Correlation between urinary fractionated metanephrines in 24-hour and spot urine samples for evaluating the therapeutic effect of metyrosine: a subanalysis of a multicenter, open-label phase I/II study

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Abstract. We recently conducted an open-label phase I/II study to evaluate the efficacy and safety of preoperative and chronic treatment with metyrosine (an inhibitor of catecholamine synthesis) in pheochromocytoma/paraganglioma (PPGL) in Japan. We compared creatinine-corrected metanephrine fractions in spot urine and 24-hour urine samples (the current standard for the screening and diagnosis of PPGLs) from 16 patients to assess the therapeutic effect of metyrosine. Percent changes from baseline in urinary metanephrine (uMN) or normetanephrine (uNMN) were compared between spot and 24-hour urine samples. Mean percent changes in uMN or uNMN in spot and 24-hour urine were –26.36% and –29.27%, respectively. The difference in the percent change from baseline between uMN or uNMN in spot and 24-hour urine was small (~2.90%). The correlation coefficient was 0.87 for percent changes from baseline between uMN or uNMN measured in spot and 24-hour urine. The area under the receiver operator characteristic (ROC) curve of uMN or uNMN measured in spot urine vs. 24-hour urine (reference standard) to assess the efficacy of metyrosine treatment was 0.93. Correlations and ROCs between 24-hour urinary vanillylmandelic acid, adrenaline, and noradrenaline and 24-hour uMN or uNMN were similar to those between spot uMN or uNMN and 24-hour uMN or uNMN. No large difference was observed between spot and 24-hour urine for the assessment of metyrosine treatment by quantifying uMN or uNMN in Japanese patients with PPGLs. These results suggest that spot urine samples may be useful in assessing the therapeutic effect of metyrosine.

Key words: Metanephrine, Normetanephrine, 24-hour urine sample, Spot urine sample, Metyrosine

PHEOCHROMOCYTOMAS AND PARAGANGLIOMAS (PPGLs) are rare neuroendocrine tumors that produce and secrete excess catecholamines [1]. Abnormal levels of catecholamines and their metabolites in urine or plasma may be indicative of the presence of neuroendocrine tumors, such as neuroblastoma [2-4], pheochromoco-
cytoma [5], or PPGLs [6]. Thus, catecholamines and their metabolites, such as vanillylmandelic acid (VMA) [5], metanephrine (MN), and normetanephrine (NMN) [7], are used as markers for the diagnosis of neuroendocrine tumors [8, 9].

Current clinical practice guidelines [7] recommend the measurement of free MN and NMN in plasma or urinary metanephrine fractions (uMN and uNMN) for the diagnosis of neuroendocrine tumors. In Japan, the measurement of free MN and NMN in plasma was recently approved by regulatory authorities, and its use in clinical practice is expected. Thus, the measurement of uMN and uNMN in 24-hour urine samples is the current standard for the screening and diagnosis of neuroendocrine tumors in Japan. Quantification of uMN and uNMN in 24-hour urine samples may also be useful for evaluating the efficacy of pheochromocytoma treatment. However, 24-hour urine sample collection cannot be performed easily or conveniently for several reasons. For example, this sampling method is subject to storage issues leading to the loss of specimens, or improper storage of the urine, which can affect the integrity of the sample and accuracy of the measurements [10, 11]. For this reason, patients tend to require hospitalization for 24-hour urine sample collection. Moreover, such sample collection may lead to unnecessary exposure to hospital-acquired infections and is best avoided if possible. As long-term monitoring is often needed for patients with PPGLs, more convenient quantitative assessments are required, not only for diagnosis, but also to evaluate biochemical treatment responses and long-term monitoring for the management of PPGLs.

