

Cervicovaginal Inflammation Is Associated With Cervical Neutrophil Infiltration and Activation In Women at High-risk of Delivering Preterm

Belen Gimeno-Molina, Erna Bayar, Yun S. Lee, Ingrid Muller, Marina Botto, Anne Dell, Stuart Haslam, Paola Grassi, Gang Wu, David A. MacIntyre, Phillip R. Bennett, Pascale Kropf, Lynne Sykes
March of Dimes Prematurity Research Centre, Imperial College London, United Kingdom

INTRODUCTION

- **Infection** is one of the most common causal aetiologies in spontaneous preterm birth (sPTB).
- The vaginal microbial composition (Community State Type, CST) can influence the risk of sPTB.
- ***Lactobacillus* depletion (CST IV)** increases the risk of sPTB.
- ***L. crispatus* (CST I)** is protective¹⁻⁴.
- The mechanism of microbial induced inflammation at the cervico-vaginal interface is not fully understood.

HYPOTHESIS:

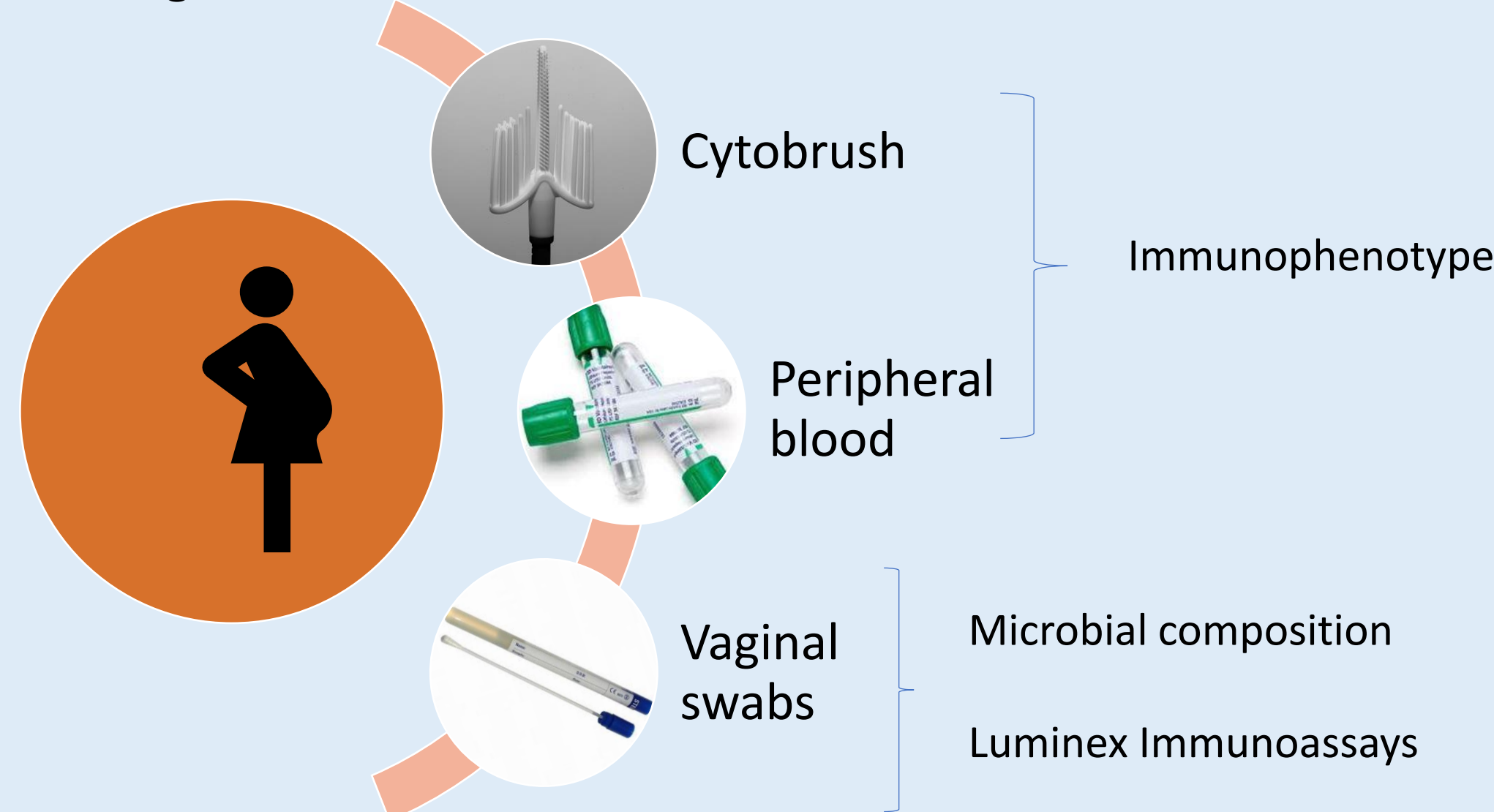
Cervical neutrophils play a key role in microbial-induced inflammation and sPTB

