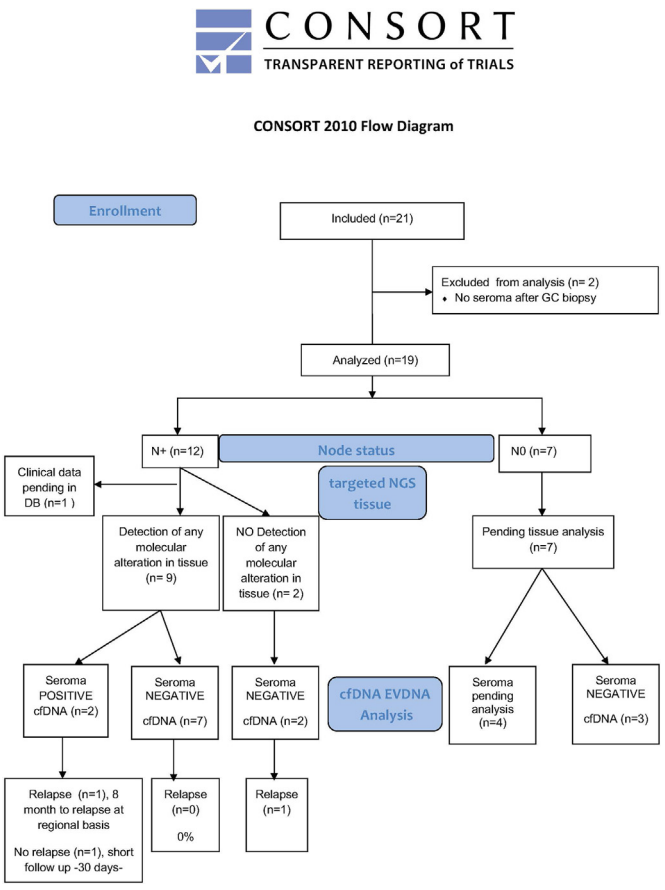

PP.78

THE JOURNAL OF LIQUID BIOPSY 1 (2023) 100010 100091

PULMONARY MICRORNA PROFILING IN A RAT MODEL OF VENTILATOR-INDUCED LUNG INJURY

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Introduction: Diffuse alveolar damage (DAD) is the histological correlate of the acute respiratory distress syndrome (ARDS). MicroRNAs (miRNAs) regulate gene expression and have been described in different models of acute lung injury. The objective was to determine if DAD development is associated to a specific phenotype and miRNA expression profile in a rat model of ventilator-induced lung injury (VILI).

Methods: Male Sprague–Dawley rats were anesthetized and ventilated for 2.5 h using two ventilatory strategies: 1) protective strategy with low tidal volume (Vt, Vt= 9 ml/kg; PEEP=5 cm H_2O, n= 10), and 2) injurious strategy with high tidal volume (Vt= 25ml/kg; PEEP= 0 cm H_2O, n = 16). We assessed pulmonary histological changes, gas exchange, inflammation in serum and lung (ELISA), apoptosis (TUNEL), and alveolo-capillary permeability in the lung (IgM and total protein in bronchoalveolar fluid, BALF). We measured miRNA expression in rat lungs by NGS and performed a pathway enrichment analysis by REACTOME and miRNET network. Statistical analysis: one-way ANOVA and pearson correlation, p value < 0.05 was considered statically significant.

Results: Seven of 16 rats in the high Vt group and none in the low Vt group developed DAD. Compared with rats without DAD, rats with DAD showed: 1) higher peak inspiratory pressure (PIP) and lower compliance 2) lower PaO_2/FiO_2 ratio; 3) increased concentration of cytokines in lung tissue and serum, 4) enhanced alveolo-capillary permeability; (5) increased apoptosis and lower caspase-3 activity in lungs; and (6) different miRNA expression profile in the lungs ; (7) miR 132-5p expression correlated with some cytokines concentration in serum and lungs, total protein in BALF, caspase-3 activity in lungs and PIP at the end of the experiment. Pathway enrichment analysis showed that the miRNAs differentially expressed were involved in pathways related to the immune response, inflammation, and apoptosis.

Conclusions: The DAD phenotype was characterized by higher PIP, impairment of oxygenation, acute systemic and pulmonary inflammation, increased permeability and apoptosis in the lung, and differential miRNA expression in the lung as compared to those without DAD. The miRNAs differentially expressed between groups are involved in pathways relevant in the pathophysiology of VILI and ARDS.

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AN END-TO-END CFRNA-SEQ PIPELINE FOR EARLY CANCER DETECTION FROM LIQUID BIOPSY

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Introduction: Liquid biopsies have become increasingly important diagnostic tools for early cancer detection due to their high sensitivity and minimal invasiveness.

Plasma cell-free RNA (cfRNA) is a rich source of information, including biomarkers for early cancer detection.

cfRNA-based biomarker identification requires a robust and reproducible in vitro workflow for RNA isolation and next generation sequencing (NGS), combined with high-performance in silico pipelines for NGS data processing and analysis. As the cfRNA-based liquid biopsy field is still developing, technical limitations remain to be overcome for the generation and analysis of high quality cfRNA-Seq data. Resolving these issues will improve the robustness and reproducibility of this approach and generate high confidence biomarkers for early cancer diagnosis.

Here we describe a combined in vitro and in silico pipeline that overcomes these challenges to generate and analyse high quality cfRNA-Seq data.

Methods: We obtained plasma from various donors and tested different protocols at key steps in the in vitro pipeline, including blood collection, RNA isolation, DNase treatment and library preparation. These approaches were evaluated quantitatively and qualitatively at the RNA and cDNA library levels, including sequencing of the cDNA libraries.

We built a custom in silico pipeline to process the cfRNA-Seq data. Using a variety of Quality Control (QC) statistics, including several innovative metrics developed in-house, we performed an extensive comparative analysis of the cfRNA profiles obtained across the in vitro conditions tested.

Results: We identified the optimal protocol at each stage of the in vitro pipeline for the generation of high quality cfRNA-Seq libraries. Data analysis using our in silico pipeline revealed that the optimised pipeline steps improve cfRNA-Seq data quality to a level suitable for confident biomarker identification.

Conclusions: We have developed a pipeline that overcomes the technical challenges that have prevented the generation and analysis of high quality cfRNA-Seq data.

Bioinformatics pipeline optimization was crucial to obtain relevant QC metrics that allowed selection of the optimal protocol at each stage.

This pipeline has great potential for the identification of high confidence biomarkers for early cancer detection, which will accelerate treatment and result in more favourable patient outcomes.

Liquid Biopsy in Early Detection for Cancer

PP.80

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ENHANCED BLOOD SAMPLING FOR ULTRA-SENSITIVE DETECTION OF CTDNA AND CTCS IN NEOADJUVANT-TREATED EARLY BREAST CANCER PATIENTS

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