

Plasma free metanephrines measured by LC-MS/MS: a new diagnostic tool for the diagnosis of neuroblastoma

Sebastiano Barco ¹, Maria Valeria Corrias ², Stefania Sorrentino ³, Alberto Garaventa ³, Gino Tripodi ¹ and Giuliana Cangemi ^{1*}

¹Laboratory of Analyses, ²Laboratory of Experimental Therapies in Oncology, and ³Pediatric Oncology, IRCCS Istituto Giannina Gaslini, Genoa, Italy

*giulianacangemi@gaslini.org

Introduction

PFM are well established diagnostic biomarkers of pheochromocytoma (1). Their quantification is challenging because of their polar nature, their low molecular weight and the very low physiological concentration in human plasma (in the order of ng/L). A complete kit by LC-MS / MS (Clinmass[®]) was recently introduced by Recipe (Munich, Germany) based on SPE separation or on-line SPE with deuterated internal standards, 5-level calibrators and 3 controls. In this work we describe the analytical performance of this new test on our LC-MS / MS system (ThermoFisher TSQ Quantiva coupled to UHPLC Ultimate 3000) and its diagnostic performance in patients with NB at the onset. NB is the most common extra-cranial solid tumor in pediatrics and the determination of urinary catecholamines (in particular the two metabolites homovanillic, HVA, and vanillylmandelic, VMA acids) represents the first level diagnostic standard, followed by imaging (CT, MRI and MIBG) and hysto-pathological confirmation (2, 3). The diagnostic role of plasma metanephrines in NB has never been investigated on an adequate number of cases until now (4).

Materials and Methods

A partial validation of the method has been performed following EMA guidelines for linearity, LLOQ, intra- and inter-run accuracy and reproducibility. Fifty-four plasma samples of patients with NB at the onset (4 stage 1, 16 stage 3, 24 stage 4 and 10 stage 4S) and 49 age-matched controls (0-5 years) were analyzed. The samples of patients with NB were collected between 2012 and 2016 from different Italian centers and biobanked at Giannina Gaslini Institute, a tertiary care pediatric hospital, Italian national reference center for the biochemistry of NB. The study has been approved by the Local Ethical Committee and informed consent was obtained from all patients' guardians.

Results

The method is linear over a wide range of concentrations (2.83-5094 ng/L for 3-MT, 2.76-19860ng/L for NMN, 2.91-20970ng/L for MN) and has been shown to be highly accurate and reproducible (intra- and inter-assay CV% <5% and RE 95-105 for all the analytes) with a rapid runtime (4.5 min). Reference ranges were obtained in controls with a non-parametric test (CLSI 28-A3, non-normal distribution) and the results were as follows: 3MT: N.D.-9.3; MN: N.D.-75.4; NMN: 11.4-169.6 ng/L. Results were comparable with literature data. Differently from MN, 3-MT and NMN showed very high sensitivity and specificity (3MT: 90.5 and 100% with AUC = 0.939 and NMN: 79.555 and 95.7% with AUC = 0.893 respectively) and were able to discriminate between NB and controls (Mann Whitney p <0.001). The concentration of 3-MT has been shown to increase from stage I to IV. Interestingly, 3/54 NB patients only resulted negative for all the 3 plasma markers and were also negative for urinary HVA and VMA. Accordingly, 3/54 patients that were negative for urinary HVA and VMA resulted also negative for PFM.

Discussion

The new quantitative LC-MS/MS assay for PFM was easily applicable to our LC-MS/MS system and its analytical performance allows us to routinely use it in the laboratory workflow. Its diagnostic performance on NB patients was tested in a cohort of well characterized samples of NB patients and matched controls. The analyte with the best performance was 3-MT followed by NMN. MN did not show any significant result and the differences displayed with 3-MT and NMN could be explained by the different biochemical pathways to which these three metabolites belong. In conclusion, PFM showed an excellent performance for the diagnosis of NB and can also be very useful in addition, or as an alternative, to urinary catecholamine profile. Their eventual prognostic role in identifying patients with an unfavorable outcome should be investigated in a larger cohort of patients. The results of this cohort study should be confirmed with a prospective study with extensive case studies.