AIMS:

- To characterise the immunophenotype of cervical and peripheral blood neutrophils.
- To determine if the immunophenotype of cervical neutrophils is altered depending on the local microbial composition

METHODS

- Pregnant women (n=61) at high-risk of PTB were recruited from the PTB prevention clinic at QCCH.
- Matched sets (n=81) of cervicovaginal swabs, cytobrush and peripheral blood were collected across pregnancy.
- Paired peripheral and cervical immune cell subsets and activation markers were assessed by Aurora Cytek™.
- Microbial composition was determined by surveying the V1-V2 region of 16S rRNA gene on Illumina MiSeq. Luminex immunoassays were used to detect Complement proteins from cervicovaginal fluid



1 Detection of peripheral and cervical neutrophils

Brightfield imaging of cytobrush samples revealed presence of epithelial cells, neutrophils and bacteria (Figure 1A), and by Giemsa staining (Figure 1B) and use of specific markers (CD15, CD16, CD63, Figure 1C-E) we can confirm the detection of cervical neutrophils.

Granulocytes were purified from whole peripheral blood. Peripheral granulocytes and cytobrush samples were stained with antibodies to recognise cell surface markers antibodies specific for the detection of neutrophils (CD45, CD15, CD14) (Figure 2). Cervical neutrophils were detected in 86% of samples (n=70/81).

