



EXOSOMES IN POLYCYTHEMIA VERA: "MINI PLATELETS" WITH THROMBOGENIC POTENTIAL

A.Shacham Abulafia^{1,2}, G. Spectre^{1,2}, K.Revital Geiger¹, Z.Sarsor³, N.Ron^{2,3}, E.Beery³, E. Ziv¹, P.Raanani^{1,2,3}, *O. Uziel^{1,2,3} & *U.Rozovski^{1,2,3}

¹Institute of Hematology, Rabin Medical Center, Petah-Tikva; ²Sackler School of Medicine, Tel Aviv University, Tel Aviv; ³Felsenstein Medical Research Center, Rabin Medical Center, Petah-Tikva, Israel



Background

Polycythemia vera (PV) is a chronic myeloproliferative neoplasm, characterized by proliferation of the erythroid, myeloid and megakaryocytic cell lineages in the bone marrow and increased blood cells in the peripheral blood. Clonal proliferation is driven by JAK2^{V617F} mutation, resulting in a dysregulation of the JAK-STAT pathway and subsequent activation of downstream signaling pathways. An increased risk of thrombotic events ($\approx$ 45% of deaths) and disease progression to myelofibrosis or acute myeloid leukemia ($\approx$ 13% of deaths) have been identified as the major complications of the disease. While different pathogenic pathways may explain these complications, and although genetic predisposition may partially explain these links, whether and how PV-clonal cells induce pro-thrombotic and oncogenic environment is still under investigation. Secreted by all types of cells, exosomes are nano-scaled particles which carry a molecular cargo that reflects their cell of origin. Exosomes travel in blood, may reach distant sites and affect various physiological and pathological processes. These features led us to think that PV-derived exosomes contribute to the thrombotic manifestations and oncogenic potential of patients with PV.

Objective

- To study *in-depth* the role of PV-derived exosomes in the processes of thrombosis.
1. Determine whether *PV-exosomes* carry mutated *JAK2* transcripts.
 2. Determine the prothrombotic potential of *PV-exosomes* by using thrombin generation assay.
 3. Determine whether *PV-exosomes* induce endothelial dysfunction by measuring membrane resistance.
 4. Determine whether *PV-exosomes* induce platelets activation by using flow cytometry.

