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Development of an assay by liquid chromatography coupled to tandem mass spectrometry for thiopurines drugs in whole blood and application for their Therapeutic Drug Monitoring

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Context

The cytotoxic and immunosuppressive thiopurine drugs 6-TG and 6-MMP, are mainly used in acute lymphocytic leukemia, disorders of immune regulation and inflammatory bowel disease.

Quantification methods for their Therapeutic Drug Monitoring are usually reported in various blood matrices, but barely with Dried Blood Spots (DBS).

The suitability of our in-house validated LC-MS/MS and the use of DBS are explored by a cross-validation with a well-established external assay

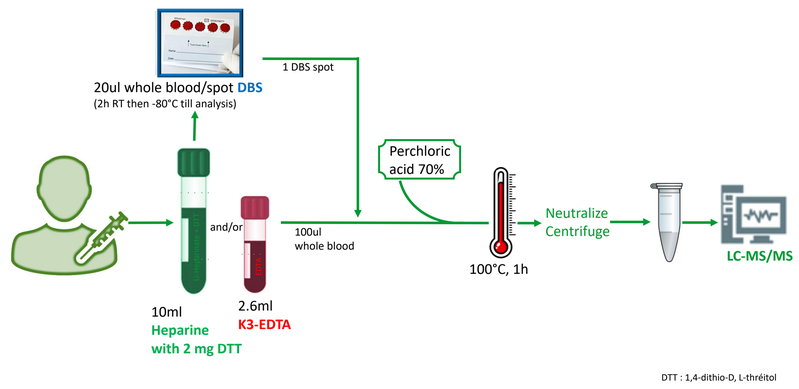
6-TG: 6-Thioguanine nucleotide, 6-MMP: 6-Methyl-mercaptopurine

Objective

- Development and validation of in-house thiopurines assay
- Correlations between concentrations of 6-TG and 6-MMP determined in blood collected on EDTA, versus on heparin with 0.02% (m/v) dithiothreitol (Hep-DTT) from external reference method, and versus DBS

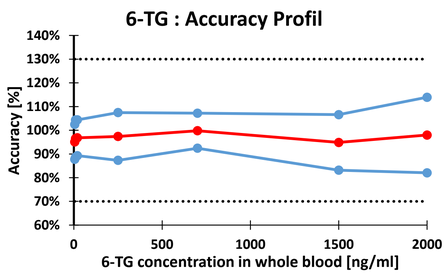
Methods

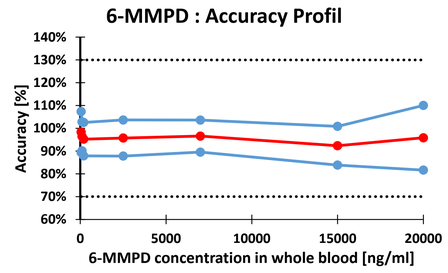
- 34 patient samples.
- The DBS were obtained by spotting 20 µl of the Li-Hep/DTT samples on a Whatman FTA DMPK-B Card.
- Hematocrit and red blood cell were measured from Hep-DTT-blood to normalize the thiopurines levels [a]
- Linear regression, correlation and Bland-Altman test were performed

