Preanalytical considerations and out-patient vs in-patient tests of plasma metanephrines to diagnose pheochromocytoma

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Abstract

Context: Sampling of blood in the supine position for diagnosis of pheochromocytoma and paraganglioma (PPGL) results in lower rates of false-positives for plasma normetanephrine than seated sampling. It is unclear how in-patient *versus* out-patient testing and other preanalytical factors impact false-positives.

Objective: Identify preanalytical precautions to minimize false-positive results for plasma metanephrines.

Design: Impacts of different blood sampling conditions on plasma metanephrines were evaluated, including out-patient *versus* in-patient testing, sampling of blood in semi- versus fully recumbent positions, use of cannulae *versus* direct venipuncture and differences in outside temperature.

Setting: Ten tertiary referral centers

Patients: 3147 patients tested for PPGL, including 278 with and 2869 without tumors.

Interventions: None.

Outcome measures: Plasma metanephrines and rates of false-positive results.

Results: Out-patient rather than in-patient sampling resulted in 44% higher plasma concentrations and a 3.4-fold increase in false-positive results for normetanephrine. Low temperature, a semi-recumbent position and direct venipuncture also resulted in significantly higher plasma concentrations and rates of false-positive results for plasma normetanephrine than alternative sampling conditions, though with less impact than out-patient sampling. Higher concentrations and rates of false-positive results for

plasma normetanephrine with low than warm temperatures were only apparent for out-patient sampling. Preanalytical factors were without impact on plasma metanephrines in patients with PPGL.

Conclusions: Although in-patient blood sampling is largely impractical for screening patients with suspected PPGL, other pre-analytical precautions (e.g., cannulae, warm testing conditions) may be useful. In-patient sampling may be reserved for follow-up of patients with difficult to distinguish true- from false-positive results.

Key words: paraganglioma, normetanephrine, preanalytics, false-positives, temperature, venipuncture

Introduction

Since excessive catecholamine secretion from a pheochromocytoma or paraganglioma (PPGL) can result in dangerous increases in blood pressure it is important to consider these neuroendocrine tumors in patients with hypertension who present with other features or conditions associated with enhanced likelihood of disease (1). Hypertension is, however, common and the numerous other features and conditions associated with PPGL are also mostly non-specific so that the tumors are frequently searched for but rarely found (2). Consequently, during biochemical screening for PPGL, the vast majority of positive results are false-positive (3, 4); this complicates identification of these elusive but dangerous tumors.

The biochemical tests of first choice for diagnosis of PPGL include plasma free or urinary normetanephrine and metanephrine (together termed metanephrines), the respective O-methylated metabolites of norepinephrine and epinephrine (5). Additional measurements of methoxytyramine enable detection of dopamine-producing tumors (6), but this is only accurate for plasma since in urine dopamine and its metabolite are largely derived from renal decarboxylation of L-dopa or other precursors (7-9).

Measurements of metanephrines and methoxytyramine in plasma show higher diagnostic accuracy than in urine, particularly for patients at higher risk for PPGL (9). Among the three metabolites, normetanephrine is the most critical since this is the metabolite most consistently increased (9). Nevertheless, findings of increases in plasma metanephrine or methoxytyramine can improve the positive predictive value of test results and provide added diagnostic value for identifying tumors that produce epinephrine and/or dopamine (10). To minimize false-positive test results, upper cut-offs of reference intervals for metanephrine and methoxytyamine are preferably set well above the 97.5 percentiles of reference populations, whereas for normetanephrine it is preferable to set upper cut-offs lower in order to minimize false-negative results (11). Age-specific reference intervals for normetanephrine help to further minimize false-positive results (11, 12); nevertheless, false-positive results for this metabolite remain the most troublesome.

