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¹Clinical Research, NeuroResearch Clinics Inc., Cape Coral, FL, USA; ²Stein Orthopedic Associates, Plantation, FL, USA; ³DBS Laboratories, Duluth, MN, USA **Background:** The purpose of this paper is to present the results of state call analysis of spot baseline urinary norepinephrine and epinephrine across in carelation with spot baseline urinary serotonin and dopamine findings previously cablished to the authors. Our research indicates a need for physicians and decision-maker a funderstand to lack of validity of this type of spot baseline monoamine testing when using it in the decision-making process for neurotransmitter deficiency disorders.

Methods: Matched-pairs *t*-tos were performed for group of subjects for whom spot baseline urinary norepinephrine and binephrine as tys were performed on samples collected on different days then paired by subjects.

Results: The reported laborate stest results for urinary serotonin, dopamine, norepinephrine, and epinephrine, but I on different days from the same subjects, differed significantly and were not reproduct to.

Conclusion: Spot to the monoamine assays, in subjects not suffering from a monoamine-section of the production of peripheral or central period of the period o

Key ords: neurotransmitter testing, epinephrine, norepinephrine, dopamine, serotonin

Introduction

A previously published paper by the authors of this paper discussed the reproducibility of spot baseline urinary serotonin and dopamine assays. This companion paper discusses the reproducibility of spot baseline urinary norepinephrine and epinephrine assays, and explores the feasibility and validity of using spot urinary norepinephrine or epinephrine assays in subjects not suffering from a monoamine-secreting tumor as a basis for decision-making. The paper then correlates the novel spot baseline norepinephrine and epinephrine findings reported here with our earlier reports relating to spot baseline urinary serotonin and dopamine.

Urinary neurotransmitter testing samples can be generated in several ways. "Spot urine" is a single urine sample obtained at a specific time. A 24-hour urine sample is a collection of all urine excreted in a defined time period, and is used when the total daily excretion of a substance by the kidneys into the urine is to be studied. One application of the 24-hour urine test is in the diagnosis of monoamine-secreting tumors.



Correspondence: Marty Hinz 1008 Dolphin Dr, Cape Coral, FL 33904, USA Tel +1 218 626 2220 Fax +1 218 626 1638 Email marty@hinzmd.com Collection of a 24-hour urine sample is burdensome, and requires the subject to carry sample collection materials during all daily activities.^{2,14}

Urinary monoamines exist in two states, ie, "the endogenous state", found when no amino acid precursors of the monoamines are being administered, and "the competitive inhibition state", found when significant amounts of both serotonin and dopamine amino acid precursors are being administered simultaneously. Obtaining urine samples in the endogenous state is known as "baseline testing". The focus of this paper is spot urine measurements obtained in the endogenous state, which is also known as "baseline urinary neurotransmitter testing". Spot baseline urinary neurotransmitter testing samples obtained in the endogenous state are of no value in patients not suffering from a monoamine-secreting tumor, such as pheochromocytoma or carcinoid syndrome, due to a lack of reproducibility of the testing involved. Previously published peer-reviewed literature has established the validity and utility of OCT interpretation of monoamine assays in the competitive inhibition state when performed under proper conditions. 1,3-5

Materials and methods

Results of statistical analysis of spot baseline urinary ne rotransmitter testing of serotonin and dopamine assays hav been discussed and published previously by the this paper. Novel statistical results of spot ba neurotransmitter testing of norepinephrine depin assays from a database accumulated two authors norepinep of this paper are reported here. Uri epinephrine samples obtained diffe at days from the same subject were statistically analyzed using a matched-0.05 was considered to reveal pairs t-test. A P value a significant different bety on groupings. JMP (SAS Institute, Cary, soft re was sed to perform the sis. statistical ana

Processey, many from and assay of the urine samples collected for the addy were as follows. Urine samples were collected six hours, nior to bedtime, with 4 pm being the most frequent collection time point. The samples were stabilized in 6 N HCl to preserve urinary dopamine and urinary serotonin. Samples were shipped to DBS Laboratories, Duluth, MN. Urinary norepinephrine and dopamine were assayed utilizing commercially available radioimmunoassay kits (3 CAT RIA IB88501 and IB89527; Immuno Biological Laboratories Inc, Minneapolis, MN). DBS Laboratories is accredited as a high complexity laboratory by Clinical Laboratory Improvement Amendments to perform these assays.