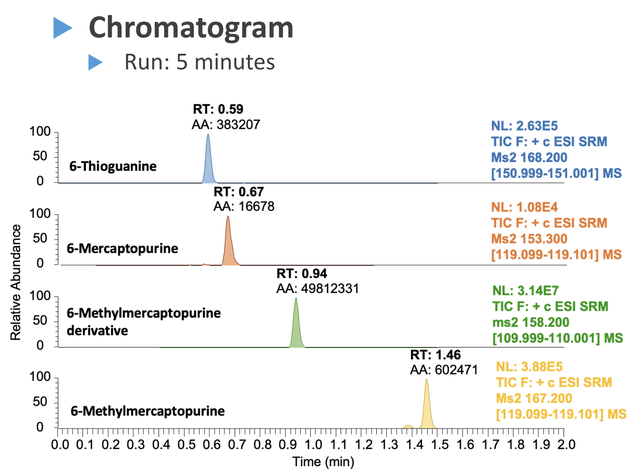


Results

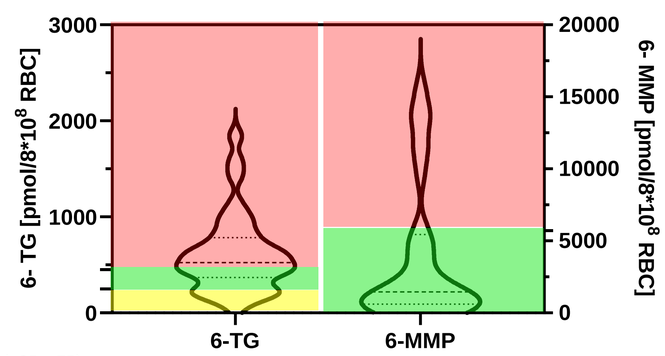
Validation LC-MS/MS method







Dosages of Thiopurines (n=34)



..... Percentiles 25 and 75 (p25, p75)

– – – – Median (p50)

Sub-therapeutic range [a,b]

Therapeutic range for 6-TGN, No toxicity for 6-MMP

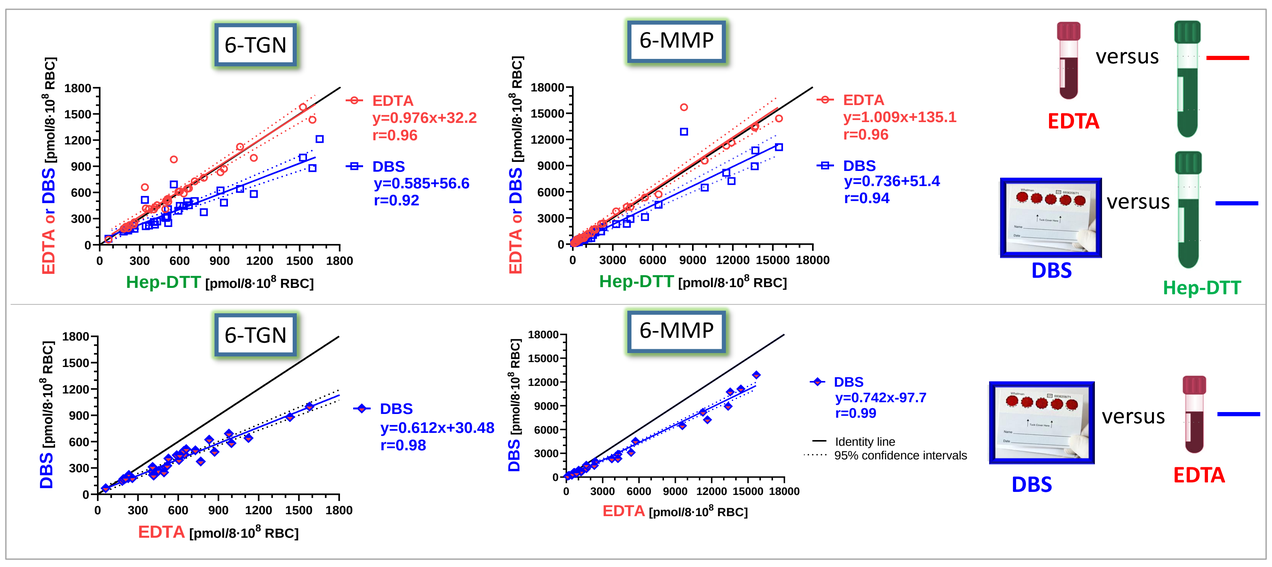
Toxicity (myelotoxicity for 6-TGN, hepatotoxicity for 6-MMP)

