Development of a non-invasive maternal blood test for congential heart disease

Petra Adamova[1,2], Andrew Powell[1,2], Robyn Lotto[1,3], Attilio Lotto[1,3], Iain M. Dykes[1,2]

1. Liverpool Centre for Cardiovascular Science

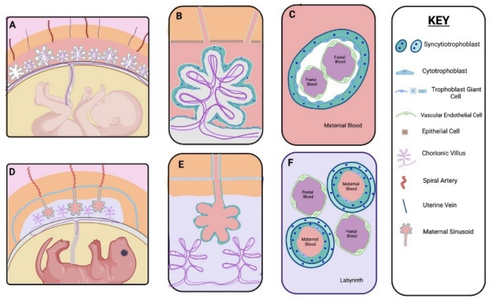
2. School of Pharmacy & Biomolecular Sciences, Liverpool John Moores University

3. School of Nursing & Allied Health, Liverpool John Moores University

Abstract

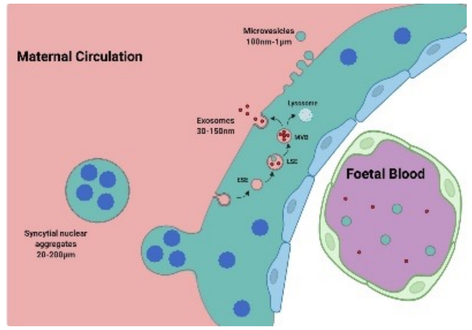
Congenital heart disease (CHD) is the most prevalent birth defect in the UK affecting ~4,600 babies per year. Early diagnosis improves clinical outcomes, yet current technology (foetal anomaly ultrasound screen at 18-20 weeks) is only ~50% accurate. There is a need for a more accurate screening method. We propose that such a test could assay maternal blood. Extracellular vesicles (EVs) are small membranous particles produced by the foetus which can cross the placental barrier and enter maternal blood. EVs are emerging as a promising blood biomarker in many diseases. We have performed a pilot study using rat blood to develop flow cytometry methods to detect EVs and quantify EV biomarker expression. We are currently recruiting patients for a clinical study in which we aim to develop an EV disease signature for the CHD Tetralogy of Fallot.

The Placenta forms a semi-permeable barrier between mother and foetus



In both humans (A-C) and rodents (D-F) the syncytiotrophoblast forms a barrier separating maternal blood (pink) from foetal blood (purple). In humans, the syncytiotrophoblast surrounds the foetal blood vessels which are located in villi projecting into the maternal blood space. In the rodent placenta, the syncytiotrophoblast surrounds the maternal blood space and separates this from foetal capillaries within a structure known as the labyrinth.

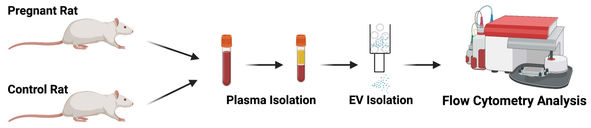
Extracellular Vesicles (EVs) mediate foetal-maternal communication



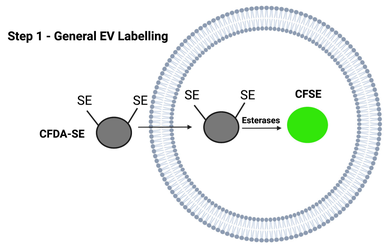
Extracellular vesicles (EVs) are small membranous particles which mediate endocrine signalling. EVs carry a cargo consisting of RNA and soluble proteins and in addition are enriched in specific transmembrane and membrane-associated proteins and extracellular glycans at the vesicle surface. The placenta is a source of EVs and also permits transport of EVs between foetus and mother.

Isolation and detection of EVs from blood

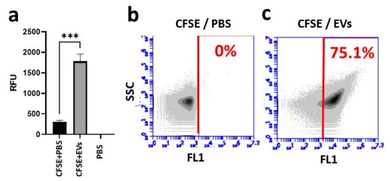
- We performed a pre-clinical pilot study using rat blood to develop EV isolation and assay methodologies
- Blood was taken from pregnant and non-pregnant rats, cell-free plasma was prepared from which EVs were isolated by size exclusion chromatography
- We developed flow cytometry methods for EV detection and EV biomarker quantification



Dye labelling permits EV detection by flow cytometry

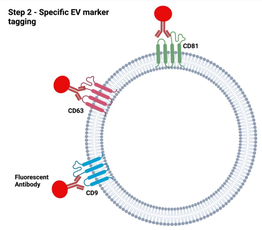


CFSE is a dye which permeates the membrane and is then activated within the EV to produce a fluorescent signal. This allows us to separate EVs from other similarly sized particles in blood such as LDL cholesterol. make CFSE bigger. It could fill the EV as you are not showing anything else here.

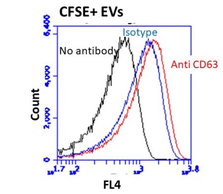


(A) CFSE is only fluorescent in the presence of EVs and not in the PBS control
(B,C) When this was analysed using the flow cytometer we observed the appearance of a population of green fluorescent particles (C) not present in PBS (B). This is shown by an increase in fluorescence (FL1). We calculated that 75% of particles within the EV-enriched sample are labelled with CFSE.

Fluorescent antibodies permit detection of surface EV biomarkers.



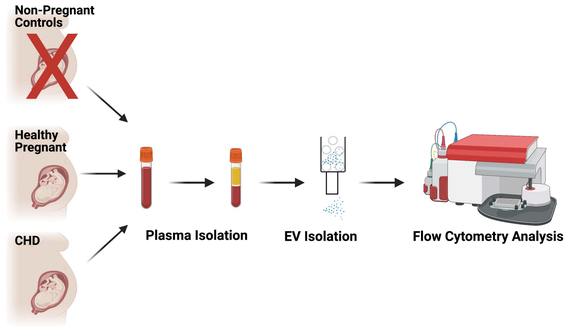
Fluorescent antibodies detect specific EV surface biomarkers such as the tetraspanins CD9, CD63 and CD81. We combined these with CFSE staining to identify EV markers expressed by the CFSE+ population.



We focussed on the tetraspanin CD63. The results demonstrated that the CFSE+ population express CD63 (red line). The blue line shows a negative control (isotype). We are currently optimising methods to improve the signal to noise ratio.

Can we identify an EV biomarker diseases signature for CHD?

The ultimate aim of our work is to improve prenatal diagnosis of CHD by identifying maternal blood biomarkers for CHD.



We have obtained NHS ethical approval for the ExoChase study. Here, we will recruit 20 pregnant women diagnosed with a common CHD (Tetralogy of Fallot). We will use methods developed in our pre-clinical rat model to compare blood from these patients to blood from women with a normal pregnancy and to non-pregnant women.

We are currently recruiting patients at Liverpool Womens' Hospital. Blood will be drawn at their routine 18-20 week scan.

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p.adamova@2020.ljmu.ac.uk @AdamovaPetra9 www.linkedin.com/in/petra-adamova/