Blood sampling for metanephrines comparing venipuncture vs. indwelling intravenous cannula in healthy subjects

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Abstract

Background: To diagnose pheochromocytoma or sympathetic paraganglioma, guidelines recommend blood sampling after at least 30 min of supine rest and using an indwelling intravenous cannula is preferred. Although blood sampling by venipuncture is more convenient and cost-effective, it is unknown whether venipuncture affects plasma concentrations of free metanephrines (MNs). We therefore investigated whether there is a difference in plasma concentrations of free MNs collected by venipuncture or by an intravenous cannula.

Methods: We included 22 healthy participants (12 men and 10 women, median age 26 years). We collected blood using an indwelling cannula and venipuncture to determine plasma concentrations of free MNs and catecholamines, and calculated the median of the individually calculated absolute and relative differences.

Results: Plasma concentrations of free MN, normetanephrine (NMN) and epinephrine were higher with blood sampling using venipuncture compared to that when using an indwelling cannula. The median (interquartile range [IQR]) difference was MN 0.020 (–0.004 to 0.040) nmol/L, median percentage difference 20.5% (–2.4 to 35.2%), NMN 0.019 (–0.004 to 0.077) nmol/L, median percentage difference 4.6% (–1.1 to 25.4%) and epinephrine 0.022 (0.007–0.079) nmol/L, median percentage difference 24.9% (7.8–83.3%). When the two sampling conditions were compared, plasma-free 3-methoxytyramine (3-MT), norepinephrine and dopamine concentrations did not differ.

Conclusions: Blood sampling by venipuncture resulted in statistically significant higher concentrations of MN, NMN and epinephrine compared to sampling by means of an indwelling cannula. However, differences were small. For most patients it seems justifiable to collect blood via venipuncture.

Keywords: blood sampling condition; paraganglioma; pheochromocytoma; plasma-free metanephrines.

Introduction

Pheochromocytomas and sympathetic paragangliomas (PPGL) are rare neuroendocrine tumors arising from chromaffin cells of the adrenal gland or sympathetic paraganglia. They can autonomously synthesize and secrete catecholamines (epinephrine, norepinephrine and dopamine). Symptoms and signs of PPGL such as hypertension, headache, tachycardia, sweating and pallor are due mainly to the hypersecretion of these catecholamines, which can lead to severe and potentially fatal cardiovascular complications [1, 2].

Measurement of the free 3-O-methylated metabolites of catecholamines, i.e. metanephrine (MN), normetanephrine (NMN) and 3-methoxytyramine (3-MT), represents the cornerstone of the biochemical diagnosis of PPGL [3]. Determination of plasma concentrations of free MNs has a reported sensitivity of 90–100% and specificity of 79–98% [2].

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One factor that may result in false-positive test results is the method of blood sampling. Previous research has shown that plasma concentrations of free MN and NMN are approximately 30% higher when collected from patients in seated compared to supine positions, due to increased activity of the adrenal medulla and sympathetic nervous system in the sitting position [4]. For this reason, blood sampling after 20–30 min of supine rest is recommended to provide optimal test accuracy. Some researchers challenge the necessity of supine rest, as it is less patient-friendly and possibly less cost-effective [5]. Additional expenses may result from the recommended practice of collecting venous blood through an indwelling intravenous cannula, which requires specially trained personnel. The advice to use an indwelling intravenous cannula originates from the hypothesis that blood collection by direct venipuncture may elicit an acute emotional stress reaction with concomitant release of catecholamines into the circulation, and consequently a rise in plasma concentrations of free MNs. However, as yet no studies have compared how blood sampling via an intravenous cannula or direct venipuncture affects the level of plasma concentrations of free MNs.

We therefore aimed to determine whether a difference in plasma concentrations of free MNs exists between blood collected by direct venipuncture or by an indwelling intravenous cannula.