References

- 1) Darr B, Kuhn M, Bode C et al. *Endocrine*. 2017 Jun;56(3):495-503.
- 2) Swift CC, Eklund MJ, Kravaka JM et al. *Radiographics*. 2018 Mar-Apr;38(2):566-580.
- 3) Brodeur GM, Pritchard J, Berthold F, et al. *J Clin Oncol*. 1993;11(8):1466-1477.
- 4) Francini LC, Vazquez-Montes M, Buclin T et al. *Blood Cancer*. 2015 Apr;62(4):587-93.
- 5) European Medicines Agency. Guideline on Bioanalytical Method Validation. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/08/WC500109686.pdf, 2015

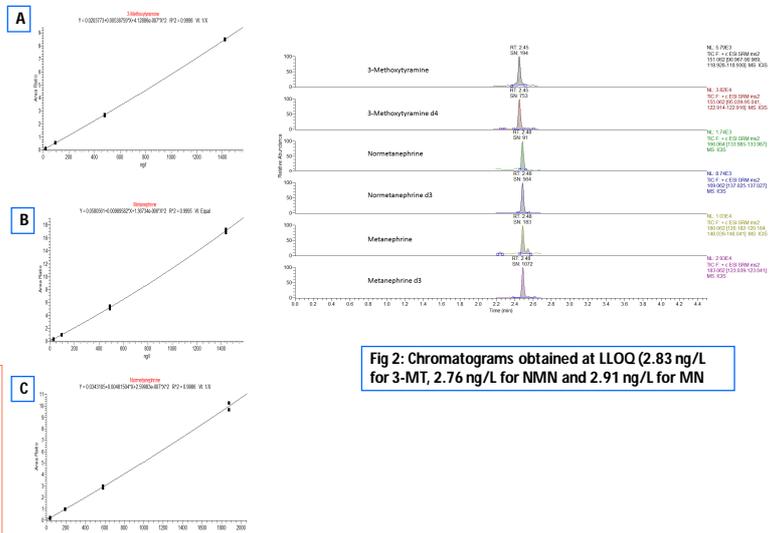


Fig 1: Representative calibration curves of 3-MT (A) MN (B) and NMN (C).

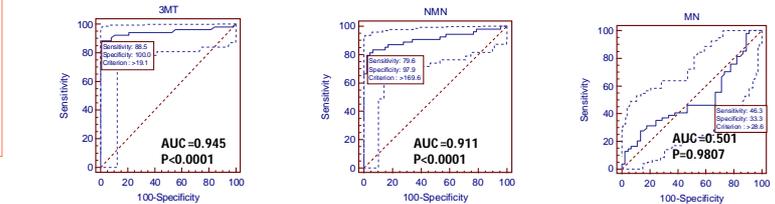


Fig 3: ROC curve analysis of the diagnostic performance of PFM for NB

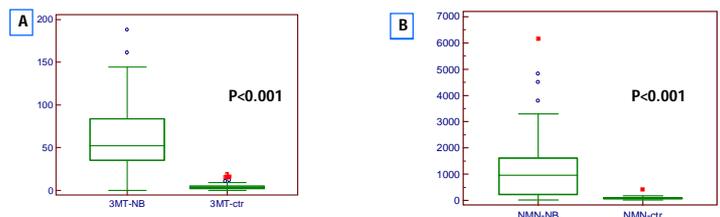


Fig 4: Results of Mann Whitney test for 3-MT (A) and NMN (B). The two biomarkers allow to discriminate between NB and controls

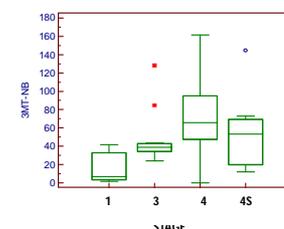


Fig 5: Results of the Kruskal Wallis test for 3-MT: the biomarker significantly increases from stage I (localized) to IV (metastatic)

Fig 2: Chromatograms obtained at LLOQ (2.83 ng/L for 3-MT, 2.76 ng/L for NMN and 2.91 ng/L for MN)