Results

In order for laboratory testing to be valid it needs to be reproducible. The following is a discussion of the statistical reproducibility of spot baseline urinary neurotransmitter testing of norepinephrine and epinephrine performed on a group of subjects in whom two urine samples were obtained on different days. The matched-pairs *t*-test was used to evaluate these spot baseline samples. To complete the serotonin and catecholamine discussion, previously published data by the authors relating to spot baseline urinary neurotransmitter testing of serotonin and dopamine is the ded, because norepinephrine and epinephrine production and palance are related to balanced levels of serotonin and dopamine.

Spot baseline nor pinephrine matched-pairs, t-1 st

inephra data are ovel. The laboratory The following no values are repositive ug of nore nrine per g of creatinine. From a matched-pair, roup of n = 54, the mean and standard) for both t baseline norepinephrine urinary assa groups was determined. For Group 1, the mean norepifound to be 64.66 (±148.98). For Group 2 ine value wa seline nor pinephrine testing performed on a different (spot assay), the mean norepinephrine value was be 42.01 (± 173.39). All data greater than the value ound in calculating the sum of two SDs plus the mean were emoved from consideration, revealing a group of n = 44. his matched-pair values group was then analyzed using the matched-pairs t-test, revealing a P value of 0.0399. These findings indicate that spot baseline urinary norepinephrine levels do differ in a statistically significant manner when spot baseline assays are performed on different days from the same subject. This supports the assertion that spot urinary norepinephrine values are not uniform or reproducible from day to day. The epinephrine group (n = 44) comprised 21 females aged 48.22 (±13.34) years and 23 males aged 46.31 (±14.63) years.

Spot baseline epinephrine matched-pairs *t*-test

The following epinephrine data are also novel. The laboratory values are reported in μg of epinephrine per g of creatinine. From a matched-pairs group of n = 135, the mean and the SD for both spot baseline epinephrine urinary assay groups was determined. For Group 1, the mean epinephrine value was found to be 6.55 (± 5.5). For Group 2 (spot baseline testing performed on a different day after the first assay), the mean epinephrine value was found to be 10.4 (± 14.12).

All data greater than the value found in calculating the sum of two SDs plus the mean were removed from consideration, leaving a group of n = 122. This matched-pair values group was then analyzed using the matched pairs t-test, revealing a P value of <0.0001. These findings indicate that spot baseline urinary epinephrine levels do differ in a statistically significant manner when spot baseline assays are performed on different days from the same subject. This supports the assertion that spot urinary epinephrine values are not uniform or reproducible from day to day. The epinephrine group (n = 122) comprised 63 females aged 59.09 (± 11.87) years and 59 males aged 45.89 (± 18.72) years.

Spot baseline serotonin matched-pairs *t*-test

A 2010 peer-reviewed paper by the authors presented results of a novel spot serotonin matched-pairs t-test (n = 134). Spot baseline—baseline grouping of urinary serotonin samples obtained on different days from the same patient revealed a P value of 0.0080. This indicates that spot baseline urinary serotonin levels differ in a statistically significant manner when they are performed on different days from the same subject. This supports the assertion that spot urinary serotonin values are not uniform or reproducible from day. ¹

Spot baseline dopamine matched-pirs *t*-test

A 2010 peer-reviewed paper by zer auth of this paper dopamine presented results of a novel sa t-test (n = 138). Spot baselin —base e grouping of urinary dopamine samples obtained on different lays from the same patient revealed a P rue of 00049. This indicates that spot baseline urinary doming vels differ in a statistically sigwhen y are proformed on different days nificant mann me su ect. Th ports the assertion that spot urinar opami lues are not uniform or reproducible from day

Results of the four matched-pairs *t*-tests shown in Table 1 reveal that there are significant differences between spot baseline urinary neurotransmitter testing performed on different days from the same subject for all four monoamines under scrutiny.

Simply asserting that testing differs significantly and is not reproducible from day to day in the same subject may not have the impact of reviewing the data used for the statistical analysis. The data in the accompanying tables illustrate that the urinary neurotransmitter testing results are not

Table I Matched-pairs t-test values. A P value <0.05 indicates that a significant difference between the test I grouping and test 2 grouping exists on different days in the same individual. Spot baseline monoamine assays are not uniform and reproducible from day to day in the same subject, and therefore the testing is not reproducible or valid

| | n | P value |
|----------------|-----|----------|
| Norepinephrine | 44 | 0.0399 |
| Epinephrine | 122 | < 0.0001 |
| Serotonin | 134 | 0.0080 |
| Dopamine | 138 | 0.0049 |

reproducible from day to day and that spot a seline urinary neurotransmitter testing is not a solid foundar in for medical decision-making. Tables 2–5 contact the daired results of 160 spot baseline a mary new otransmitter tests. All values are reported in ag of the dairine pag of creatinine.