Materials & Methods

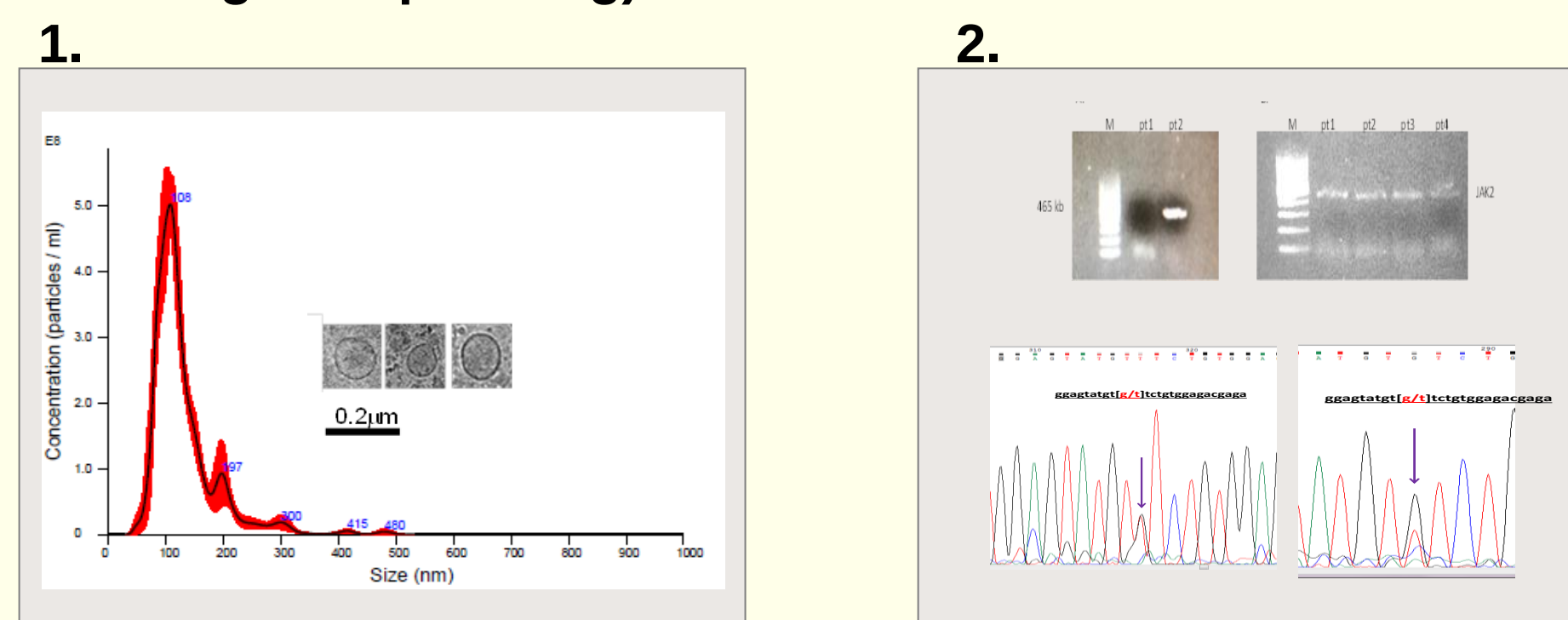
- We isolated microparticles by serial ultracentrifugation and confirmed the presence of exosomes by NTA (Malvern) and imaged these exosomes by EM.
- We then extracted RNA and generated cDNA from the exosomal-RNA (Invitrogen).
- We amplified a 465 bp long DNA segment within exon14 of JAK2 by PCR and used Sanger sequencing to determine the JAK2 mutational status.
- We used thrombin generation assay to assess the thrombotic potential of PV-exosomes.
- Trans Epithelial Electric Resistance (TEER) assay was used to assess endothelial dysfunction.
- The activation of normal platelets by PV derived exosomes was measured by flow cytometry by following the expression of PAC-1, CD-62 and CD-63 in response to various agonists.

Results

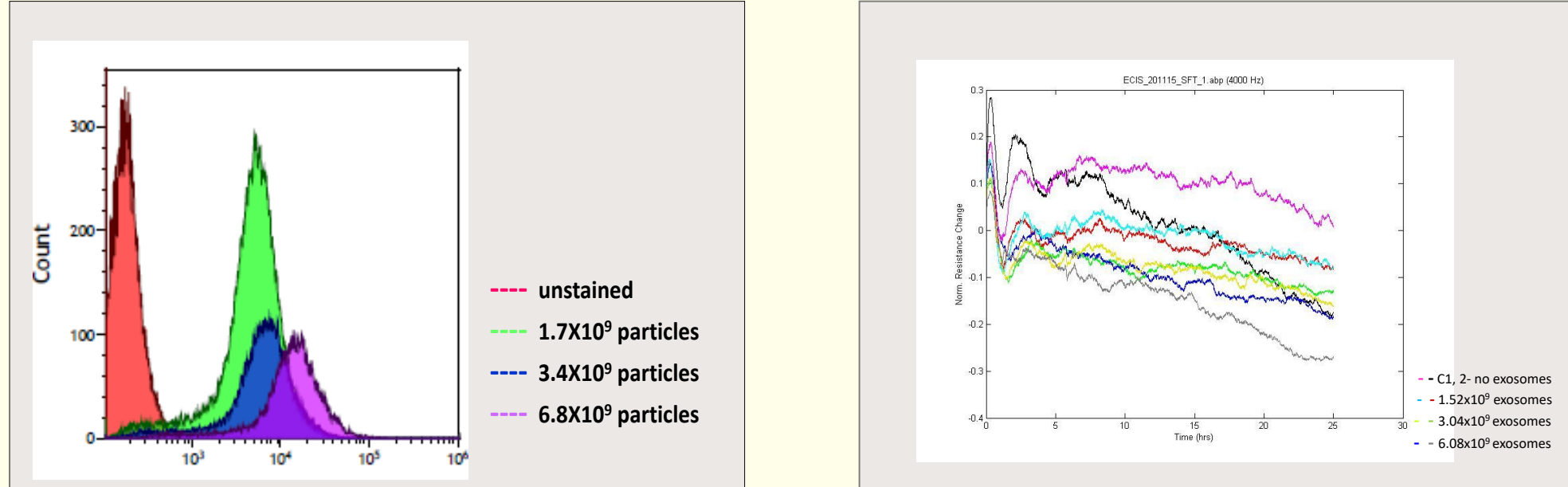
We showed that PV-exosomes carry mutated *JAK2* transcripts in all patients with PV and the presence of wild-type JAK2 in all exosomes of healthy individuals. Next, we found that FM-1-43 labelled JAK2^{mutated}-exosomes were taken up by endothelial cells in a dose- and time- dependent manner and induced endothelial dysfunction; resistance was dramatically decreased in the presence of PV-exosomes, indicating that the tight junction between adjacent endothelial cells was impaired. Additionally, an increase in all parameters of the thrombin generation in the presence of PV-exosomes compared with exosomes from healthy individuals was obtained. We also detected an upregulation of three markers of platelet activation by flow cytometry in response to PV- exosomes compared with their healthy-derived counterparts (CD62, CD63, PAC1).