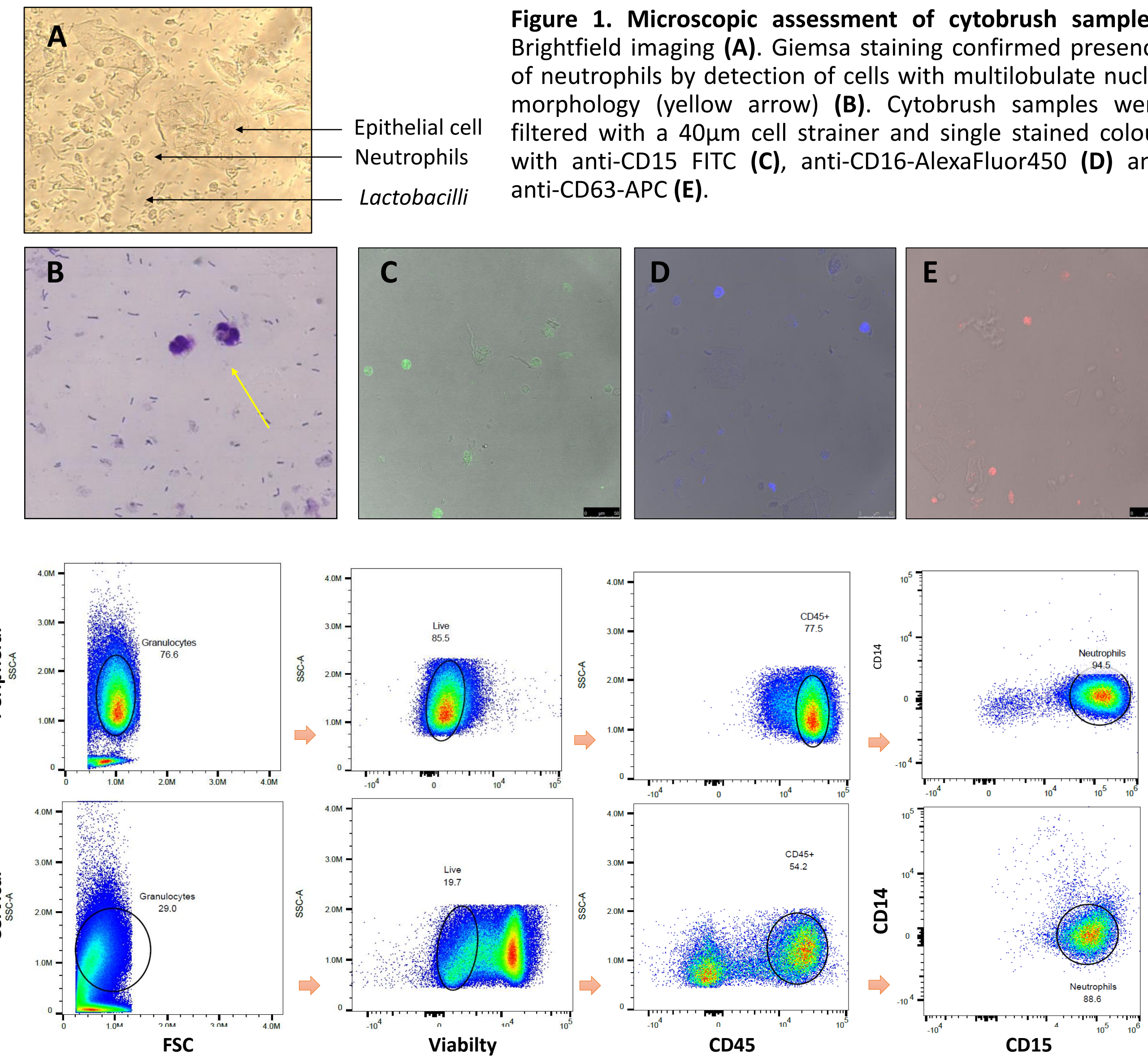