Several studies have evaluated the correlation between MN in spot urine samples and 24-hour urine samples. A previous study concluded that levels in single-voided specimens were closely correlated to those in 24-hour specimens [12]. Another study reported that total MN measurements in random 1-hour and 24-hour urine samples were useful for diagnosing pheochromocytomas [13]. Another study showed that total MN measurements in urine samples could be used to diagnose benign PPGLs with a sensitivity of 74% [14]. Two other studies suggested that a spot urine MN and NMN assays could be sensitive and specific screening and diagnostic tools for pheochromocytoma [15] and for managing incidentaloma [16].

α-Methyl-paratyrosine (metyrosine) is a tyrosine hydroxylase inhibitor that inhibits catecholamine synthesis and is used for the management of PPGLs in patients where other treatments have been ineffective [3]. Two retrospective analyses [17, 18], in which patients with PPGLs were prepared preoperatively with metyrosine and phenoxybenzamine, concluded that the combination of metyrosine and α-blockade resulted in better blood pressure control, less blood loss, less use of antihypertensive medication or pressors during surgery, and the need for less intraoperative fluid replacement.

We recently conducted an open-label, multicenter phase I/II study to evaluate the efficacy and safety of preoperative and chronic treatment with metyrosine in PPGLs in Japan [19]. In our study, we determined the treatment efficacy of metyrosine by assessing whether uMN or uNMN in 24-hour urine samples decreased by more than 50% from baseline. Here, we describe a subanalysis in which we aimed to compare metanephrine fractions in spot urine with metanephrine fractions in 24-hour urine samples (as a reference standard) to assess the therapeutic effect of metyrosine. Additionally, 24-hour urinary catecholamine fractions (urinary adrenaline [uA] and urinary noradrenaline [uNA]), and urinary VMA (uVMA), which were used for biochemical diagnosis, were compared with metanephrine fractions.

Materials and Methods

Study design

The study design has been described in detail elsewhere [19]. The main study was a prospective, multicenter, open-label phase I/II study (Japic CTI-152999) conducted in Japan [19]. The present study was a retrospective subanalysis of data collected during the main study [19]. The institutional review boards of all participating centers approved the study and informed consent was obtained from all patients.

Patients

Patients were ≥12 years of age; had inoperable tumors that required chronic medication therapy; were surgical candidates requiring preoperative treatment; had a diagnosis of PPGLs; had baseline uMN and uNMN levels ≥3 times the upper limit of normal; were treated with α-blockers; and presented symptoms of excess catecholamines.

Patients who were newly treated or temporarily treated with a drug or who consumed foods that could affect urinary catecholamines and their metabolites; with impaired intestinal absorption; with severe or uncontrollable complications; with an estimated glomerular filtration rate (eGFR) <30 mL/min; and with a left ventricular ejection fraction <40% were excluded from the main study [19].

Study interventions and measures

Metyrosine treatment and dose adjustments were described in detail previously [19]. After the dose was established, two consecutive 24-hour urine samples were
collected to measure uMN, uNMN, uVMA, uA, and uNA. Levels of uMN, uNMN, and creatinine were also measured in spot urine samples, and the creatinine-corrected values (µg/g creatinine) of uMN and uNMN were calculated.

Patients were hospitalized while the urine examinations were completed. For 24-hour urine samples, patients were instructed to accurately record the start/end time of urine sample collection and the total volume of urine collected. The 24-hour urine samples were collected at the following time points: 3 days during the observation period, Days 6–8, Days 28–29, Days 56–57, Days 84–86, 3 days after fixed administration at the increased or decreased dose, and every 12 weeks (continuous dosing) after Day 84. In cases of adrenalectomy, 24-hour urine samples were collected as recommended (2 days before surgery, the day of surgery, and Days 5–7 after surgery). For 24-hour urine sample collection, an acid UriMeasure Tablet (Kanto Chemical Co., Inc., Tokyo, Japan) was added to prevent decomposition of urinary catecholamines and metabolites, and the samples were stored at room temperature.

