NEUTROPHIL EXTRACELLULAR TRAP FORMATION IN PRIMARY CILIARY DYSKINESIA: INSIGHTS FROM PMA-INDUCED NETOSIS

Karla J. Rosario-Pacheco[1] BS, Sonya J. Malavez-Cajigas[1] MS, Mercedes Lacourt-Ventura[1] MS, Jose Muñiz[1] BS, Wilfredo de Jesús-Rojas[1] MD and Marcos J. Ramos-Benitez[1] PhD

Basic Sciences, Ponce Health Sciences University, Ponce Research Institute, Ponce, Puerto Rico

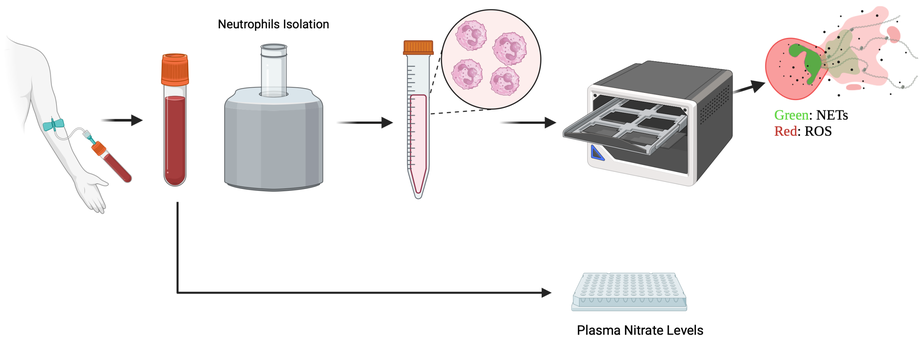
ABSTRACT

Neutrophil Extracellular Traps possess a contradictive role in the immune system. Although Primary Ciliary Dyskinesia (PCD) is known to be susceptible to neutrophil infiltration, their role and contribution to disease pathogenesis is largely unexplored. To investigate this, we isolated neutrophils from PCD patients along with matched controls and stimulated them with different concentrations of Phorbol 12-myristate 13-acetate (PMA), a NETosis inducer. Significant differences were observed using 100nM of PMA, which caused a decreased NETs capacity in PCD patients compared to healthy controls. We also found a trend towards a positive correlation between NETs capacity and plasma nitrate levels. Our preliminary results highlight the importance of continuing the study of NETs in the context of PCD.

INTRODUCTION

- PCD is a rare genetic disease characterized by impaired mucociliary clearance and recurrent infections, which leads to neutrophil infiltration and the formation of NETs in the airways.
- Patients with PCD have been shown to have a decreased capacity to produce nitric oxide (NO), which plays a regulatory role in NETosis by modulating the activation of neutrophils.
- Previous studies suggested patients with PCD display reduced NETs formation in response to PMA, a common NETosis stimulant. However, the underlying mechanisms leading to the difference in NETosis capacity remain unclear.
- We hypothesize that NO levels are associated with NETosis capacity in these patients. Thus, we examined neutrophils from patients with PCD following PMA stimulation and measured plasma nitrate levels as a surrogate of nitric oxide (NO) production.

METHODS



RESULTS

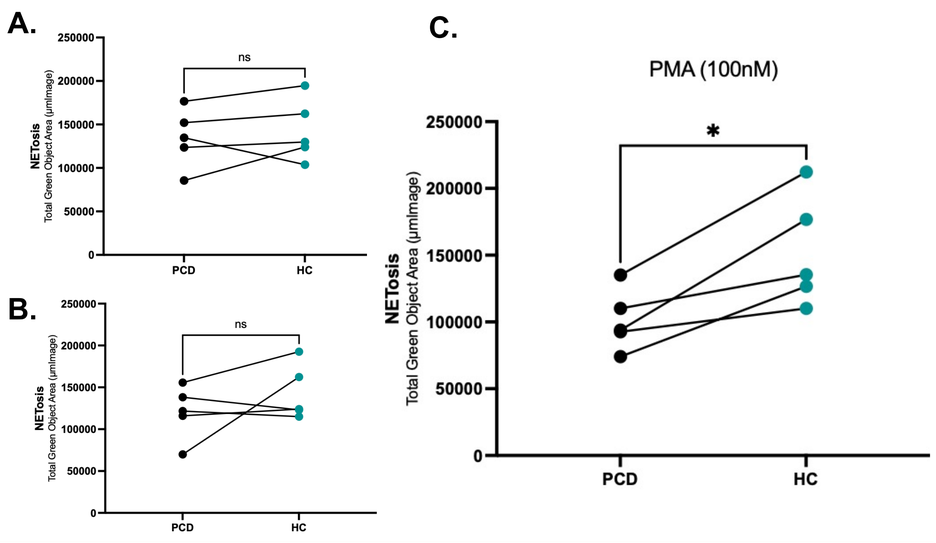


Figure 1 | NETosis capacity in PCD patients using different concentrations of PMA

A. No significant differences were found in NETosis capacity between PCD patients and matched controls at 25nM PMA. **B.** Similar to 25nM, no significant differences were found in NETosis capacity between PCD patients and matched controls at 50nM PMA. **C.** Contrary to lower PMA concentrations, 100nM demonstrated significant differences ($P<0.05$), showing a higher NETosis capacity in PCD patients compared to controls.