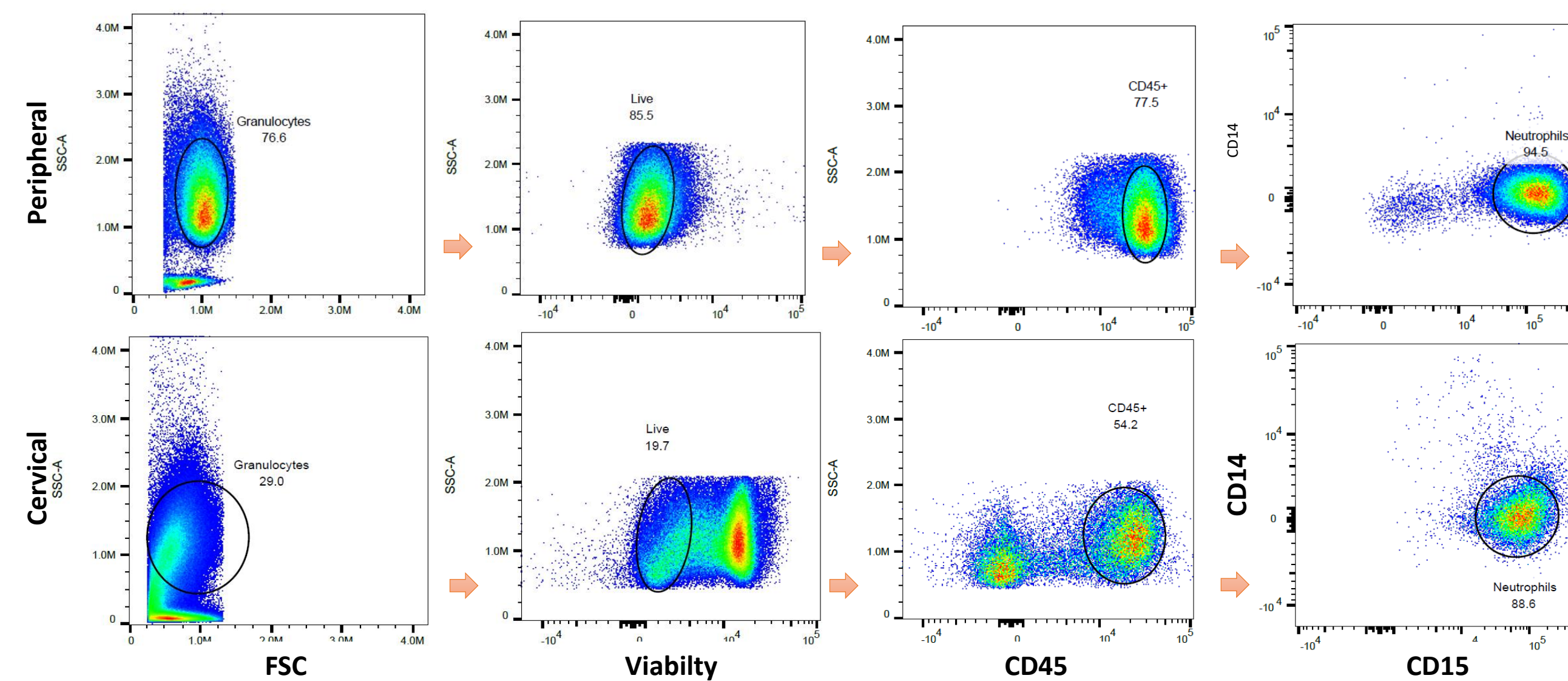