Materials and methods

Study design and study subjects

We conducted a single-center pilot study at the University Medical Center Groningen, The Netherlands. We enrolled healthy subjects between December 2016 and January 2017. We included at least 20 subjects, with a margin of 10% for possible dropout [6]. Inclusion criteria were age ≥18 years, normal blood pressure (i.e. <140/90 mmHg, without antihypertensive medication) and no documented history of cardiovascular disease (including hypertension, diabetes mellitus, cerebrovascular events, coronary artery disease and peripheral vascular disease). Use of drugs (except oral contraceptives) was not allowed. The Institutional Review Board approved the study protocol as an amendment of the Salivary, Plasma Metanephrines and Anxiety levels in Pheochromocytomas (STRESS) study, registration number NTR5066. All subjects gave written informed consent.

Methods

Blood samples were collected in the non-fasting state. Subjects were not allowed to smoke or drink caffeine-containing beverages for at least 12 h in advance. Blood pressure was measured in a seated position using an automated oscillometric device. We inserted an 18-gauge intravenous cannula (B. Braun Introcan Safety®) in the antecubital vein, with the subject in the supine position. After 30 min, a blood sample was collected in supine position via the indwelling intravenous cannula, and then as quickly as possible, in less than 5 min, we collected a second blood sample via direct venipuncture in the contralateral arm. All samples were collected using a Becton Dickinson Vacutainer® system with 10 mL EDTA coated tubes (both 1×10 mL BD Vacutainer EDTA K_2E). Blood samples arrived at the laboratory within 60 min after withdrawal and were immediately centrifuged at 2500×*g* for 11 min, after which the plasma was transferred into a cryovial (Sarstedt®) and stored at −80 °C until assessment.

Biochemical analysis

Plasma concentrations of free MNs and catecholamines were determined using high-pressure liquid chromatography-tandem mass spectrometry (LC-MS/MS) with online solid-phase extraction, essentially as described by de Jong et al. [7] and van de Merbel et al. [8]. Dopamine-HCl, norepinephrine, epinephrine, 3-methoxytyramine, DL-metanephrine-HCl, DL-normetanephrine-HCl and L-DOPA-d3, all of analytical purity, were purchased from Sigma Aldrich (MI, USA). Stable deuterated isotopes for dopamine-d4-HCl, norepinephrine-d6-HCl and epinephrine-d3 were purchased from CDN Isotopes (Pointe-Claire, Canada), 3-methoxytyramine-d4-HCl and DL-metanephrine-d3-HCl from Cambridge Isotopes (MA, USA) and DL-normetanephrine-d3-HCl from Medical Isotopes (NH, USA). All samples were determined in one assay run. Intra-assay coefficients of variability for both plasma concentrations of free MNs and catecholamines were <5%. To show the accuracy of the method, samples from the quality assurance program (QAP) for plasma-free MNs of the Royal College of Pathologists of Australasia (RCPA) were analyzed. From Survey 2019, six samples were analyzed. They revealed excellent agreement with target values reported by the RCPAQAP. Errors ranged from −8.3 to 4% for 3-MT, from −12 to 3.7% for NMN and from −6.6 to 6.5% for MN.

Statistical analysis

Data are presented as medians with interquartile ranges (IQRs) or mean with ±standard deviation (SD). The differences (expressed as the absolute concentration and as a percentage) between the blood sampling conditions were calculated within each subject as the value obtained using sampling by direct venipuncture minus the value obtained using the indwelling intravenous cannula. We then calculated the median of these within absolute and percentage differences. The Wilcoxon signed-rank test was used to statistically test the differences in MNs and catecholamines found when comparing the two sampling methods.

To calculate the possible impact on diagnostic specificity, we used the open-access database of Eisenhofer et al. [9] with 2056 patients screened for PPGL, including 236 in whom PPGL was detected, using an indwelling intravenous cannula for blood sampling. The concentrations of plasma-free MNs in patients in whom a PPGL was excluded were adjusted based on the difference observed in the present study after sampling by means of direct venipuncture.