6-TGN: n = 6, 18% | n = 6, 18% | n = 22, 65%

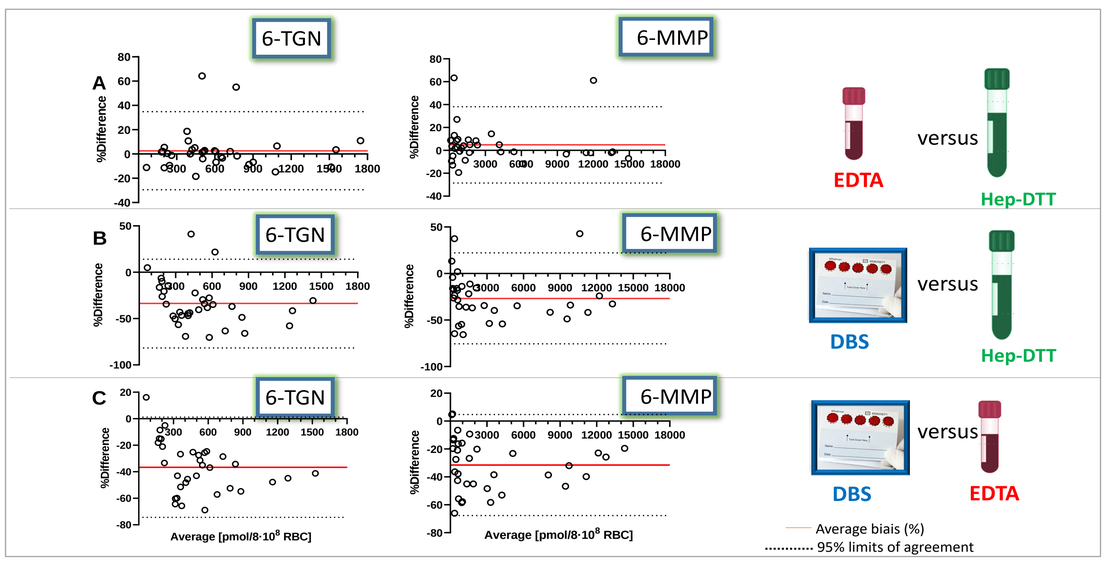
6-MMP: n = 26, 76% | n = 8, 24%

- Thiopurine quantification with CHUV method. All patient samples were found to lie within the validated concentrations range of the assay (5-2'000 ng/ml for 6-TG, and 50-20'000 ng/ml for 6-MMP).

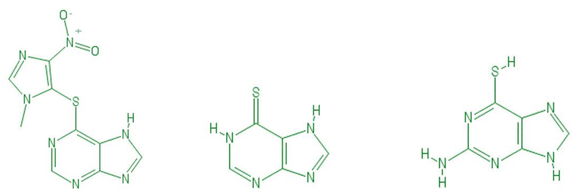
Linear regressions and correlations



Bland-Altman tests



- An excellent correlation between EDTA and hep-DTT results could be observed (slope 0.98, r=0.96 and 1.01, r=0.96, for 6-TG and 6-MMP respectively).
- Despite a good correlation between DBS and EDTA samples (r> 0.98), a significant bias is observed for 6-TG and 6-MMP with lower values in DBS (slopes of 0.61 and 0.74, respectively). Similar correlations and biases are found for hep-DTT samples.



Conclusions

- Excellent concordance was found between the in-house and reference methods. The accuracy of our in-house method is strengthened by the good performance of our laboratory at the external QC program for thiopurines [c]
- 10-ml collecting tubes of heparin with addition DTT prepared by the external laboratory and used so far can be conveniently replaced by standard 2.6-ml EDTA tubes in our LC-MS/MS method
- DBS compared to both blood anticoagulant matrices provide lower thiopurines levels. At present, DBS approach for 6-TG, 6-MMP should be used for research purpose only
- Thiopurines DBS stability and potential adsorption on the solid support of DBS card will be further investigated.

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References. [a] T. Dervieux, *et al*. Simultaneous determination of 6-thioguanine and methyl 6-mercaptopurine nucleotides of azathioprine in red blood cells by HPLC, Clin. Chem. 1998. [b] Dubinsky MC, *et al.* Pharmacogenomics and metabolite measurement for 6-mercaptopurine therapy in inflammatory bowel disease. Gastroenterology. 2000. [c] KKGT: Kwaliteitsbewaking Klinische Geneesmiddelanalyse en Toxicologie, The Hague, The Netherlands. http://kkgt.nl/?lang=en.