Spot urine samples were collected from the first-void urine of each pooled urine sample. If this was not possible, a spot urine sample was collected from the second-void or subsequent urine. Then, samples containing 2 mL of pooled urine and 3 mL of spot urine were obtained for the measurement of metanephrine fractions. Collected samples were stored at ≤15℃ until retrieval. Urinary concentrations were determined by high-performance liquid chromatography with electrochemical detection (CoulArray, Thermo Fisher Scientific Inc., Waltham, MA, USA) for uMN, uNMN, and uVMA. Urinary concentrations were determined by high-performance liquid chromatography with fluorescence detection (HLC-725CA II, Tosoh Corporation, Tokyo, Japan) for uA and uNA.

For uMN or uNMN, whichever of the two parameters had the higher ratio of baseline to the upper limit of the reference value was chosen for evaluation in each patient (shown as uMN or uNMN). Similarly, for uA or uNA, whichever of the two parameters had the higher ratio of baseline to the upper limit of the reference value was chosen for evaluation in each patient (shown as uA or uNA).

**Study assessments**

In this study, we evaluated the achievement of 50% reduction in uMN or uNMN from baseline at each time point at which both spot and 24-hour urine levels were measured, along with the achievement of 50% reduction in 24-hour uVMA and uA or uNA. Percent changes from baseline in uMN or uNMN, uA or uNA, and uVMA determined from 24-hour urine samples, and uMN or uNMN determined from spot urine samples were evaluated. uMN or uNMN in a spot urine sample were compared with uMN or uNMN in a 24-hour urine sample to assess the treatment effect of metyrosine. Additionally, the 24-hour uMN or uNMN were compared with 24-hour uA or uNA and 24-hour uMN or uNMN were compared with 24-hour uVMA.

**Statistical analysis**

The sample size for this main study was at least 10 patients because the population of patients eligible for the main study was thought to be very small. This analysis was based on the data of the full analysis set, which was defined as the population of patients included in the safety analysis set who were evaluated for efficacy at least once.

The Pearson product-moment correlation coefficient was calculated for metanephrine fraction values determined from spot and 24-hour urine samples. Similarly, the Pearson product-moment correlation coefficient of change from baseline was calculated for uMN or uNMN values determined from spot and 24-hour urine samples. Summary statistics were calculated for the difference in percent change from baseline for uMN or uNMN values determined from spot and 24-hour urine samples.

The reference standards for this subanalysis were the results of 50% reduction in uMN or uNMN from baseline in 24-hour urine samples. Using these results, we calculated the receiver operating characteristic (ROC) curves of spot uMN or uNMN, 24-hour uA or uNA, and 24-hour uVMA. Similarly, we calculated the area under the curve (AUC). We examined whether the factors eGFR, age, sex, and body weight of each patient had an effect on uMN or uNMN values determined from spot and 24-hour urine samples. The differences in uMN or uNMN values determined from spot and 24-hour urine samples in relation to these factors were then plotted.

A statistical significance level was not established as no formal statistics were performed on account of the small sample size. All statistical analyses were performed using SAS Ver.9.3 (SAS Institute Inc., Cary, NC, USA).

**Results**

Spot and 24-hour urine samples were collected from 16 patients at 11 sites. Details of the baseline patient characteristics have been described previously [19]. Briefly, the sample comprised 11 men and five women, aged 12 to 86 years, with a mean blood pressure of 126.4/71.1 mmHg, and renal function ranging from normal (n = 5, eGFR ≥90 mL/min), mildly reduced (n = 6,
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60 mL/min ≤ eGFR < 90 mL/min), to moderately reduced (n = 5, 30 mL/min ≤ eGFR < 60 mL/min). Nine patients were diagnosed with pheochromocytoma and seven with paraganglioma. Eight patients had metastatic PPGL.

The Pearson product-moment correlation coefficient of uMN or uNMN values determined from creatinine-corrected spot and 24-hour urine samples was 0.94 (Supplementary Fig. 1).

Table 1 shows the percent changes from baseline in uMN or uNMN in spot and 24-hour urine samples. Mean percent changes in uMN or uNMN in spot and 24-hour urine samples were –26.36% and –29.27%, respectively. The difference in the percent change from baseline in uMN or uNMN, as calculated by subtracting the percent change obtained for spot urine from that obtained for 24-hour urine, was small (–2.90%).