Although use of appropriate reference intervals can minimize both false-negative and false-positive results, it is the implementation of appropriate preanalytical precautions that is of paramount importance for minimizing false-positive results. In particular, sampling of blood after a period of supine rest is critical to reduce sympathetic nerve activity, thereby substantially reducing numbers of false-positive results (12-15). Other factors that may increase false-positive results for plasma normetanephrine include cold *versus* warm outside temperature (16) and use of direct venipuncture *versus* an indwelling cannula for blood sampling (17). For urinary metanephrines rates of false-positive results have been shown to be much higher for in-patient than out-patient urine collections (18). However, that study included critically ill patients and whether measurements of plasma metanephrines are impacted by out-patient compared to the in-patient testing environment remains unclear.

On the basis of the aforementioned considerations the present analysis took advantage of data collected from two prospective studies that involved measurements of plasma free metanephrines and methoxytyramine for blood samples collected at 10 European tertiary referral centers. By taking into account differences in sampling procedures between centers, we assessed the relative contributions of various factors to rates of false-positive results. The overall objective was to identify procedures that may be employed at or after screening to reduce likelihood of false-positive results.

Methods

Clinical protocols and patients

Patients were recruited under the umbrella of the European Network for the Study of Adrenal Tumors (ENSAT) according to two clinical protocols: 1. the Prospective Monoamine-Producing Tumor (PMT) study, as detailed previously (9); and 2. the ENSAT-hypertension (ENSAT-HT) study. The former study focused principally on the use of plasma free metanephrines for diagnosis of PPGL, while the latter study focused more broadly on identification of omics-based signatures for diagnostic stratification of endocrine and primary hypertension. Both studies involved mass spectrometric measurements of plasma metanephrines performed at a single center. All patients provided signed informed consent under either of the two protocols that were approved by Ethics committees at the 10

study centers: 1. Technische Universität Dresden (DR); 2. Ludwig Maximilian University of Munich (MU); 3. Radboud University Medical Centre, Nijmegen (NI), 4. University of Würzburg (WU); 5. National Institute of Cardiology, Warsaw (WW); 6. University of Glasgow (GL); 7. Hôpital Européen Georges Pompidou, Paris (PA); 8. University of Padua (PD); 9. University of Torino (TU); and 10. University Hospital Zurich (ZU).

In total 3147 patients were included in the study, these encompassing 2034 and 1113 respective patients from PMT (2011-2019) and ENSAT-HT (2017-2019) protocols (Table 1). Patients taking drugs known to increase plasma metanephrines (e.g., tricyclic antidepressants) were excluded. Among the 10 centers, two (MU & NI) recruited different patients into both protocols. The presence of PPGL in 278 patients was established by histopathological examination of surgically resected tumors or in cases that remained unoperated (e.g., due to metastatic involvement) by combinations of biochemical tests results and functional imaging studies. As described previously (9), exclusion of PPGL for patients enrolled in the PMT protocol involved a combination of follow-up of at least two years, an alternative diagnosis or negative imaging studies. For patients enrolled into the ENSAT-HT protocol, exclusion of PPGL was based on an alternative diagnosis or negative test results by independent biochemical testing for endocrine forms of hypertension.

Preanalytical conditions of blood sampling

Standard operating procedures (SOPs) that required blood samples to be taken after a minimum of 20 minutes of supine rest were provided to all centers, but were not always followed. SOPs also included requirements for samples to be taken after an overnight fast, collections of blood onto ice or cold packs and storage of plasma at -80^{o}C before shipment on dry ice to the central laboratory for analyses.

Patients were admitted for overnight stays and investigated as elective in-patients at two study centers (WW and PA) for the express purposes of testing for secondary forms of hypertension or specifically PPGLs (Table 1). At all other centers blood was collected on an out-patient basis. Sampling was by a previously inserted intra-venous cannula (indwelling i.v.) at three centers (DR, PA, and NI under the PMT protocol) and by direct venipuncture at all other centers. Blood collections were carried out in the supine position at all centers, though at three centers (WU, PA, and NI under

the ENSAT protocol) this involved a semi- rather than a fully recumbent position. The minimum duration in the supine position before blood sampling varied considerably between centers from 5 to 60 min.