The uripe tamples and execute the collected approximately six hour prior abedtime, who 4 pm being the most common time of collections are view of all samples collected at other times of the day revealed results that were similar to the aforementioned fire lings. Spot baseline urinary monoamine samples of the significantly from day to day in the same subject, regardless with time collected, and were not reproducible.

D, zussion

In the scientific world, there are two highly polarized views regarding the validity of spot baseline urinary neurotransmitter testing. One view advocates that baseline urinary neurotransmitter testing has no value in patients not suffering from a

Tables 2a, b Serial spot baseline—baseline norepinephrine assays from the same subject. Some of the norepinephrine data used to determine the norepinephrine matched-pairs *t*-test values found in Table 1. Comparison of norepinephrine 1 with norepinephrine 2 from the same subject (by row) illustrates the lack of test reproducibility. The number of days column is the number of days between urinary sample collection dates

| a) Sort: High-low by NE-I | | | b) Sort: High-low by NE-2 | | |
|---------------------------|--------|--------|---------------------------|--------|--------|
| Days (n) | NE-I | NE-2 | Days (n) | NE-I | NE-2 |
| 217 | 595.42 | 270.20 | 272 | 145.46 | 861.92 |
| 58 | 479.59 | 8.50 | 225 | 7.67 | 581.60 |
| 28 | 416.86 | 132.37 | 32 | 386.01 | 540.17 |
| 41 | 399.75 | 49.38 | 79 | 151.44 | 482.38 |
| 32 | 386.01 | 540.17 | 217 | 595.42 | 270.20 |
| 19 | 381.86 | 10.62 | 29 | 232.14 | 261.01 |
| 42 | 357.80 | 61.73 | 189 | 132.09 | 233.98 |
| 41 | 301.00 | 203.70 | 41 | 301.00 | 203.70 |
| 50 | 268.04 | 31.36 | 28 | 0.97 | 195.92 |
| 29 | 232.14 | 261.01 | 64 | 214.24 | 186.00 |

Abbreviation: NE, norepinephrine.

Tables 3a, b Serial spot baseline—baseline epinephrine assays from the same subject, including epinephrine data used to determine the epinephrine matched-pairs t-test values found in Table I. Comparison of EPI-I with EPI-2 from the same subject (by row) illustrates lack of test reproducibility. The number of days column is the number of days between urinary sample collection dates

| a) Sort: High-low by EPI-I | | | b) Sort: High-low by EPI-2 | | | |
|----------------------------|-------|-------|----------------------------|-------|-------|--|
| Days (n) | EPI-I | EPI-2 | Days (n) | EPI-I | EPI-2 | |
| 43 | 36.06 | 3.90 | 77 | 8.98 | 29.09 | |
| 22 | 24.83 | 9.58 | 272 | 13.09 | 16.37 | |
| 364 | 22.81 | 11.99 | 104 | 8.43 | 15.34 | |
| 27 | 21.37 | 3.22 | 35 | 8.62 | 15.01 | |
| 46 | 20.44 | 14.76 | 42 | 14.80 | 14.99 | |
| 49 | 18.80 | 6.69 | 46 | 20.43 | 14.76 | |
| 380 | 18.59 | 13.83 | 98 | 6.39 | 14.10 | |
| 185 | 16.49 | 4.87 | 380 | 18.59 | 13.83 | |
| 42 | 14.80 | 8.43 | 225 | 6.16 | 13.42 | |
| 41 | 12.86 | 6.57 | 22 | 24.83 | 13.02 | |

Abbreviation: EPI, epinephrine.

monoamine-secreting tumor.^{1,3–5} The other view advocates that it is very beneficial, and that it has numerous applications in medical decision-making, including diagnostic, therapeutic, and biomarker applications.^{6–12} The purpose of this writing is to educate medical practitioners regarding the selection of laboratory testing for neurotransmitter diseases so that they do not use invalid testing methods.