The Pearson product-moment correlation coefficient of the percent changes from baseline of uMN or uNMN values determined from spot and 24-hour urine samples, using 24-hour urine as the reference standard, was large (0.93) (Fig. 1B). Figs. 2A and 3A show the percent changes from baseline in uMN or uNMN vs. uA or uNA and those in uMN or uNMN vs. uVMA, respectively, in the 24-hour urine samples. Figs. 2B and 3B show the

Fig. 1 A) Correlation between percent changes from baseline in urinary metanephrine (uMN) or normetanephrine (uNMN) levels in spot and 24-hour urine samples. B) Area under the receiver operating characteristic curve of uMN or uNMN values measured in spot urine and 24-hour urine to assess the efficacy of metyrosine treatment (using 24-hour urine as the reference standard). Either uMN or uNMN, whichever had a higher ratio at baseline according to the upper limit of the reference value, was used for the efficacy assessment.

CORR, correlation; AUC, area under the curve

Table 1 Percent changes from baseline in uMN or uNMN in 24-hour and spot urine samples

| Summary statistics | 24-hour urine sample (N = 143) | Spot urine sample (N = 143) |
|--------------------|-------------------------------|-----------------------------|
| Percent change from baseline (%) in uMN or uNMN<sup>a</sup> | Mean (SD) –29.27 (38.36) | Median –34.07 |
| | Min, max –99.04, 107.41 | | –99.07, 135.99 |
| The difference<sup>b</sup> in change from baseline in MN or NMN between 24-hour and spot urine samples (%) | Mean (SD) –2.90 (22.41) | Median 0.03 |
| | Min, max –67.27, 107.91 | |

<sup>a</sup> Either uMN or uNMN, whichever had a higher ratio at baseline according to the upper limit of the reference value, was used for the efficacy assessment.

<sup>b</sup> The difference is calculated by subtracting the percent change obtained for the spot urine sample from that obtained for the 24-hour urine sample.

uMN, urinary metanephrine; uNMN, urinary normetanephrine; SD, standard deviation
AUC for assessing the efficacy of metyrosine. The correlation coefficient of the percent changes from baseline was 0.77 for uMN or uNMN vs. uA or uNA and 0.84 for uMN or uNMN vs. uVMA in the 24-hour urine samples. Additionally, using uMN or uNMN as the reference standard, the AUCs of uA or uNA (0.91) and that of uVMA (0.88) to uMN or uNMN were large.

Fig. 4A–D shows the effects of eGFR, age, sex, and body weight on the uMN or uNMN values determined from spot and 24-hour urine samples. Within-subject variability did not seem to depend on eGFR, age, sex, or body weight. In female patients and those who were not
overweight, measurement values of spot urine samples tended to be higher than those of 24-hour urine samples.

**Discussion**

In this subanalysis of a phase I/II study, we compared metanephrine fractions in spot urine with metanephrine fractions in 24-hour urine samples to assess the therapeutic effect of metyrosine, unlike previous reports [12-16] that focused on the diagnostic capability for PPGLs. Additionally, we evaluated the correlation between the percent changes from baseline in uMN or uNMN in spot and 24-hour urine samples, and the correlation between the percent changes from baseline in uA or uNA, and uVMA vs. uMN or uNMN in 24-hour urine samples for assessing the therapeutic effect of metyrosine. We based our analysis on a 50% reduction in uMN or uNMN from baseline at each time point at which both spot and 24-hour urine levels were measured. As there is no reference standard for the evaluation of the effects of metyrosine, this general convention was used; thus, the present results should be interpreted with care.

We found that there was a small mean difference (−2.90%) in the percent change from baseline between uMN or uNMN in spot and 24-hour urine samples. The correlation coefficient between the two collection meth-
ods for the assessment of uMN or uNMN was 0.87. Additionally, the correlation coefficient was 0.77 for uMN or uNMN vs. uA or uNA and 0.84 for uMN or uNMN vs. uVMA in the 24-hour urine samples. These results are in line with the findings of previous studies [15, 16], suggesting that spot urine MN or NMN assays are sensitive and specific screening and diagnostic tools for PPGLs and managing incidentaloma.