Data on out-side temperatures on specific days of sampling at each study center were retrieved according to location from the meteostat.net database. The module "meteostat", available with the package installer "pip", with its functions "Stations", "Point" and "Daily" was used to write a program in python (https://www.python.org) to enable automated retrieval of this information. In cases where the study location was not part of the database or did not deliver sufficient information, the coordinates were used to gather temperature data from surrounding weather stations. For a small proportion of cases (178/3154), temperatures on the precise days of testing could not be retrieved using the program and were instead established using the average temperature at the location during the month of testing.

Measurements of plasma metanephrines and methoxytyramine

Plasma free nometanephrine, metanephrine and methoxytyramine were measured by liquid chromatography with tandem mass spectrometry according to a previously described method (19, 20). Upper cut-offs (UC) of reference intervals for plasma normetanephrine were age-specific as described elsewhere (11), and according to the equation, $UC = 3.792\times10^{-4} \times age^3 + 98.9$, and varied from 99 pg/mL at age 5 to a maximum of 200 pg/mL at age 65 and beyond. Upper cut-offs for metanephrine and methoxytyramine were 84 pg/mL and 17 pg/mL.

Statistics

Statistical analyses were performed using the JMP statistics software package version 15 (SAS Institute Inc, Cary, NC). Univariable analyses employed the non-parametric Wilcoxon test. Multivariable analyses included standard least square and logistic models to evaluate impacts of preanalytical factors and other variables (e.g., age, sex) on plasma metabolites and false-positive results respectively. For standard least square models, logarithmic transformations were used to normalize metabolite data; the Tukey honestly significant difference post hoc test was used for

covariates involving more than two categories (e.g., study centers). Data in figures involving plasma metabolites are provided as geometric least square means corrected for the covariates employed in those models. False-positive results were calculated according to age-specific reference intervals for plasma normetanephrine and other cut-offs for metanephrine and methoxytyramine described above and established according to previously published data (11). False positive rates (FPR) were calculated according to the formula, FPR = FP/(FP+TN), where FP indicates numbers of false positives and TN indicates numbers of true negatives.

Results

Center-related differences in plasma metabolites and false-positive results

As detailed in the supplement (21), plasma concentrations of normetanephrine, metanephrine and methoxytyramine showed considerable variation among patients across centers (Supplemental table 1). This variation appeared related to differences in preanalytics (Table 1). Rates of false-positive results were over 7-fold higher for plasma normetanephrine than metanephrine or methoxytyramine (Table 2). False-positive rates differed markedly according to study center for normetanephrine ($P<0.0001$), only weakly for methoxytyramine ($P=0.0126$) and not at all for metanephrine ($P=0.1296$). In line with center-specific differences in plasma normetanephrine, rates of false-positives for this metabolite were lowest for patients at PA (2.3%) and WW (2.3%) and highest for patients at WU (13.8%) and under the ENSAT-HT protocol at NI (13.4%).

Impacts of preanalytics on plasma catecholamine metabolites

For patients without PPGL, multivariable analyses indicated significantly higher plasma concentrations of normetanephrine for blood sampling according to in-patient *versus* out-patient blood collections, semi-supine *versus* supine positions, by way of an indwelling i.v. *versus* venipuncture and associated with lower than higher outside temperatures (Table 3). Impacts of these variables on the two other metabolites were largely limited to higher plasma concentrations of metanephrine and methoxytyramine according to respective blood sampling by venipuncture *versus* an indwelling

cannula and for out-patient *versus* in-patient collections. Plasma concentration of metabolites showed no differences according to the different preanalytical variables in patients with PPGL.

Among patients without PPGL plasma concentrations of metabolites were positively related to age (P<0.0001) and plasma concentrations of metanephrine were higher (P<0.0001) in males than females (Table 3). In contrast, among patients with PPGL, age was negatively related to plasma normetanephrine (P=0.0028) and methoxytyramine (P=0.0415), but positively related to plasma metanephrine (P=0.0019). Multivariable models confirmed higher plasma concentrations of methoxytyramine (P<0.0001), and to a limited extent metanephrine (P=0.0011), in patients studied under PMT than ENSAT-HT protocols.