Results

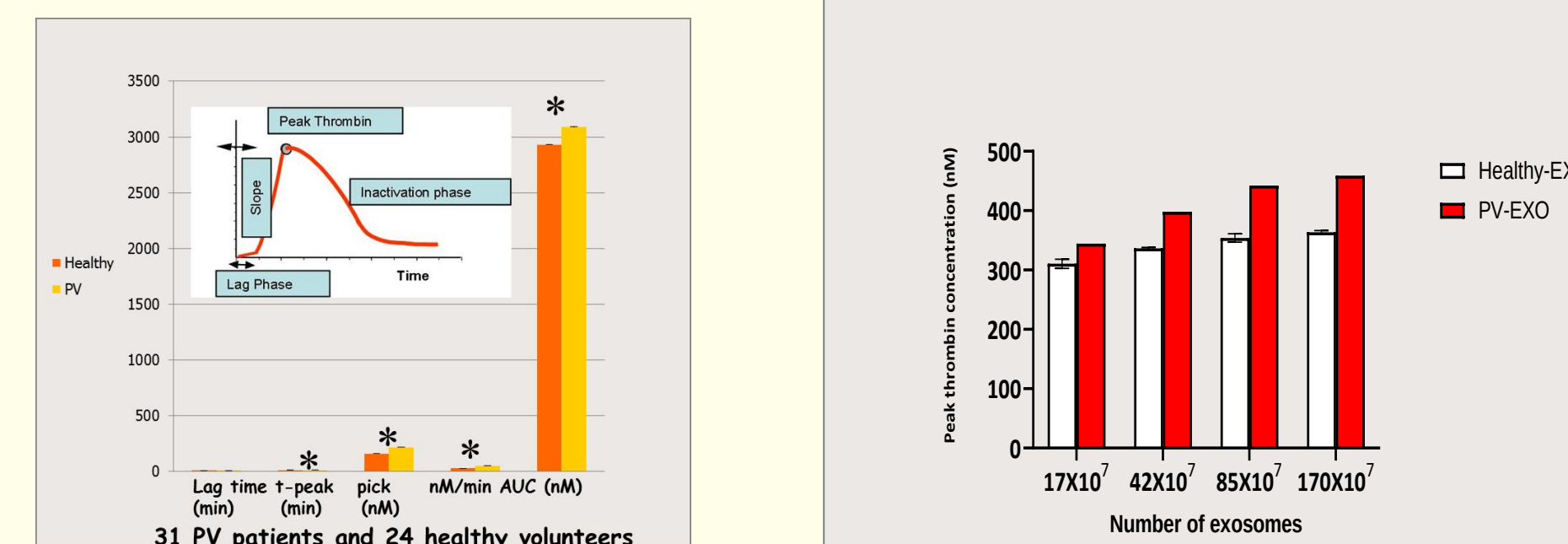
1. Microparticles isolated from PV patients are exosomes (NanoSight Tracking Analysis, EM)
2. JAK2^{V617F} is packaged in PV- derived exosomes (PCR and Sanger sequencing)