Furthermore, using 24-hour urine as the reference standard, the area under the ROC curve of spot to 24-hour urine assessing the efficacy of metyrosine treatment was large (0.93). The areas under the ROC curve of uA or uNA (0.91) and of uVMA (0.88) to uMN or uNMN were also large. This suggests that the measurement of uMN or uNMN in spot urine samples could potentially be as useful in assessing the efficacy of metyrosine as uA or uNA and uVMA in 24-hour urine, but further studies are necessary. We consider that these findings are very relevant given the lack of studies reporting on the potential use of these parameters for the assessment of the therapeutic effect of metyrosine.

Dopamine is the precursor of NA, A, MN, NMN, and VMA. Urinary dopamine was increased in some patients in our phase I/II study [19], whereas other catecholamine derivatives (i.e., uNA, uA, uMN, uNMN, and uVMA) were not. This finding is consistent with the findings reported by Kuchel et al. [20]. They reported that treating malignant pheochromocytoma with metyrosine led to initial increased in the serum levels of DOPA, DOPA sulfate, and dopamine sulfate, and that progressive increases in urinary dopamine were observed during metyrosine treatment. Notably, this effect, together with increases in dopamine metabolites (e.g., dihydroxyphenylethanol and plasma dopamine sulfate), occurred without causing changes in serum dopamine levels [20]. Thus, we consider that both serum and urinary dopamine levels should be carefully assessed in patients treated with metyrosine because urinary dopamine increases in the absence of serum dopamine increases could lead to false evaluations of urinary dopamine.

Currently, there is no consensus or standard guidelines stating which biochemical tests should be used to confirm and locate or exclude a suspected PPGLs [21]. However, it is recommended that screening for PPGLs be performed by testing for plasma-free MNs and/or urinary fractionated MNs and catecholamines [22, 23]. Although several studies have concluded that fractionated uMN and uNMN provide superior diagnostic sensitivity over uA and uNA, uVMA, or total MNs [24-27], other studies have concluded that plasma-free MNs have higher sensitivity and specificity [28, 29] for excluding or confirming pheochromocytoma. The present findings are not proof that spot urine samples can replace 24-hour urine sample collection, but they support previous findings indicating that spot urine samples may have a similar diagnostic capability as 24-hour urine samples. Additionally, spot urine samples may be useful in assessing the therapeutic effect of metyrosine.

It has been reported that measurements of total MNs and catecholamines in 24-hour urinary samples yield fewer false-positive results [30]. Several factors, such as issues with the reliability of the timed urine sample collection, difficulties in sample storage, and inpatient sampling make 24-hour urine sample collection inconvenient and costly [10, 11, 21]. In contrast, spot urine sampling has several advantages, such as relative ease of collection, noninvasiveness, the wide availability of the test, ease of implementation, and cost-effectiveness [21, 31]. Taken together, the convenience of spot urine sampling, the existing evidence showing that the spot urine sample has similar diagnostic yields to those of the 24-hour urine sample [31, 32], and the development of new, simplified methods for the quantification of catecholamines and MNs in spot urine [33, 34] will likely lead to a less frequent use of 24-hour urine testing for diagnostics and treatment assessments in routine clinical practice.

Within-subject variability did not seem to be influenced by eGFR, age, sex, or body weight on uMN or uNMN values determined from 24-hour urine samples and spot urine samples. However, women and patients with lower body weight, rather than men and overweight patients, seemed to have higher values of uMN or uNMN in spot urine than in 24-hour urine. Because the uMN and uNMN values of spot urine were creatinine-corrected, these values may have been higher in patients with less muscle mass and therefore less urinary creatinine excretion.