For patients without PPGL, largest impacts of preanalytical variables on plasma normetanephrine and methoxytyramine were observed for in-patient *versus* out-patient sampling, as indicated by 41% higher (P<0.0001) plasma concentrations of both metabolites with testing in the out-patient than the in-patient setting (Figure 1, panels A&E). Impacts of other preanalytical variables were only significant for plasma normetanephrine and metanephrine. Plasma concentrations of normetanephrine were respectively 13.3% and 8.5% higher (P<0.0001) with semi- than fully-recumbent sampling and outside temperatures below compared to above 13.8^{o}C (Figure 1, panels B&C). Plasma concentrations of normetanephrine and metanephrine were also respectively 5.6% higher (P=0.0063) and 17.3% higher (P<0.0001) according to collections of blood by venipuncture than by an indwelling cannula (Figure 1, panels D&F).

As indicated in supplemental figure 2 (21), there was considerable overlap in plasma concentrations of normetanephrine among patients with and without PPGL. The extent of overlap and proportions of false-positive results were increased by higher plasma concentrations of normetanephrine in the group of patients without PPGL.

Impacts of preanalytics on false-positive results

In the line with the above considerations, multivariable logistic analyses indicated significant impacts of out-patient *versus* in-patient testing on rates of false-positives for plasma normetanephrine and

methoxytyramine, as well as other impacts on false-positives for normetanephrine with semi- *versus* fully-recumbent sampling, use of venipuncture *versus* an indwelling i.v. and low temperatures (Table 4). Odds ratios indicated a 3.7-fold higher probability of false-positives for plasma normetanephrine with out-patient than in-patient sampling, a result in line with univariable analysis indicating a 7.8% false-positive rate for out-patient sampling that was 3.4-fold higher (P<0.0001) than the 2.3% false-positive rate for in-patient sampling. Odds ratios also indicated 2.4-fold higher probability of false-positives for plasma normetanephrine for sampling in the semi- *versus* fully-recumbent positions that was in line with univariable analyses that indicated 7.3% false-positive rate for sampling in the semi-recumbent position that was again higher (P=0.0002) than the 4.0% false-positive rate for the fully-recumbent position. Similarly, odds ratios of 1.65 for sampling by venipuncture *versus* an indwelling i.v. and of 1.70 for sampling at temperatures lower than 13.8^{o}C were in agreement with higher rates of false positives by venipuncture (5.7% vs 3.2%, P=0.0035) and lower temperatures (6.3% vs 3.8%, P=0.0020).

Impacts of temperature relative to in-patient *versus* out-patient testing

When data were examined separately for patients tested as out-patients *versus* in-patients, significant impacts of outside temperature on both plasma concentrations and false-positive results for normetanephrine were only observed for out-patient sampling (Figure 2). For out-patient sampling, the linear regression equation indicated a 25% increase in plasma normetanephrine for a 30^{o}C drop in temperature compared to at most a 9% drop in patients sampled as in-patients. Similarly, false-positive test results for the same temperature drop indicated an increase from 3.8% to 11% at 0^{o}C for out-patient sampling compared to an increase from 1.5% to only 3.0% at 0^{o}C for in-patient sampling. Thus, the difference in rates of false-positive results for in-patient *versus* out-patient sampling was most pronounced at cold temperatures and minimal at warm temperatures.

Discussion

The present study establishes markedly different rates of false-positive results for plasma metanephrines among different centers that relate to differences in preanalytical blood sampling conditions, including at one center a large increase in false-positive rates that followed changes in

procedures. The analysis provides new data that extends previous studies examining influences on plasma metanephrines of seated blood sampling (12-15), seasonal temperature differences (16, 22, 23), medications (24, 25), venipuncture (15, 17) and physical exercise (15, 26) by clarifying how some of these and other factors can contribute to increases in false-positive results for plasma metabolites.

