LC-MS/MS measurement of Urinary 2,3-dinor-11β-Prostaglandin $F_{2\alpha}$ in patients with Systemic Mastocytosis: Improved diagnostic accuracy compared to ELISA

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Systemic mastocytosis (SM) is a myeloproliferative neoplasm resulting in the accumulation of clonally derived mast cells in different tissues. When these mast cells are triggered, the released cellular contents cause symptoms such as itching, flushing, lightheadedness, tachycardia, gastrointestinal distress, or even loss of consciousness. Current diagnostic criteria for SM include bone marrow biopsy, genetic testing and flow cytometric analysis. Due to the intermittent nature of non-specific symptoms an alternative screening tool is desired by clinicians and patients.

Urine concentrations of mast cell secretory molecules and their metabolites have been used for screening and therapeutic monitoring of patients with SM. Three compounds are routinely ordered in this context, N-methylhistamine (NMH), 11-beta prostaglandin $F_{2\alpha}$, (BPG) and leukotriene E_4 (LTE4). Urinary NMH and LTE4 are measured using LC-MS/MS, while BPG is measured using an ELISA method (EIA kit, Cayman Chemical). We report an LC-MS/MS method for an 11-beta prostaglandin $F_{2\alpha}$ isoform with improved clinical sensitivity over BPG as measured by ELISA.

Methods:
BPG and the 2,3-dinor isoform of BPG (2,3-BPG) were measured in urine samples using an AB Sciex API 5000. A commercial internal standard for the 2,3-BPG was not available. D_4-labeled internal standard (D_4-BPG) was added to waste urine specimens, controls, and standards, followed by addition of acetonitrile and vacuum filtration. Online sample clean-up was accomplished using a Turboflow Cyclone column (ThermoFisher Scientific) and chromatographic separation of BPG and 2,3-BPG was performed using a C8 analytical column (Waters Xbridge, 60°C). Unique transitions for BPG, 2,3-BPG and D_4-BPG were monitored in

negative MRM mode (AB Sciex API 5000 MS/MS). The total analysis time was 11.8 minutes. Concentrations were normalized to creatinine (enzymatic method, Roche Diagnostics).

All patient information was accessed in compliance with the Institutional Review Board. Urine samples from patients with a clinically ordered NMH and BPG were collected for additional testing. A total of 203 patients, (37 positive, 166 negative for SM) were assessed for 2,3-BPG by MS/MS, as well as for LTE4. SM diagnoses were confirmed by an allergist according to blinded chart review. The clinical sensitivity and specificity for SM diagnosis was determined using either 2,3-BPG MS/MS assay or the current ELISA assay alone or in combination with the other SM markers. Patients were not removed from the analysis based on medication status.

Results:

Initial method comparison of 16 samples with an average BPG of 2467 pg/ml when analyzed by the ELISA method found that a majority of urine samples assessed did not have detectable concentrations of BPG when analyzed by MS/MS. However, 2,3-BPG is a known cross reactant with the ELISA assay and was readily measured in all urine samples. Comparison of 2,3-BPG concentration as measured by MS/MS to BPG measured by ELISA revealed a strong linear correlation (slope = 1.27, R^2 = 0.78).

Inter-assay precision for 2,3-BPG in pooled urine controls was 5.7% and 9.0% at concentrations of 450 pg/mL, and 700 pg/mL, respectively (n=5). Accuracy was assessed using spiked purified compound with average recovery of 103±10% (n=7). Analytical range was linear between 78-5000 pg/mL.

The SM patients had significantly increased urinary concentrations of 2,3-BPG (median, 883; range 218-6595 pg/mg creatinine) compared to control group (median, 740; range 161-2348 pg/mg creatinine; P=0.0111). BPG was also significantly higher among SM patients (median, 708; range 173-11046 pg/mg creatinine) compared to the control group (median, 613; range 17-6259 pg/mg creatinine; P = 0.0062). ROC curve analysis identified an optimal cutoff of 1382 pg/mg creatinine for 2,3-BPG (AUC 0.62) and 1887 pg/mg creatinine for BPG (AUC 0.61). Applying the calculated cutoffs to our data set revealed a sensitivity of 38% and specificity of

86% for 2,3-BPG and a sensitivity of 27% and specificity of 95% for BPG for SM detection (**Table 1**).

Table 1: Sensitivity and Specificity for Systemic Mastocytosis Detection by BPG (ELISA) and 2,3-BPG (MS/MS)

	Sensitivity	Specificity
BPG	27%	95%
2,3-BPG	38%	86%
	Sensitivity	Specificity
BPG + NMH	62%	87%
2,3-BPG + NMH	70%	78%
	Sensitivity	Specificity
BPG + NMH and LTE4	70%	75%
2,3-BPG + NMH and LTE4	76%	65%

Conclusions:

We have developed an LC-MS/MS assay for 2,3 dinor-11β-Prostaglandin $F_{2\alpha}$ which improves the clinical sensitivity and specificity of the existing commercial ELISA assay for 11β-Prostaglandin $F_{2\alpha}$ for systemic mastocytosis diagnosis.