Figure 2. Gating strategy for flow cytometric analysis of peripheral blood and cytobrush samples. Exclusion of cell doublets (not shown). Selection of granulocytes. Selection of live cells. Selection of immune cells (CD45+). Selection of neutrophils (CD15+, CD14-/low.)

2 Cervical and peripheral neutrophils exhibit a different immunophenotype

Cervical neutrophils showed reduced expression of migration markers CD11b (p=0.0005) and CD62L (p<0.0001), compared to peripheral neutrophils, probably due to the shedding of this markers from the surface after migration. Neutrophils perform degranulation and phagocytic activities as a defense mechanism. Markers implicated in this activities have a higher expression in cervical neutrophils (CD63 and CD66b; p<0.001).

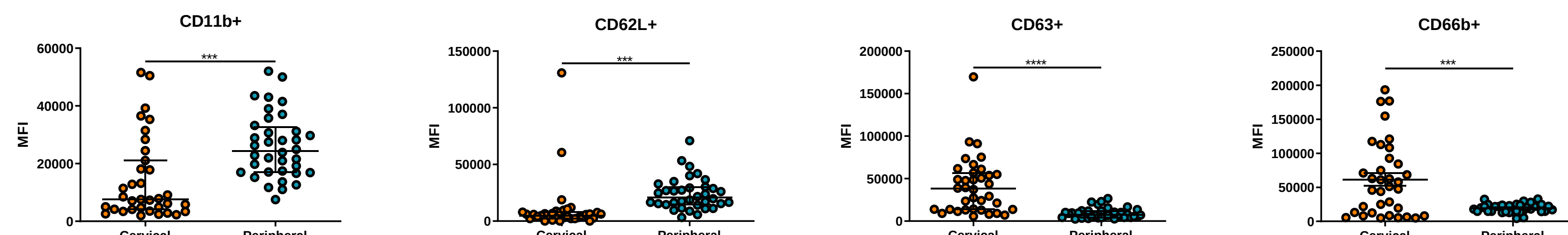


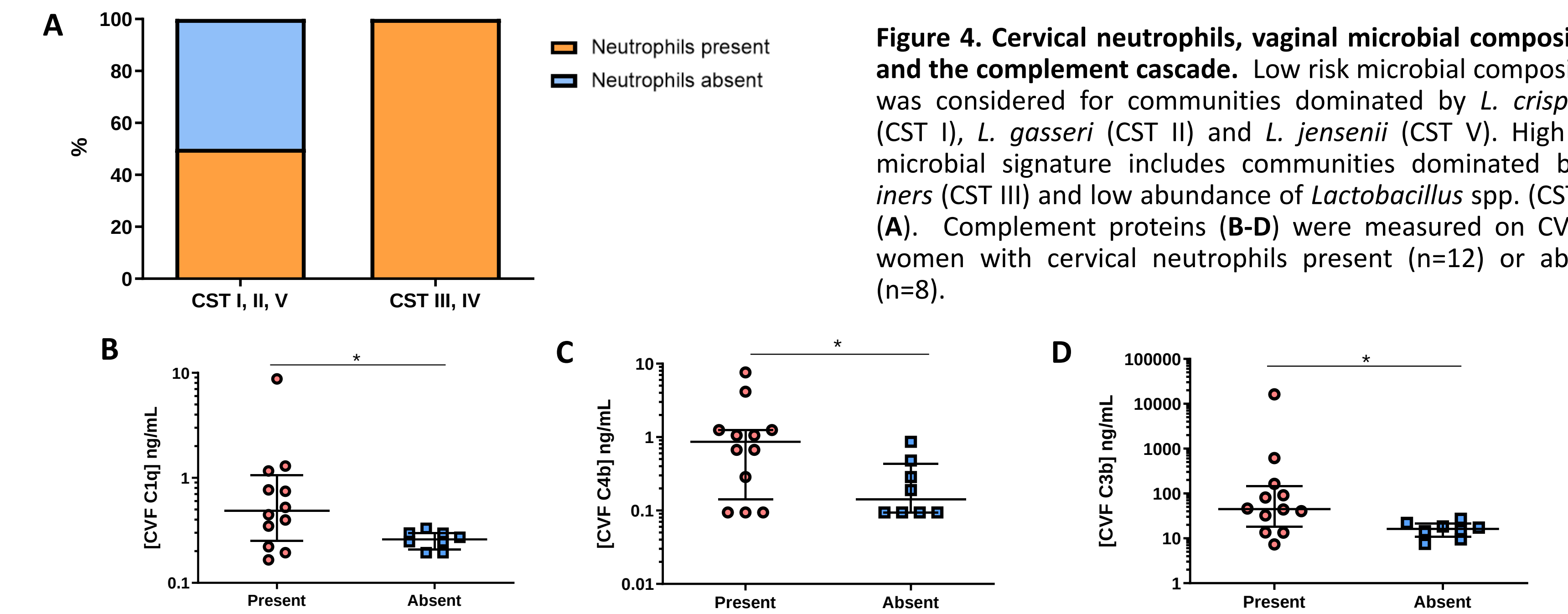
Figure 3. Activation status of maternal peripheral and cervical neutrophils. MFI expression of CD11b, CD62L, (migration) CD63 (degranulation) and CD66b (phagocytosis) markers of matched cervical and peripheral neutrophils (n=36). Statistical analysis was performed using Wilcoxon matched-pairs signed rank test.

RESULTS

3 Detection of cervical neutrophils is influenced by vaginal microbial composition and is associated with increased complement proteins

Cervical neutrophils were detected in all samples where vaginal composition was dominated by *L. iners* (CST III) or anaerobic, non-lactobacilli dominant bacteria (CST IV) (Figure 4A).

The complement system bridges the adaptive and innate immune system. Complement proteins play a key role in inflammation and host defense against bacterial infection. The classical, lectin and alternative pathways converge on C3. The presence of cervical neutrophils was associated with increased complement proteins such as C3b, C1q and C4b. (Figure 4 B-D).



CONCLUSION

- **Cervical neutrophils** are more **activated** than peripheral neutrophils and their presence at the cervical vaginal interface is associated with **high-risk microbial profiles** and activation of the **complement cascade**.
- These findings support a role for neutrophils in microbial driven preterm birth.
- **Potential impact:** understanding the pathophysiology of preterm labour could lead to the development of a point of care test, and development/management of new therapeutics approaches, such as use of live biotherapeutics (*poster ref 1308*) or complement modulators.