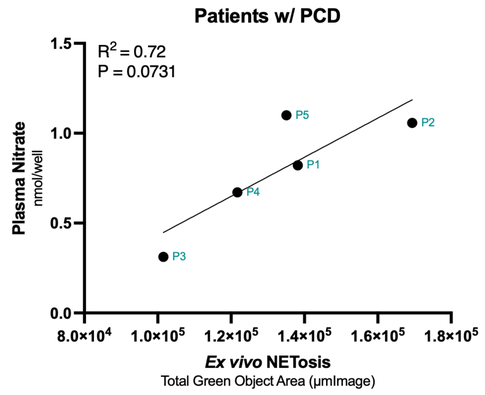


Figure 2 | Plasma nitrate levels and NETosis capacity among PCD patients

The graph shows the association between nitrate levels measured in plasma of patients with PCD and the capacity of neutrophils from those patients to release NETs in response to PMA. It establishes a possible association between lower NETosis capacity after PMA stimulation and NO production among PCD patients. Pearson correlation coefficient was used to assess the linear relationship, with a 95% confidence interval.

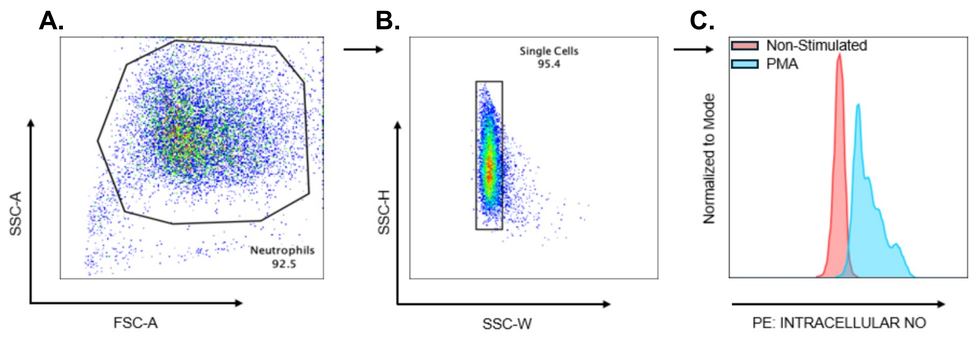


Figure 3 | Intracellular NO Assay following PMA stimulation in Healthy Controls

These graphs demonstrate a flow cytometry analysis measuring intracellular NO generation in healthy controls following PMA stimulation. These preliminary results suggest PMA is capable of inducing NO production.

DEMOGRAPHICS

Patient	Age	Sex	Ethnicity	Mutation
PCD 1	56	M	Hispanic	*RSPH4A*
PCD 2	3	M	Hispanic	*RSPH4A*
PCD 3	22	F	Hispanic	*RSPH4A*
PCD 4	9	M	Hispanic	*RSPH4A*
PCD 5	11	M	Hispanic	*RSPH4A*

CONCLUSIONS

- PMA can induce NO in neutrophils, therefore, the low capacity for NETosis in response to PMA stimulations observed in PCD patients may be attributed to variations in NO synthesis capacity.
- Due to this, PMA's utility for *ex vivo* stimulations in PCD studies should be considered, since other physiologically relevant stimuli have been shown to not induce NO in neutrophils. Thus, affecting our interpretation of the NETosis capacity of neutrophils in PCD.
- This research highlights the need to further explore the complex nature of NETs in PCD.

ACKNOWLEDGEMENTS

Funding: This research was funded by Research Centers in Minority Institutions (RCMI) Center for Research Resources Grant and the Molecular and Genomics Core #U54MD007579. Hispanic Clinical and Translational Research Education and Career Development (HCTRECD) program #5R25MD007607-22, The Hispanic Alliance for Clinical and Translational Research - Mentor Mentee Program Grant #5U54GM133807-03 and PR-INBRE Developmental Research Project Program (DRPP) #5P20GM103475-21
IRB Protocol number: 2303138796

Immunology & Molecular Pathogenesis

CILIA4PR

PONCE RESEARCH INSTITUTE
PONCE MEDICAL SCHOOL FOUNDATION INC.

REFERENCES

Scan the QR code to see the references!