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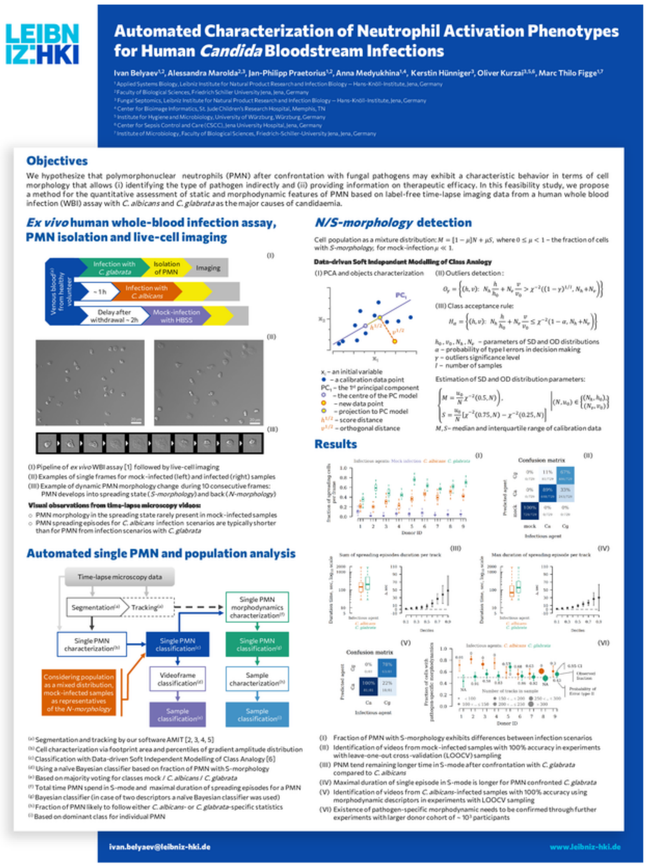

Automated Characterization of Neutrophil Activation Phenotypes for Human *Candida* Bloodstream Infections

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Objectives

We hypothesize that polymorphonuclear neutrophils (PMN) after confrontation with fungal pathogens may exhibit a characteristic behavior in terms of cell morphology that allows (i) identifying the type of pathogen indirectly and (ii) providing information on therapeutic efficacy. In this feasibility study, we propose a method for the quantitative assessment of static and morphodynamic features of PMN based on label-free time-lapse imaging data from a human whole blood infection (WBI) assay with *C. albicans* and *C. glabrata* as the major causes of candidaemia.

Ex vivo human whole-blood infection assay, PMN isolation and live-cell imaging

(I)

(II)

(III)

(I) Pipeline of *ex vivo* WBI assay [1] followed by live-cell imaging
(II) Examples of single frames for mock-infected (left) and infected (right) samples
(III) Example of dynamic PMN morphology change during 10 consecutive frames: PMN develops into spreading state (*S-morphology*) and back (*N-morphology*)

Visual observations from time-lapse microscopy videos:

- PMN morphology in the spreading state rarely present in mock-infected samples
- PMN spreading episodes for *C. albicans* infection scenarios are typically shorter than for PMN from infection scenarios with *C. glabrata*

Automated single PMN and population analysis

[a] Segmentation and tracking by our software AMIT [2, 3, 4, 5]
[b] Cell characterization via footprint area and percentiles of gradient amplitude distribution
[c] Classification with Data-driven Soft Independent Modelling of Class Analogy [6]
[d] Using a naïve Bayesian classifier based on fraction of PMN with S-morphology
[e] Based on majority voting for classes mock / *C. albicans* / *C. glabrata*
[f] Total time PMN spend in S-mode and maximal duration of spreading episodes for a PMN
[g] Bayesian classifier (in case of two descriptors a naïve Bayesian classifier was used)
[h] Fraction of PMN likely to follow either *C. albicans*- or *C. glabrata*-specific statistics
[i] Based on dominant class for individual PMN

N/S-morphology detection

Cell population as a mixture distribution: $M = [1-\mu]N + \mu S$, where $0 \leq \mu < 1$ – the fraction of cells with *S-morphology*; for mock-infection $\mu \ll 1$.

Data-driven Soft Independent Modelling of Class Analogy

(I) PCA and objects characterization

x_c – an initial variable
● – a calibration data point
PC_n – the n^{th} principal component
○ – the centre of the PC model
● – new data point
○ – projection to PC model
$h^{1,2}$ – score distance
$v^{1,2}$ – orthogonal distance

(II) Outliers detection:

$$O_\beta = \left\{(h,v):\ N_\beta \frac{h}{h_\beta} + N_\beta \frac{v}{v_\beta} > \chi^{-2}((1-\gamma)^{1/f}, N_\beta + N_\beta)\right\}$$

(III) Class acceptance rule:

$$H_\alpha = \left\{(h,v):\ N_\beta \frac{h}{h_\beta} + N_\beta \frac{v}{v_\beta} \leq \chi^{-2}(1-\alpha, N_\beta + N_\beta)\right\}$$

$h_\beta, v_\beta, N_\beta, N_\alpha$ – parameters of SD and OD distributions
α – probability of type I errors in decision making
γ – outliers significance level
f – number of samples

Estimation of SD and OD distribution parameters:

$$\begin{cases} M = \frac{N_\beta}{N} \chi^{-2}(0.5, N), \\ S = \frac{N_\beta}{N} [\chi^{-2}(0.75, N) - \chi^{-2}(0.25, N)] \end{cases} \Bigg| \{(N_\alpha, h_\alpha), (N_\alpha, v_\alpha)\} \in \begin{cases} (N_\alpha, h_\alpha) \\ (N_\alpha, v_\alpha) \end{cases}$$

M, S – median and interquartile range of calibration data

Results

(I) Fraction of PMN with S-morphology exhibits differences between infection scenarios
(II) Identification of videos from mock-infected samples with 100% accuracy in experiments with leave-one-out cross-validation (LOOCV) sampling
(III) PNM tend remaining longer time in S-mode after confrontation with *C. glabrata* compared to *C. albicans*
(IV) Maximal duration of single episode in S-mode is longer for PMN confronted *C. glabrata*
(V) Identification of videos from *C. albicans*-infected samples with 100% accuracy using morphodynamic descriptors in experiments with LOOCV sampling
(VI) Existence of pathogen-specific morphodynamic needs to be confirmed through further experiments with larger donor cohort of ~ 10^2 participants

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