Phenotyping uracil and 5-fluorouracil metabolism using LC-MS/MS to prevent the toxicity and adjust the dose of fluoropyrimides

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Background: Plasma concentrations of 5-fluorouracil (5-FU) exhibit wide inter-individual variability related to the activity of dihydropyrimidine dehydrogenase (DPD). The European Medicines Agency safety committee has recently recommended that patients should be tested DPD deficiency before starting cancer treatment with medicines containing fluoropyrimidines. In France, testing is mandatory since December 2018 and it is performed by measuring uracil (U) plasma concentration. In addition, 5-FU therapeutic drug monitoring in cancer patients is by the French Society of Pharmacology and Therapeutics [Bull. Cancer (2018) **105** (9):790 803].

Methods: We developed a HPLC-MS/MS method for the simultaneous determination of U, UH_2, 5-FU and FUH_2 in human plasma. Over a period of 10 months, we analyzed samples prior to fluoropyrimidine treatment in 526 cancer patients, as part of routine care.

Results:
After protein precipitation, liquid-liquid extraction and chromatographic separation using a Cortecs®T3 column, compound detection and quantification were performed using an AB-Sciex QTRAP®4500MD triple quadrupole mass spectrometer. The run time was 6.0 min. The distribution of plasma uracil concentrations was log-normal, with median (25-75th percentiles) of 10.6 (8.4-13.8) µg/L and extreme values of 3.9 and 81.6 µg/L. Plasma uracil concentration was ≥16 µg/L in 78 patients and > 30 µg/L in 7. A DPYD hypofunctional allele was detected in only 2 of these 7 cases. In 3 cases, the time between sample collection and centrifugation was unacceptably long (between 5 and 16 hours), which can explain artificially high U levels (UH_2 is unstable and degrades into U at ambient temperature in whole blood). In 2 cases, the delay could not explain the increased U level observed in the absence of DPYD polymorphism.

Conclusion:
The method presented is an rapid and efficient tool for DPD deficiency detection prior to
treatment initiation with a fluoropyrimidine, as well as for individual dose adjustment afterwards. Our experience confirms that it is essential that blood samples are centrifuged within 1.5h if tubes are kept at ambient temperature, or 4h if they are stored at +4°C.

Keywords: Pharmacogenetics, drug adverse effects, oncology, therapeutic drug monitoring