Specificity was calculated based on the percentage of true-negative over the total of true-negative plus false-positive test results in patients without PPGL. Analysis was performed using SPSS version 23. A two-sided p-value of ≤0.05 defined statistical significance.

Results

Subjects

The study group consisted of 22 healthy subjects, 12 men and 10 women, with a median age of 26 (IQR 22–28, range 19–51) years. The mean (±SD) systolic blood pressure was 121 ± 10 mmHg and mean diastolic blood pressure was 75 ± 8.0 mmHg.

Plasma concentrations of free metanephrines

As shown in Table 1 and Figure 1A, the plasma concentrations of free MN and NMN were higher after collection via direct venipuncture than via the indwelling intravenous cannula: MN 0.144 (0.116–0.188) nmol/L vs. 0.133 (0.092–0.167) nmol/L, respectively (p = 0.006) and NMN 0.397 (0.311–0.440) nmol/L vs. 0.344 (0.268–0.422) nmol/L, respectively (p = 0.015). Plasma concentrations of free 3-MT did not differ between these two sampling methods. Values for each healthy subject are shown in the Supplementary file. The median of the individually calculated differences between the plasma concentrations collected by direct venipuncture and by indwelling intravenous cannula was 0.020 (–0.004 to 0.040) nmol/L for plasma-free MN, and 0.019 (–0.004 to 0.077) nmol/L for plasma-free NMN. The median of the individually calculated percentage differences was 20.5% (–2.4 to 35.2%) for plasma-free MN, and 4.6% (–1.1 to 25.4%) for plasma-free NMN. In nine out of 22 subjects, the differences between the sampling conditions were lower than the intra-assay coefficients of variations of plasma MN, NMN and 3-MT.

Plasma concentrations of catecholamines

The plasma concentration of epinephrine was significantly higher after sampling by direct venipuncture than by indwelling intravenous cannula: 0.131 (0.087–0.164) nmol/L vs. 0.081 (0.065–0.127) nmol/L, respectively (p = 0.002) (Table 1, Figure 1B). Plasma concentrations of norepinephrine and dopamine did not differ between collection methods. The median of the individually calculated absolute difference for plasma epinephrine was 0.022 (0.007–0.079) nmol/L, and the median of the individually calculated percentage differences was 24.9% (7.8–83.3%). In seven subjects, the difference between the sampling conditions was lower than the intra-assay coefficients of variability of plasma epinephrine, norepinephrine and dopamine measurements.

Impact on specificity

Plasma concentrations of free MNs according to the extensive open-access database of Eisenhofer et al. [9] were adjusted to the observed increment of plasma MN of 20.5% and NMN of 4.6%, after sampling by venipuncture. The number of false-positive results was 140, and the number of true-negative results was 1680, which resulted in a specificity of 1680/1820 × 100 = 92.3%. The number of false-positive results provided by Eisenhofer et al. was 93, and their number of true negatives was 1727, and thus a specificity of 1727/1820 × 100 = 94.9%, which is higher compared to 92.3% (p = 0.000) [9].

Table 1: Median plasma concentrations of free metanephrines and catecholamines in blood samples collected via an indwelling intravenous cannula and direct venipuncture, and the median (IQR) of individual differences.

	IV cannula	Venipuncture	Median difference venipuncture – IV cannula	p-Value
MN, nmol/L	0.133 (0.092–0.167)	0.144 (0.116–0.188)	0.020 (–0.004 to 0.040)	0.006
NMN, nmol/L	0.344 (0.268–0.422)	0.397 (0.311–0.440)	0.019 (–0.004 to 0.077)	0.015
3-MT, nmol/L	0.012 (0.008–0.016)	0.014 (0.010–0.016)	0.000 (–0.004 to 0.004)	0.910
Epinephrine, nmol/L	0.081 (0.065–0.127)	0.131 (0.087–0.164)	0.022 (0.007–0.079)	0.002
Norepinephrine, nmol/L	2.043 (1.619–3.020)	1.932 (1.711–2.785)	–0.024 (–0.353 to 0.203)	0.506
Dopamine, nmol/L	0.075 (0.057–0.124)	0.069 (0.058–0.136)	0.001 (–0.009 to 0.013)	0.519

[a]Individual differences are calculated (venipuncture – IV cannula); subsequently, the median of these differences is determined. MN, metanephrine; NMN, normetanephrine; 3-MT, 3-methoxytyramine; IV, intravenous; IQR, interquartile range.

