

IHE Internship project 9:

What is the origin of increased cell-free DNA in disease?

Introduction: Obviously the majority of cell-free DNA (around 60%) is from hematological cells, which we have shown in a few allogeneic stem cell patients and has also been published by Dennis Lo et al. (Lui et al. Clin Chemistry 2002;48:3). A possible other source could be endothelial cells. During sepsis the amount of cell free DNA is increased, as measured by nucleosome ELISA and by RQ-PCR, and this correlates with severity and fatality. Other groups suggest that this increased DNA is derived from apoptotic lymphoid cells. In pregnancy, the total cell-free DNA concentration is increased. The total cell-free DNA is even more increased in complications of pregnancy, especially in pre-eclampsia and HELLP. For both situations it is relevant to know the origin of the non-hematological cell-free DNA.

Approach

- 1) Identification of genes which are differentially methylated between endothelial cells and leukocytes. Quantitation of these targets in cell free DNA of normal donors, sepsis patients or pregnant women by RQ-PCR.
- 2) Quantitation of rearranged IgH and TCR genes in cell free DNA in sepsis patients compared to healthy volunteers

Plan of investigation:

- 1) IgH / TCR PCRs on cell free DNA. Design consensus IgH primer in FR3 which can be used in combination with JH primer and JH probe, and consensus Vbeta – Jbeta primers (together with Rob Dee / Denise Stalder).
- 2) MLPA kits on HUVEC to detect possibly hypermethylated genes in endothelial cells. If present, then if possible an MLPA on cell free DNA. If not, then bisulfite sequencing around the CpG islands and develop RQ-PCR. Furthermore, testing MLPA on other possible sources for cell-free DNA in collaboration with PA.
- 3) In parallel:
 - a. Search for endothelial specific genes (e.g. CD146/S-Endo1, VE-Cadherin, VWF, Tie2).
 - b. Identification of CpG islands within the promoter of these genes cq literature study on methylation studies of these genes.
 - c. Testing by PCR before and after BstU1 /Hha1 digestion (or other methylation dependent restriction enzymes) with leukocytes and HUVEC.
 - d. Bisulfite sequencing from endothelial cells and leukocytes.
 - e. Design of methylation-specific RQ-PCRs.
 - f. Testing of samples.

Duration: 6 – 9 months. Students from the University or HLO who are looking for a dynamic and interesting internship and are interested in the above project are encouraged to contact the project leaders, Ellen van der Schoot and Aicha Ait Soussan by e-mail: e.vanderschoot@sanquin.nl and a.aitsooussan@sanquin.nl