Abstract citation ID: gfae069.850

#3031

# Cell free DNA methylation analysis reveals cerebral cell death during hemodialysis

Line Heylen[1,2], Kobe De Ridder[3], Eline Hoes[2], Xinran Song[3], Wouter Wilms[1], Cathy Dedeyne[1], Joris Penders[1,2], Koen Ameloot[1], Steven Thiessen[1], Ben Sprangers[1,2], Aleandra Gilio[1] and Bernard Thienpont[3]

[1]Ziekenhuis Oost-Limburg—Limburg Clinical Research Center, Genk, Belgium
[2]Hasselt University, Hasselt, Belgium
[3]KU Leuven, Leuven, Belgium

**Background and Aims:** Patients treated with hemodialysis demonstrate a significant cognitive decline, likely explained by cerebrovascular disease and hypoperfusion. Despite the significant morbidity and mortality associated, no method exists to detect subclinical cerebral injury. This prevents early diagnosis and precludes mitigation strategies. Following cell death, cell free DNA (cfDNA) is released into the peripheral blood. Since DNA methylation is cell-type specific, cfDNA methylation analysis can reveal the cellular origin of cfDNA and cell-type specific changes in cell death. Here, we evaluate if subclinical cerebral ischemia during hemodialysis is detectable through cfDNA methylome analyses.

**Method:** Chronic hemodialysis patients with a high cardiovascular risk profile were studied during a single hemodialysis session. Brain tissue oxygenation was measured continuously by near-infrared spectroscopy using two sensors on the forehead. Plasma samples were taken immediately before and after hemodialysis. cfDNA was extracted from the blood plasma and converted into whole genome bisulfite sequencing libraries using tailored protocols. Next, in-solution target enrichment was applied to analyze 4,989 genomic loci, selected for showing specific methylation in one of 15 tissues or cell types. After sequencing, reads were aligned to the hg38 reference genome using BisMark, and deconvoluted using EMeth-Laplace to disclose the relative contribution of the 15 selected tissues or cell types.

**Results:** 41 hemodialysis patients participated in the study, with a mean age of 75 $\pm$ 8 years (46% male). The median ultrafiltration volume was 2.3 L (IQR 1.6-3.0 L). After targeted bisulfite sequencing and QC-based filtering, 35 patients remained for cfDNA methylation analysis. In 30 patients, cfDNA concentrations were increased following dialysis. Deconvolution analysis revealed a relative increase in granulocyte-derived cfDNA from 36.7% to 55% before versus after dialysis. Consequently, for most other tissues and cell types we observed a relative decrease in their cfDNA contribution. Interestingly, a subset of patients ($n = 13$) showed an increase in neuron-derived cfDNA from 0% to 0.26%, while no neuron-derived cfDNA was detectable in the remaining 22 patients. Near-infrared spectroscopy showed a significantly reduced cerebral oxygenation in these 13 patients ($P < 0.0001$) at 2 separate time windows during hemodialysis treatment (at 20-60 and 207-251 minutes into the session) (Fig.).

**Conclusion:** In patients with a high cardiovascular risk profile, cfDNA in peripheral blood increased after hemodialysis. cfDNA methylome analyses suggested this to be due to an increased contribution of granulocytes, indicating elevated levels of NETosis. In addition, we observed more neuron-derived cfDNA after dialysis in about 1 in 3 patients. This was most apparent in patients with a reduced brain tissue oxygenation during hemodialysis. Together, these findings suggest that hypoperfusion during hemodialysis can evoke neuronal cell death, detectable through methylome analyses of cfDNA isolated from a minimally invasively obtainable bodily fluid. Follow-up studies are ongoing to assess if this translates to progressive cognitive impairment.

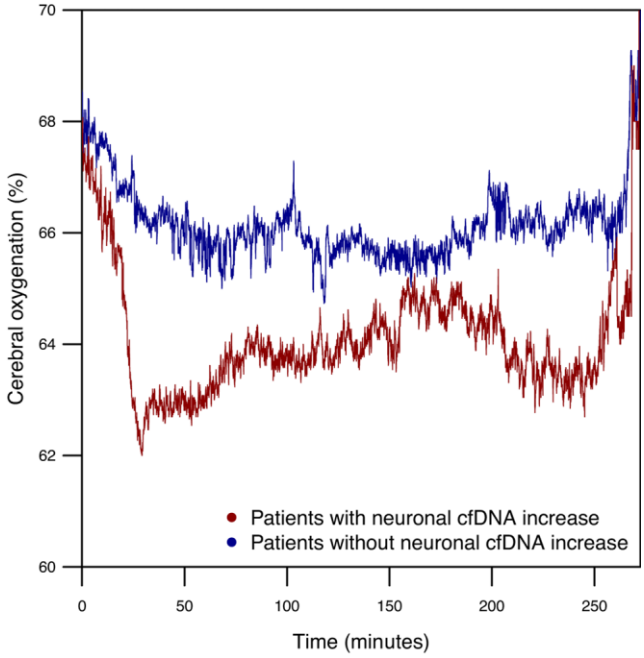


**Figure 1:** Cerebral oxygenation is significantly lower during hemodialysis in patients who demonstrate an increase in neuronal cell free DNA (cfDNA) after a single hemodialysis session compared to patients who do not have an increase in neuronal cfDNA.