We specifically establish that plasma concentrations and rates of false-positive results for plasma normetanephrine are markedly lower for blood collected in an in-patient rather than an out-patient setting and further clarify the relative importance of different preanalytical factors on plasma concentrations and false-positive results for the measured catecholamine metabolites. While a fully-recumbent sampling position and use of an indwelling cannula are within the domain of clinical control for out-patient screening, outside temperature is not but as outlined later can be considered at both screening or any necessary follow-up of positive screening results.

The lower plasma concentrations and rates of false-positive results for patients screened for PPGL in an in-patient *versus* an out-patient setting can in part be explained by the more extended rest associated with the former situation than the often busy, rushed and potentially stressful nature of out-patient blood draws. The findings that outside temperature was inversely related to plasma concentrations and false-positive results for normetanephrine, but not metanephrine are in agreement with previous reports of seasonal variations in normetanephrine and false-positive results (16, 22). Our additional findings that this influence is considerably weakened for patients tested in an in-patient *versus* an out-patient setting suggests that acclimation of the sympathetic nervous to warmer inside temperatures over a longer period may contribute to the reduced plasma concentrations and rates of false-positive results in an in-patient compared to an out-patient setting. Thus, on colder days a longer period of acclimation to warmer indoor temperatures might be considered before blood sampling for out-patient screening or during follow up of previously positive test results.

Although the observation of higher rates of false-positive results for out-patient *versus* in-patient settings might be expected, this finding should also be considered in light of another report that highlighted paradoxically higher rates of false-positive results for urinary metanephrines in

hospitalized patients compared to those screened for PPGL in an out-patient setting (18). Findings in that study of urinary outputs of metanephrines that were often indistinguishable between patients with and without PPGL are, however, explained by the seriously ill nature of many of these patients, including some in intensive care units. As documented previously (27), such situations can considerably confuse interpretation of biochemical tests of catecholamine excess. In contrast to the above studies involving acutely ill hospitalized patients, the present study largely involved patients screened for PPGL on the basis of the usual indications for clinical suspicion: hypertension, signs and symptoms of catecholamine excess, findings of an incidentaloma and risk of disease due to previous history or hereditary condition (28). Such patients when screened for PPGL are less likely to have an activated sympathoadrenal system than those who are acutely ill.

Among patients with positive results for plasma normetanephrine that are not high enough to clearly indicate a PPGL, it can be useful to employ the clonidine suppression test for follow-up confirmation or exclusion of disease (24). Of relevance to the present report, it was recently shown in two independent studies that about half of all patients who undergo this test have normalized plasma concentrations of normetanephrine on the day of testing before administration of clonidine (29, 30). These findings may now be explained by the rest and sympathoadrenal acclimation associated with the extended in-patient setting in one of the studies (29), and the more usual day-care setting for the other study (30). Thus, simply bringing patients back for day-care follow-up measurements of plasma metanephrines may be sufficient to exclude a PPGL in patients with borderline positive test results.

Decreased concentrations and false-positive results for plasma normetanephrine with blood sampled via an indwelling cannula *versus* venipuncture is in agreement with small but significant decreases observed previously (17). We now show that this extends to plasma metanephrine. Although most metanephrine is produced within adrenal chromaffin cells independently of secreted epinephrine, a small proportion is produced from extra-adrenal metabolism of epinephrine after secretion (31). Since the stress associated with venipuncture results in increased plasma catecholamines (32), it is possible that lowered plasma normetanephrine and metanephrine associated

with sampling via an indwelling cannula reflects lower levels of stress-related sympathoadrenal activation with this procedure than with a needlestick.

