

## Dairy fatty acids intake is protective against the occurrence of vasospasm after subarachnoid haemorrhage, while omega6 fatty acids exert an opposite role

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Aneurysmal subarachnoid hemorrhage (aSAH) is a severe disease that can be followed within the first four days by a delayed cerebral infarction (DCI) due to vasospasm (Vs). This is an important cause of complications and death after the primary aSAH, statistically occurring in one third of patients. To date the outcome of this DCI cannot be predicted, which precludes any upstream correcting clinical support. Scarce but significant studies also highlighted that this second event may be influenced by a nutritional component, such as an imbalance into the omega-6 and omega-3 fatty acids status. Thus, the aim of this study was to identify a metabolite signature that predicts the occurrence of vasospasm and also to underscore the importance of a nutritional component in DCI.

A cohort of 28 individuals has been established from patients suffering from aSAH and admitted in emergencies at La Timone hospital, Marseille-France. Plasma and cerebrospinal fluid (CSF) were sampled in the first 24hr (T1) and also between 48 to 72hr after admission (T2), e.g. prior to any DCI occurrence. Samples were processed for fatty acids profiling and mass-spectrometry metabolomics untargeted analysis. Dietary intake were assessed using a food frequency questionnaire filled up by patient's relatives. Fatty acids profiling was also used to evaluate the relative intake of various fats.

About 50% of patients developed DCI from day 4 to 15 after the first aSAH. Among those who were DCI diagnosed, the dietary assessment indicated higher omega-6 fatty acids intake. This was relevant with fatty acids analysis, showing higher C20 :4n-6/(C20 :5n-3+C22 :6n-3) ratio in plasma of DCI diagnosed patients ( $P < 0.05$ ). This analysis also revealed higher levels of fatty acids occurring from dairy products consumption in patients who did not developed DCI, such a C14:0, C15:0 and the sum of C14 :0+C15 :0+C17 :0. Plasma GCMS metabolomics sampled at T1 revealed a subset of 8 metabolites which, when assembled into a composite score using a partial least-square method, predicted the occurrence of DCI and vasospasm with an accuracy of 94.7% (ROC area under the curve = 0.993). Five of these metabolites belonged to a recently identified pro-inflammatory pathway in human. Plasma sampled at T2 confirmed this finding. Conversely, CSF analyses were much less informative than did plasma. Complementary analyses using high-resolution mass spectrometry based metabolomics are underway to identify other metabolic and nutritional factors attached to DCI.

In conclusion, we highlighted the nutritional component of DCI, with both a possible deleterious effect of omega-6 to omega-3 imbalance and a favorable effect of dairy products intake. We were also able to predict with a high accuracy the occurrence of DCI by combining 8 plasma metabolites into a predictive equation from using an agnostic plasma metabolomics analysis. Although encouraging, these results must be confirmed by a wider multicentric cohort study.