_{D_N}} \quad \text{with}$$
$$f_{\phi_N} = \phi_N^T / \phi_N^{min} \quad \text{and} \quad f_{D_N} = D_N^T / D_N^{min}$$







- **either the diffusion coefficient or the phagocytosis rate was fixed:**
  - we found that $f_{D_N} < f_{\Phi_N}$
  - changing only the diffusion coefficient was more effective for C. *glabrata* infection
  - changing inly the phagocytosis rate was more effective for *C. albicans* infection

- **combined impact of both parameters yield a pair of optimal values** $(f^*_{\phi_N}, f^*_{D_N})$ with minimal distance from $(f_{\phi_N} = 1, f_{D_N} = 1)$ with $f^*_{\Phi_N} < f^*_{D_N}$

- $(f^*_{\phi_N}, f^*_{D_N})$ was lower for C. *glabrata* in all VNP

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Pathogenic fungi and their human host: Networks of interaction
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