Although the present analysis did not include comparisons of seated *versus* supine sampling, it is already well established that seated sampling results in unacceptably high rates of false-positive results for plasma free metanephrines (12, 13). Therefore, guidelines mandate sampling in the supine position (5). Nevertheless, supine sampling can be inconvenient or not possible for out-patient settings at some centers (33, 34). Indeed, while sampling in the present study was carried out supine, the nature of this did not follow SOPs at some centers. Sampling at three centers involved a semi- rather than fully-recumbent position, including one center that contributed patients to both protocols and used only a five-minute rest period for the protocol with semi-recumbent sampling. This is little better than sampling in the seated position; combined with use of venipuncture, the departure from SOPs for that center was likely responsible for the 49% higher plasma concentrations and 4.6-fold higher rates of false-positives for normetanephrine in patients studied under one than the other protocol.

The findings among patients without PPGL of higher plasma concentrations of metanephrine in males than females and of positive relationships of metabolites with age are established (11). The reciprocal age-related decreases in plasma normetanephrine and methoxytyamine in patients with PPGL, and increases in metanephrine, may appear surprising but are in agreement with higher ages of patients with pheochromocytoma that produce epinephrine and predominance of other tumors at younger ages (35).

This study has some limitations. The differences in methoxytyramine between the two protocols may reflect improved analytical accuracy in measurements over the successive time periods of the two studies associated with recognised difficulties in measuring the particularly low normal plasma concentrations of this metabolite (36). This confounder was nevertheless accounted for by inclusion in multivariable analyses. A strength is that the data were derived from prospective multicenter studies that allowed for confirmation and exclusion of PPGL. Nevertheless, the studies were not actually designed to investigate preanalytical influences on plasma metanephrines. Rather the results reflect a retrospective analysis and as such the present study suffers many of the same limitations of

retrospective studies. Thus, we did not include subjects as their own controls for a statistically more powerful paired approach to assess impacts of preanalytics. We also did collect precise time periods that each patient was maintained in the supine position before blood collections and we could not clarify other possible preanalytical influences, such as medications, or possible departures from SOPs for processing blood and storage of plasma. Also, the study did not address whether any of the preanalytical variables might be useful for acutely ill hospitalized patients (18, 27).

Although preanalytical precautions are likely of minimal relevance in acute cases of severe illness or hypertensive emergencies, the results of this study nevertheless do clarify the importance of such precautions in the vast majority of patients tested for a PPGL using plasma metanephrines. At initial screening it remains important that blood collections should be carried out after a minimum of 20 minutes in the fully supine position, ideally with patients as comfortable as possible. Additional use of an indwelling cannula for blood collections, and on cold days a prolonged warm indoor environment, may be also useful to achieve normalized plasma concentrations of metanephrines. While screening carried out on an in-patient basis is impractical and too costly for most centers, this or possibly blood collections in a day care environment along with other precautions may offer options for follow-up of patients in who initial screening yields difficult to distinguish true- from false-positive results.

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Data Availability: The data sets generated during and/or analyzed during the present study are publicly available at https://doi.org/10.5281/zenodo.6624878.

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Figure legends

Figure 1. Bar graphs of plasma concentrations of normetanephrine are shown for in-patient *versus* out-patient studies (A), for blood samples collected in the fully- *versus* semi-recumbent positions (B), at outside temperatures of higher *versus* lower than 13.8°C (C) and for sampling with an indwelling i.v. *versus* direct venipuncture (D). Bar graphs are also shown for plasma concentrations of methoxytyramine with blood samples collected in-patient *versus* out-patient studies (E) and of metanephrine for sampling with an indwelling i.v. *versus* direct venipuncture (F). All results are shown as least square geometric means with confidence intervals. The cut-off of 13.8°C for higher than lower temperatures was selected according to the Youden index for false-positive results for plasma normetanephrines.

Figure 2. Relationships of outside temperature with plasma concentrations (A) and false-positive results (B) for normetanephrine in patients studied in an out-patient setting (●) *versus* an in-patient setting (▲). Data were derived from the 2869 observations after stratification into 12 equally sized groups of 238-240 observations according to 12 temperature ranges from respective minimum to maximum day temperatures of -14.6°C to 37.1°C. Results represent means of temperatures for each range plotted against respective geometric means and means for plasma concentrations and false-positive rates for normetanephrine.

