Nanopore sequencing to elucidate the origins of circulating DNA during extracorporeal membrane oxygenation

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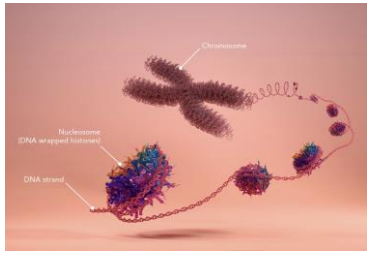
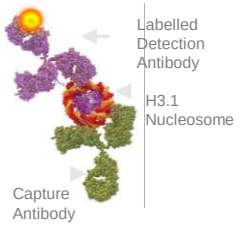
INTRODUCTION

- Circulating DNA and nucleosomes are a new class of biomarkers for severe respiratory infection and sepsis.
- Extreme cases of sepsis undergo extracorporeal membrane oxygenation (ECMO).
- The effects of ECMO on circulating DNA and chromatin biomarkers are unknown.



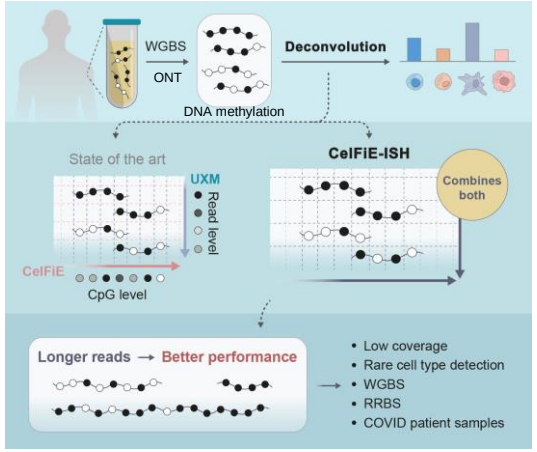
OBJECTIVES

- To understand the changes and origins of circulating DNA in patients with severe infection-related respiratory failure before, during, and after ECMO treatment.
- Use shallow whole-genome sequencing (sWGS) from Oxford Nanopore Technologies (ONT) to determine cell of origin and cfDNA fragmentation.
- ONT sWGS does not require amplification, enables both long and short DNA sequencing of both host and pathogen DNA, and is most suitable for near-patient rapid analysis in the hospital setting.

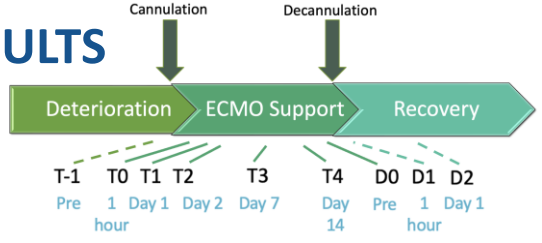


METHODS

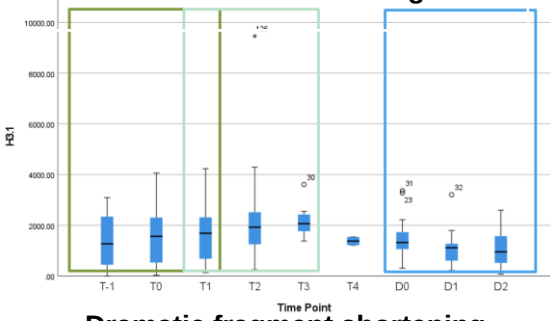
- We analyzed plasma cfDNA from 61 timepoints from 8 adult patients: one timepoint collected before ECMO, multiple timepoints during ECMO, and one or more timepoints up to 2 days after decannulation.
- Measured nucleosome levels using the H3.1 Nu.Q® assay.
- Sequenced cfDNA using ONT sWGS (median 17 million reads)[1].
- Determined Cell of Origin of circulating DNA using computational deconvolution of genome-wide DNA methylation levels[2].



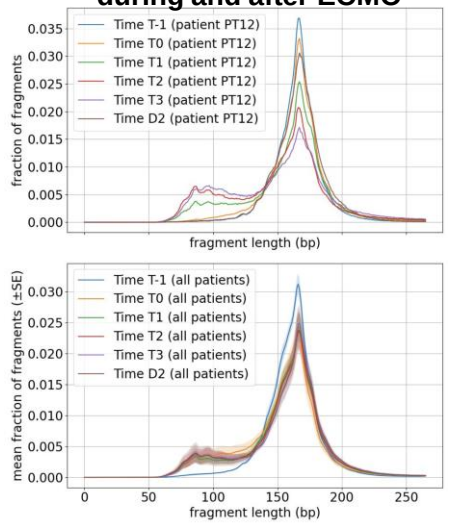
RESULTS



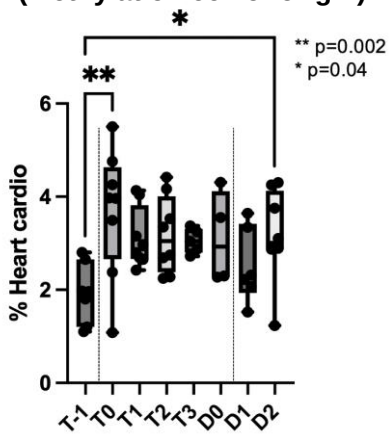
H3.1 Nucleosome levels During ECMO



Dramatic fragment shortening during and after ECMO



Increase in heart DNA during and after ECMO (Methylation cell of origin)



CONCLUSIONS

- ONT sequencing can reveal key molecular markers from circulating cell-free DNA patients with severe infections.
- The ability of ONT to profile both fragmentomic properties as well as DNA methylation allowed us to identify changes associated with severe respiratory failure and ECMO.
- The suitability of ONT sequencing for near-patient, rapid sequencing makes it a promising technology in the critical care setting.
- ONT sWGS of circulating DNA may have sufficient sensitivity to measure cardiac stress or damage[3] during ECMO.

ACKNOWLEDGEMENTS

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