The science supporting the view of the authors is a follows. It is a well-known fact that norepinephrine unpehrine, serotonin, and dopamine do not cross the clood train barrier. These monoamines are filtered at the clomery and are then metabolized by the kidneys. Significant dounts of these monoamines filtered at the gloot rulus do not each the final urine. Monoamines found at the case of patients not suffering from a monoaming secreting turn ware primarily

synthesized by structures in the kidneys.^{1,3–5,13} Spot baseline testing lacks reproducibility and is of no value in patients not suffering from a monoamine-secreting tumor.¹

Those who claim that spot baseline urinary neurotransmitter testing is valid assert that monoamines cross the blood–brain barrier, are filtered at the glomerulus, and simply excreted into the urine without further renal involvement. They conclude that spot baseline urinary neurotransmitter testing is a valid assay for peripheral and central nervous system neurotransmitter levels.^{6–12}

Spot baseline urinary neurotransmit actesting of norepinephrine, epinephrine, serotonic and doponine is not reproducible from day to day in the same subject therefore, this type of testing is not valid. An intelligenumber of assays performed on an infinite number of days over generate an infinite number of differing test results. The following are true, based on the catistics patients and a this writing:

- Spot urinary net stransmitter testing is not a reliable asset to peripheral accentral nervous system function; the majority of serotonin and catecholamine molecules tund in the trine of patients not suffering from a majoramine-creting tumor have never been in the peripher of central nervous system, having been consisted by renal structures
- Spot urinary neurotransmitter testing does not correlate with monoamine neurotransmitter-related disease states in patients not suffering from a monoamine-secreting tumor
- Spot urinary neurotransmitter testing, due to lack of reproducibility, cannot assist the health care practitioner in making informed decisions regarding the choice

Tables 4a, b Serial spot aselir paseline serotonin assays from the same subject, including some of the serotonin data used to determine the serotonin mass ad-pairs to st values found in Table 1, from a previously published paper by the authors. Comparison of serotonin 1 years serotonin 2 same subject (by row) vividly illustrates lack of testing reproducibility. The number of days column is the number of days between urinary sample collection dates

| a) Sort: High-lab serotomin | | | b) Sort: Hig | b) Sort: High-low by Serotonin 2 | | |
|-----------------------------|-------------|-------------|--------------|----------------------------------|-------------|--|
| Days | Serotonin I | Serotonin 2 | Days | Serotonin I | Serotonin 2 | |
| 42 | 98 64 | 179.65 | 272 | 307.07 | 6004.24 | |
| 28 | 5178.39 | 415.45 | 79 | 1159.95 | 5194.81 | |
| 32 | 3309.76 | 1191.05 | 41 | 2451.00 | 4049.95 | |
| 41 | 2451.00 | 4049.95 | 41 | 96.77 | 3655.97 | |
| 98 | 2157.10 | 368.47 | 103 | 9885.65 | 3246.75 | |
| 42 | 1569.16 | 432.35 | 217 | 828.22 | 2275.38 | |
| 79 | 1159.95 | 5194.81 | 204 | 276.97 | 2183.79 | |
| 29 | 1005.58 | 851.43 | 47 | 227.30 | 2000.00 | |
| 217 | 828.22 | 2275.38 | 383 | 60.32 | 1996.24 | |
| 19 | 763.47 | 31.14 | 32 | 3309.76 | 1191.05 | |

Tables 5a, b Serial spot baseline—baseline dopamine assays from the same subject, including dopamine data from a previous study used to determine the dopamine matched-pairs *t*-test values found in Table 1. Comparison of dopamine 1 with dopamine 2 from the same subject (by row) illustrates the lack of test reproducibility. The number of days column is the number of days between urinary sample collection dates

| a) Sort: High-low by dopamine I | | | b) Sort: High-l | igh-low by dopamine 2 | | |
|---------------------------------|------------|------------|-----------------|-----------------------|------------|--|
| Days (n) | Dopamine I | Dopamine 2 | Days (n) | Dopamine I | Dopamine 2 | |
| 46 | 7854.32 | 1884.93 | 41 | 1129.58 | 2891.23 | |
| 41 | 1129.58 | 2891.23 | 98 | 300.37 | 2623.79 | |
| 204 | 1034.63 | 71.76 | 6 | 138.81 | 2504.14 | |
| 28 | 785.00 | 181 | 103 | 164.50 | 2109.03 | |
| 77 | 652.35 | 1288.47 | 46 | 7854.32 | 1884.93 | |
| 27 | 498.23 | 68.80 | 28 | 785.00 | 1806.00 | |
| 58 | 419.82 | 88.41 | 77 | 652.35 | 1288.48 | |
| 168 | 405.20 | 180.51 | 314 | 197.72 | 1220.54 | |
| 28 | 387.64 | 169.78 | 47 | 785.0 | 853.00 | |
| 29 | 372.51 | 208.49 | 383 | 209.88 | 430.71 | |

of amino acids, or the dosing value for intervention with a disease state associated with monoamine neurotransmitters

- Spot urinary neurotransmitter testing, due to lack of reproducibility, does not have a place in clinical practice for identifying biomarkers of peripheral or central nervous system function and disease states
- Spot urinary neurotransmitter testing cannot determine monoamine imbalances that exist in subjects becare results are not reproducible
- Spot baseline monoamine assays cannot see as a prector of expected efficacy once amino and precessors a started due to lack of reproducibility

There is evidence that urinar monor ones, such as norepinephrine reported on 24° cur urine sancles, may be elevated in a specific group of patents with depression. ¹⁵ However, these are group findings, and lo not necessarily translate to individual testing falidity on spot testing due to the lack of reproductivities of the test from day to day in the same subject.