Table 1. Preanalytical blood sampling conditions and numbers, sex distributions, and ages of patients with and without PPGLs according to study and center

Study	PMT					ENSAT-HT						
Center	DR	MU	NI	WU	WW	GL	MU*	NI*	PA	PD	TU	ZU
Preanalytics												
Out vs In-patient	OUT	OUT	OUT	OUT	IN	OUT	OUT	OUT	IN	OUT	OUT	OUT
Sampling	IV	VP	IV	VP	VP	VP	VP	VP	IV	VP	VP	VP
Recumbency**	Fully	Fully	Fully	Semi	Fully	Fully	Fully	Semi	Semi	Fully	Fully	Fully
Minimum time**	30	15	20	20	30	20	15	5	15	60	20	20
Patients without PPGL												
Number	269	207	99	246	975	50	118	134	478	125	141	27
Sex (F)	136	123	53	116	463	24	81	72	192	33	59	13
Age (median)	57	54	54	53	52	56	51	52	49	50	50	49
Patients with PPGL												
Number	42	45	16	59	76	1	4	5	21	6	2	1
Percent of total†	13.5	17.9	13.9	19.3	7.2	2.0	3.3	3.6	4.2	4.6	1.4	3.6
Sex (F)	24	30	7	28	45	0	3	3	7	4	2	1
Age (median)	48	53	49	53	49	25	59	49	52	37	51	46

Abbreviations: DR, Dresden; MU, Munich, NI, Nijmegen, WU, Wurzburg; WW, Warsaw; GL, Glasgow; PA, Paris; PD, Padua; TU, Turino; ZU, Zurich; Out, Out-patient; In, In-patient; IV, indwelling intravenous cannula; VP, venipuncture. * MU and NI participated in both PMT and ENSAT-HT studies with contributions of different patients to each study. **All patients were sampled in the supine position, but at three centers this involved the semi-recumbent rather than the fully-recumbent position with considerable variation in periods of recumbency, which are shown as minimum times recumbent in minutes. †Percent of total patients represents the number of patients with PPGL as a percent of all patients with and without PPGL.

Table 2. False-positives (FP) and false-positive rates (FPR) according to study center

	Normetanephrine		Metanephrine		Methoxytyramine		All metabolites	
Center	FP/Total	FPR	FP/Total	FPR	FP/Total	FPR	FP/Total	FPR
PMT								
DR	13/269	4.8%	4/269	1.5%	1/264	0.4%	18/269	6.7%
MU	12/207	5.8%	3/207	1.4%	5/198	2.5%	20/207	9.7%
NI	3/99	3.0%	0/99	0.0%	1/98	1.0%	4/99	4.0%
WU	34/246	13.8%	3/246	1.2%	5/245	2.0%	42/246	17.1%
WW	22/975	2.3%	4/975	0.4%	3/967	0.3%	29/975	3.0%
ENSAT-HT								
GL	4/50	8.0%	0/50	0.0%	1/50	2.0%	5/50	10.0%
MU*	6/118	5.1%	0/118	0.0%	0/118	0.0%	6/118	5.1%
NI*	18/134	13.4%	2/134	1.5%	1/134	0.7%	21/134	15.7%
PA	11/477	2.3%	0/477	0.0%	0/477	0.0%	11/477	2.3%
PD	10/125	8.0%	0/125	0.0%	0/125	0.0%	10/125	8.0%
TU	9/141	6.4%	1/140	0.7%	2/141	1.4%	11/141	7.8%
ZU	1/27	3.7%	0/27	0.0%	0/27	0.0%	1/27	3.7%
Total	143/2868	5.0%	17/2867	0.6%	19/2844	0.7%	179/2868	6.2%

False positive rates were calculated as the numbers of FP divided by the total number of FP and true negatives

Table 3. Multivariable analyses for impacts of preanalytical and other factors on plasma metabolites among patients with and without PPGL