3. PV- derived exosomes are taken up by endothelial cells (Flow Cytometry analysis)
4. PV- derived exosomes promote endothelial dysfunction (the TEER assay)

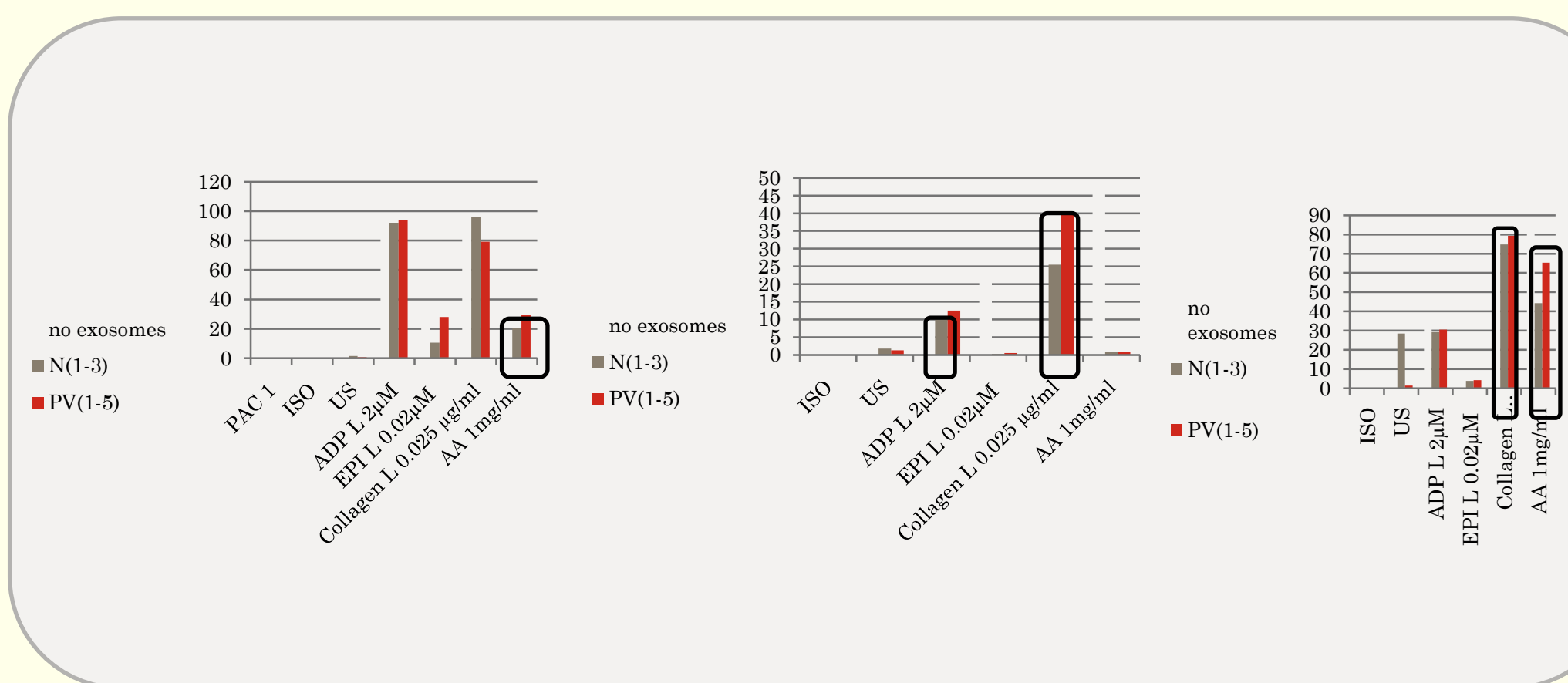


5. PV- derived exosomes promote thrombin generation (the thrombin generation assay)



Results

6. PV exosomes activate normal platelets (flow cytometry)



Conclusions

PV-derived exosomes carry mutant JAK2 transcripts, like their parental cells. These exosomes seem to contribute to the pro-thrombotic environment by different pathogenic pathways, by inducing endothelial dysfunction, increased thrombin generation and upregulating platelet activation.