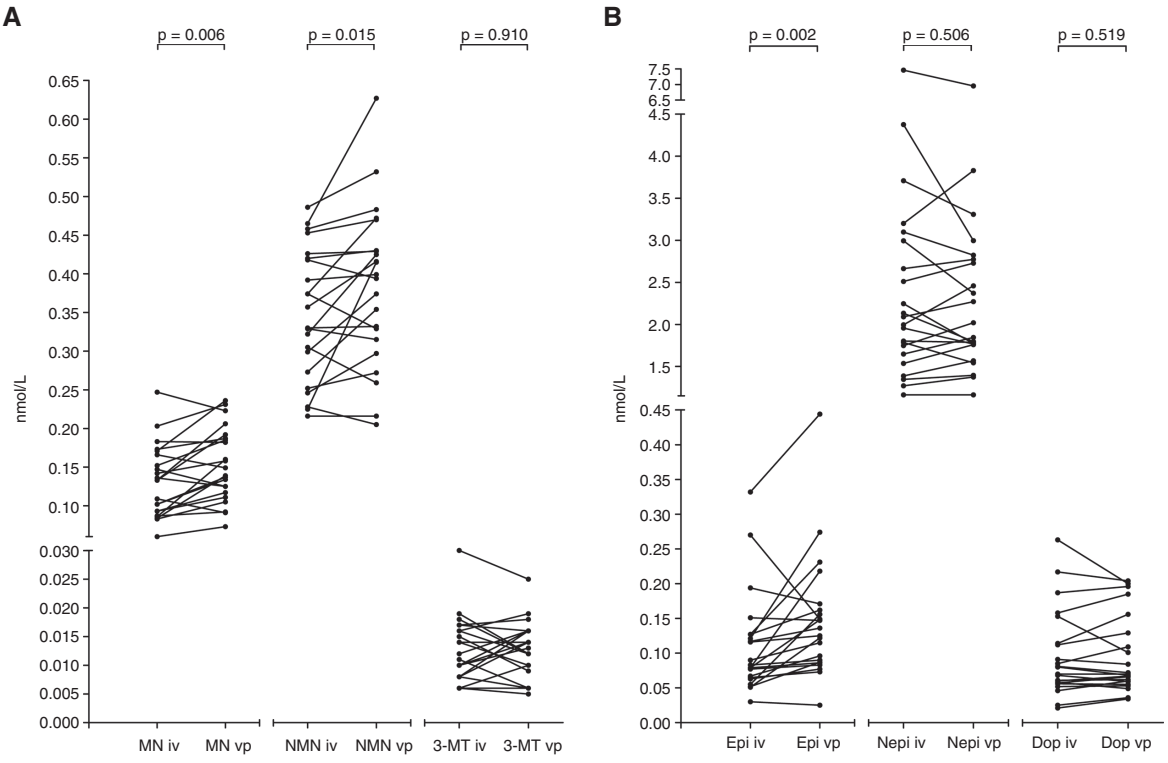

Figure 1: Plasma concentrations.
(A) Plasma concentrations of free metanephrines in blood samples collected via an indwelling intravenous cannula (iv) or via venipuncture (vp). (B) Plasma concentrations of catecholamines in blood samples collected via an indwelling intravenous cannula (iv) or via venipuncture (vp). MN, metanephrine; NMN, normetanephrine; 3-MT, 3-methoxytyramine; Epi, epinephrine; Nepi, norepinephrine; Dop, dopamine.

