Extracorporeal Photopheresis Induces NETosis in Neutrophils Derived From Patients With Graft-Versus-Host Disease

Erez Halperin MD*[1,2] Idan Goldberg MD*[1,2], Galit Granot PhD[3], Alona Telerman PhD[3], Shirly Partouche PhD[3], Tzippy Shochat MSc[4], Liat Shargian[1,2], Moshe Yeshurun MD[1,2], Pia Raanani MD[1,2], Ofir Wolach MD[1,2], Vered Yahalom MD[2,5] *Equal contribution

[1]Institute of Hematology, Davidoff Cancer Center, Beilinson Hospital, Rabin Medical Center, Petah Tikva, Israel. [2]Sackler School Medicine, Tel Aviv University, Tel Aviv, Israel. [3]Felsenstein Medical Research Center, Beilinson Hospital, Rabin Medical Center, Petah Tikva, Israel.

[4]Statistical Consulting Unit, Beilinson Hospital, Rabin Medical Center, Petah Tikva, Israel. [5]Blood Services & Apheresis Institute, Rabin Medical Center, Petah Tikva, Israel. . [5]Blood Services & Apheresis Institute, Rabin Medical Center, Petah Tikva, Israel

Introduction

- The mechanism of **extracorporeal photopheresis (ECP)** effectiveness in **acute and chronic graft versus host disease (GVHD)** is not fully understood.
- The generation of **neutrophil extracellular traps (NETosis)** has been linked to major **autoimmunity and hypercoagulability phenomena.** [1-3]
- The role **of NETosis in the clinical response to ECP** has yet to be determined.

Objective

To assess the influence of ECP on the formation of neutrophil extracellular traps (NETs) among patients with GVHD.

Methods

- We assessed several patients with GVHD who were treated with ECP at Rabin Medical Center.
- **Blood samples** were obtained at **three different time points:**
  - **Before** the onset of an **ECP** cycle
  - At the **end of the first day** of treatment
  - **24 hours following the initiation** of the ECP treatment cycle.
- **NET formation** was assessed by measurement of **neutrophil elastase activity and by immunofluorescence.**

Results

- Blood samples were collected from six patients with chronic GVHD.
- Neutrophil elastase activity increased immediately and 24 hours post treatment with ECP:
- The expression of citrullinated histone H3 was markedly increased following ECP treatment. As demonstrated in figure 2.

Table 1: **Patient characteristics and unstimulated neutrophil activity**

Serial number	Age (Gender)	Underlying Disease	NE activity before treatment	NE activity Immediately after treatment	NE activity 24 hours after treatment
1	23 (M)	B-ALL	1.22	22.99	35.14
2	71 (M)	AML	2.03	13.32	23.97
3	79 (M)	MDS	2.53	16.86	17.59
4	36 (F)	AML	2.16	7.54	6.65
5	31 (F)	AML	3.04	12.81	11.87
6	45 (M)	AML	2.3	9.42	8.85

NE- Neutrophil elastase, ALL- Acute lymphoblastic leukemia, MDS-Myelodysplastic syndrome, AML- acute myeloid leukemia, F- Female, M- Male. Neutrophil elastase units- mU/ml

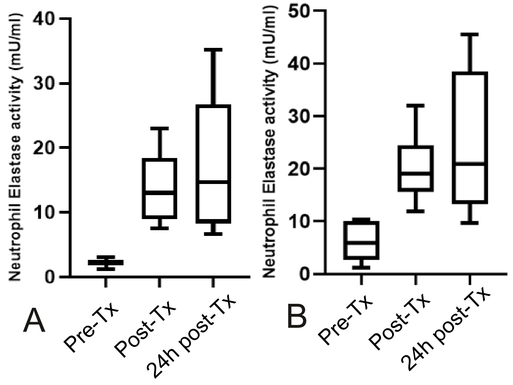


Figure 1: NE activity before, Immediately after and 24 hours after ECP. **A** Without stimulation **B** After Phorbol Myristate Acetate stimulation

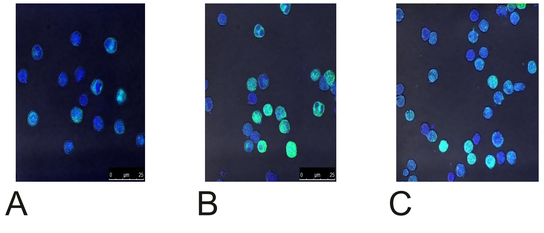


Figure 2: Immunofluorescence images of unstimulated neutrophils derived from one study participant stained with DAPI (blue) and anti-H3cit (green):

A Before. **B** Immediately after. **C** 24 hours after ECP.

Conclusions

We report the first evidence for the **augmentation of NETosis by ECP treatment** among patients with chronic GVHD.

Discussion

- There is few anecdotal evidence for the role of NETosis in GVHD. [4,5]
- Our preliminary findings shed light on the **mechanism by which ECP may affect GVHD**, and thus are **hypothesis-generating** and set the stage for further mechanistic studies.

References

1. Brinkmann et al., Neutrophil Extracellular Traps Kill Bacteria. Science (80-). 2004. 2. Barnado et al. At the Bedside: Neutrophil extracellular traps (NETs) as targets for biomarkers and therapies in autoimmune diseases. J Leukoc Biol. 2016 3. Martinod et al. Thrombosis: Tangled up in NETs. Blood. 2014. 4. An et al. Neutrophil extracellular traps (NETs) contribute to pathological changes of ocular graft-vs.-host disease (oGVHD) dry eye: Implications for novel biomarkers and therapeutic strategies. Ocul Surf [Internet]. 2019. 5. Teijeira et al. CXCR1 and CXCR2 Chemokine Receptor Agonists Produced by Tumors Induce Neutrophil Extracellular Traps that Interfere with Immune Cytotoxicity. Immunity. 2020