Con usio

This research anderscores the fallacy of the attempt to use spot baseline inary neurotransmitter testing as a potential biomarker in the deatment of patients with presumed monoamine neurotransmitter-related diseases who are not suffering from a monoamine-secreting tumor. Levels of urinary norepinephrine, epinephrine, serotonin, and dopamine, found in the urine on spot baseline testing, differ significantly from day to day in the same subject. Results are not reproducible, so spot baseline urinary neurotransmitter testing in the endogenous state in subjects not suffering from a monoamine-secreting

tumor is of no clinic wals. Health care practitioners need to understand as differ se where electing a form of testing for their care ats. It is hop cannot this writing will spark interest and scrully of the topic, leading to advancement of a solevant scient

Pisclosu e

The outhors eport no conflicts of interest in this work.

Relerences

- Hinz M, Stein A, Trachte G, Uncini T. Neurotransmitter testing of the urine, a comprehensive analysis. *Open Access Journal of Urology*. 2010;2:177–183.
- Smythe G, Edwards G, Graham P, Lazarus L. Biochemical diagnosis
 of pheochromocytoma by simultaneous measurement of urinary
 excretion of epinephrine and norepinephrine. *Clin Chem.* 1992;38:
 486–492.
- 3. Hinz M, Stein A, Uncini T. The dual-gate lumen model of renal monoamine transport. *Neuropsychiatr Dis Treat*. 2010;6:387–392.
- Hinz M. Depression. In: Kohlstadt I, editor. Food and Nutrients in Disease Management. Boca Raton, FL: CRC Press; 2009.
- 5. Hinz M, Stein A, Uncini T. Amino acid responsive Crohn's disease. *Clinical and Experimental Gastroenterology*. 2010;3:171–177.
- Alts J, Alts D, Bull M. Urinary Neurotransmitter Testing: Myths and Misconceptions. Osceola, WI: NeuroScience Inc; 2007.
- Watkins R. Validity of urinary neurotransmitter testing with clinical applications of CSM (Communication System Management) model. Asheville, NC: Sanesco International; 2009. Available at: http://www.neurolaboratory.net/lab/neurolab%20pdf.%20 files/2009%20Urinary%20NT%20White%20Paper.pdf. Accessed November 24, 2010.
- Theirl S. Clinical relevance of neurotransmitter testing. *The Original Internist*. December 2009. Available at: http://www.clintpublication.com/documents/Dec_OI_2009.pdf. Accessed November 24, 2010.
- Sanesco. Neurolab baseline sample report. Available at: http://sanesco. net/images/files/resourcelibrary/baseline_sample_report.pdf. Accessed November 24, 2010.
- Neuroscience. Assessing nutritional imbalances. Available at: https:// www.neurorelief.com/index.php?option=com_content&task=view&id= 131&Itemid=48. Accessed November 24, 2010.

- Kellermann G, Bull M, Ailts J, et al. Understanding diurnal variation. Technical Bulletin Issue 4. Osceola, WI: NeuroScience Inc; January 9, 2004. Available at: https://www.neurorelief.com/index. php?option=com_content&task=view&id=224&Itemid=48. Accessed November 24, 2010.
- Marc D, Ailts J, Ailts-Campeau D, et al. Neurotransmitters excreted in the urine as biomarkers of nervous system activity: validity and clinical applicability. *Neurosci Biobehav Rev.* 2011;35:635–644.
- Trachte G, Uncini T, Hinz M. Both stimulatory and inhibitory effects of dietary 5-hydroxytryptophan and tyrosine are found on urinary excretion of serotonin and dopamine in a large human population. *Neuropsychiatr Dis Treat*. 2009;5:227–235.
- Hughes J, Watkins L, Blumenthal J, Kuhn C, Sherwood A. Depression and anxiety symptoms are related to increased 24-hour urinary norepinephrine excretion among healthy middle-aged women. *J Psychosom Res*. 2004;57:353–358.



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