As mentioned above, there was no major difference between spot and 24-hour urine samples in percent changes from baseline in uMN or uNMN levels. Nevertheless, the measurement values for spot urine can be expected to vary by sampling time, which is not the case in 24-hour urine samples. This may affect the reliability of measurements using spot urine when assessing the efficacy of metyrosine. In this study, a large difference in percent changes from baseline was observed between spot and 24-hour urine samples in some cases. Thus, a possible variance in measured values by sampling time must be considered in the clinical setting. When the efficacy of metyrosine is evaluated using spot urine samples in clinical practice, it is appropriate to scrutinize measurement results by considering multiple measurement points and measurement changes over time.

This subanalysis had some limitations, such as its retrospective nature and small sample size, which limited the number of urine samples collected, and the fact that
plasma concentrations of MN, NMN, and other metabolites were not quantified. The absence of formal statistical testing also limited the extent of conclusions that can be drawn from current findings.

Based on the present findings, we conclude that differences were small between the spot and 24-hour urine samples for the assessment of metyrosine treatment based on the quantification of uMN and uNMN in Japanese patients with PPGL.

Acknowledgments

Authors thank all participating patients and their families, as well as all health care professionals, who enabled this study. We thank all the subinvestigators of each 11 institutions for their collaboration with this clinical trial. The authors would also like to thank Keyra Martinez Dunn, MD, of Edanz Medical Writing for providing medical writing services, which were funded by Ono Pharmaceutical Co., Ltd. (Osaka, Japan).

Disclosure

Kazuhiro Takekoshi received research funding from Blue Industries Inc. Fumitoshi Satoh received honoraria from Takeda Pharmaceutical Co. Ltd.; research funding from Fujirebio Inc., FUJIFILM Wako Pure Chemical Corporation, Japan Agency for Medical Research and Development, Nippon Boehringer Ingelheim Co. Ltd., MSD K.K., Mochida Pharmaceutical Co. Ltd., Fuji Yakuhin Co. Ltd., Kowa Pharmaceutical Co. Ltd., Shionogi & Co. Ltd., Taisho Toyama Pharmaceutical Co. Ltd., Ono Pharmaceutical Co. Ltd., and Teijin Pharma Ltd.; and scholarship donations from Novartis Pharma K.K., Novo Nordisk, Takeda Pharmaceutical Co. Ltd., Mitsubishi Tanabe Pharma Corporation, Bayer Yakuhin Ltd., Daiichi Sankyo Company Ltd., Teijin Pharma Ltd., Sumitomo Dainippon Pharma Co. Ltd., and JCR Pharmaceuticals Co. Ltd. Akiyo Tanabe received research funding from Ono Pharmaceutical Co. Ltd. Takahiro Okamoto received a scholarship donation from Taiho Pharmaceutical Co. Ltd. Atsuhiro Ichihara received honoraria from Mochida Pharmaceutical Co. Ltd. Takuyuki Katabami received a scholarship donation from Sanofi K.K. Masatoshi Nomura received honoraria from Ono Pharmaceutical Co., Ltd., Taishotoyama Pharmaceutical Co. Ltd., Novo Nordisk Pharma Co. Ltd., MSD K.K., Chugai Pharmaceutical Co. Ltd., and Sanofi K.K.; research funding from Astellas Pharma Inc., and scholarship donations from Taishotoyama Pharmaceutical Co. Ltd. and Ono Pharmaceutical Co. Ltd. Tadashi Matsuda received research funding from Ono Pharmaceutical Co. Ltd. Suguru Asada and Nobuyuki Kawata are employees of Ono Pharmaceutical Co. Ltd. Mika Tsuki, Tomoaki Tanaka, Tsuneo Imai, Masanobu Yamada and Mitsuhide Naruse have no conflicts of interest to declare.

Funding Source

This work was funded by Ono Pharmaceutical Co., Ltd. (Osaka, Japan).

Supplementary Fig. 1 Pearson product-moment correlation coefficient of uMN or uNMN values determined from spot and 24-hour urine samples.

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