Discussion

Blood sampling by direct venipuncture resulted in slightly, but significantly, higher plasma concentrations of epinephrine, MN and NMN in healthy subjects than did sampling by an indwelling intravenous cannula. In contrast, plasma concentrations of norepinephrine, dopamine and 3-MT did not differ between these two blood sampling procedures.

The specific influence of the blood sampling method on the measurement of plasma catecholamines and free MNs has not previously been studied. Previous studies on the effects of blood sampling conditions on plasma MNs reported on the combined effects of body position, preceding rest and method of blood sampling [10, 11]. However, these studies do not allow for any conclusions as to the comparative effects of direct venipuncture vs. an indwelling intravenous cannula [10, 11].

One would expect that the acute stress response, with stimulation of the sympathico-adrenal axis, elicited by direct venipuncture would result in higher plasma concentrations of catecholamines and MNs, compared to sampling via an indwelling intravenous cannula. This appeared to be the case for epinephrine MN and NMN, but remarkably, the higher plasma NMN concentration after direct venipuncture was not accompanied by an elevated plasma norepinephrine concentration. Both epinephrine and norepinephrine are taken up rapidly at neuronal and extra neuronal sites [12]. Unlike epinephrine, however, the uptake of norepinephrine has been demonstrated to be inversely related to the blood flow in the forearm [13]. During an acute stress response, the forearm blood flow increases, thereby enhancing the local removal of norepinephrine from the circulation [14]. Moreover, the increased blood flow will also dilute any locally released norepinephrine. Our findings are supported by Goldstein et al., who showed that mental stress evoked a generally increased sympathetically mediated norepinephrine release, which was accompanied by an increase of the norepinephrine concentration in arterial but not in venous blood samples [15].

Collecting blood via direct venipuncture could have a negative impact on test specificity. Using the dataset of Eisenhofer et al., we showed that the specificity was only slightly decreased, from 94.9% to 92.3%, after adjusting the plasma concentration of free MN and NMN by 20.5% and

4.6%, respectively, in the patients without PPGL [9]. This is a small decrease, which could be a logical consequence because we compared our venipuncture results with reference values established with indwelling cannula from the database of Eisenhofer et al. [9]. This increase of false-positive results was statistically significant. In case of mildly elevated plasma MNs in a patient with a low pretest probability for the presence of PPGL, one could choose to repeat the measurement after blood sampling through an indwelling cannula [16]. Based on our results we cannot draw conclusions regarding accuracy or sensitivity, although it was not our intention to verify the accuracy. Similar to blood collection in a seated position with blood sampling via direct venipuncture, more false-positive results are to be expected and the need for follow-up with sampling via an indwelling cannula will be increased.

More importantly, in clinical care, physicians should use the upper cut-offs of reference intervals established with blood sampling under the same sampling conditions as used in patients. A change in the sampling condition thus also should lead to the use of new interval ranges. Blood collection by venipuncture has several advantages, such as lower costs and the lack of need for specially trained personnel. Blood sampling using an indwelling intravenous cannula might be advisable for selected patients, e.g. those with a phobia of needles. Measuring salivary cortisol 1 day before and prior to the venipuncture might help to identify these patients.

This study has a few limitations. The study subjects were normotensive and relatively young and it remains to be established whether our results can be extrapolated to elderly or hypertensive subjects. In particular, it is unclear whether the age dependency of plasma NMN concentration might affect the results [17]. Another potential limitation is that the two blood sampling methods were obtained from different arms and with a time interval less than 5 min. It is, however, unlikely that this difference will have affected our findings.

In conclusion, plasma epinephrine, free MN and NMN concentrations are higher in blood collected via direct venipuncture than via an indwelling intravenous cannula. However, as the differences are minimal, and because of economical and practical advantages, one might choose to use direct venipuncture instead of an indwelling cannula.

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