	Normetanephrine		Metanephrine		Methoxytyramine	
	Effect*	P-value	Effect*	P-value	Effect*	P-value
In-patient (In) vs out-patient (Out)						
No PPGL	Out > In	<0.0001	NA	0.6970	Out > In	<0.0001
PPGL	NA	0.3209	NA	0.6566	NA	0.0796
Semi vs fully recumbent						
No PPGL	Semi > Fully	<0.0001	NA	0.8570	NA	0.0520
PPGL	NA	0.5430	NA	0.2747	NA	0.1604
Venipuncture (Veni) vs indwelling i.v.						
No PPGL	Veni > i.v.	0.0063	Veni > i.v.	<0.0001	NA	0.1624
PPGL	NA	0.0799	NA	0.2747	NA	0.1702
Outside temperature on day of blood sampling						
No PPGL	Negative	<0.0001	NA	0.9690	Negative	0.0422
PPGL	NA	0.9240	NA	0.8630	NA	0.6232
Minimum time of recumbency before blood sampling (5-60 min)						
No PPGL	NA	0.7177	NA	0.9086	NA	0.2640
PPGL	NA	0.8078	NA	0.1249	NA	0.2261
Age						
No PPGL	Positive	<0.0001	Positive	<0.0001	Positive	<0.0001
PPGL	Negative	0.0028	Positive	0.0019	Negative	0.0415
Sex						
No PPGL	NA	0.4048	M > F	<0.0001	M > F	<0.0001
PPGL	NA	0.8078	NA	0.2459	M > F	0.0460
Study**						
No PPGL	NA	0.1059	PMT > ENSAT	0.0011	PMT > ENSAT	<0.0001
PPGL	NA	0.4432	NA	0.1411	NA	0.0796

*Effects for categorical binary variables are displayed according to whether concentrations are larger (>) for one than the other variable of the binary pair, whereas for continuous data (i.e., temperature and age) effects are shown according to whether those variables show positive versus negative relationships with plasma concentrations of metabolites. Regression models for relationships of metabolites with age are shown in supplemental figure 1 (21). **Effects according to study reflect whether blood sampling was carried out under the PMT versus the ENSAT-HT (ENSAT) protocols. NA, not applicable since not significant. Analyses were carried out after logarithmic transformation of plasma concentrations of metabolites (pg/mL).

Table 4. Logistic multivariable analyses for impacts of preanalytical factors on false positive results for plasma metabolites among patients without PPGL

	Normetanephrine	Metanephrine	Methoxytyramine	All metabolites
Out-patient vs in-patient				
Odds ratio	3.66 (2.42-5.53)	2.69 (0.74-9.74)	6.15 (1.39-27.05)	3.65 (2.50-5.32)
P-value	P<0.0001	0.1309	P=0.0162	P<0.0001
Semi vs fully recumbent				
Odds ratio	2.35 (1.55-3.56)	0.93 (0.26-3.24)	0.66 (0.22-2.02)	2.00 (1.36-2.95()
P-value	P<0.0001	0.9077	P=0.4639	P=0.0004
Venipuncture vs i.v. cannula				
Odds ratio	1.65 (1.06-2.57)	1.49 (0.44-5.00)	4.24 (0.88-20.44)	1.67 (1.11-2.49)
P-value	P=0.0264	0.5208	P=0.0719	P=0.0126
Outside temperature ≤ 13.8°C				
Odds ratio	1.70 (1.20-2.42)	0.90 (0.34-2.37)	0.79 (0.31-3.188)	1.53 (1.11-2.11)
P-value	P=0.0029	0.8381	P=0.6187	0.0094

Odds ratios are shown with confidence intervals in parentheses according to odds of false-positive results for out-patient compared to in-patient blood collections, a semi- versus fully recumbent sampling position, venipuncture compared to use of indwelling i.v. and lower outside temperatures than 13.8°C, a cut-off selected using the Youden index. The reference population used to establish reference intervals and from this determine false-positive results has been detailed elsewhere (11).

Figure 1

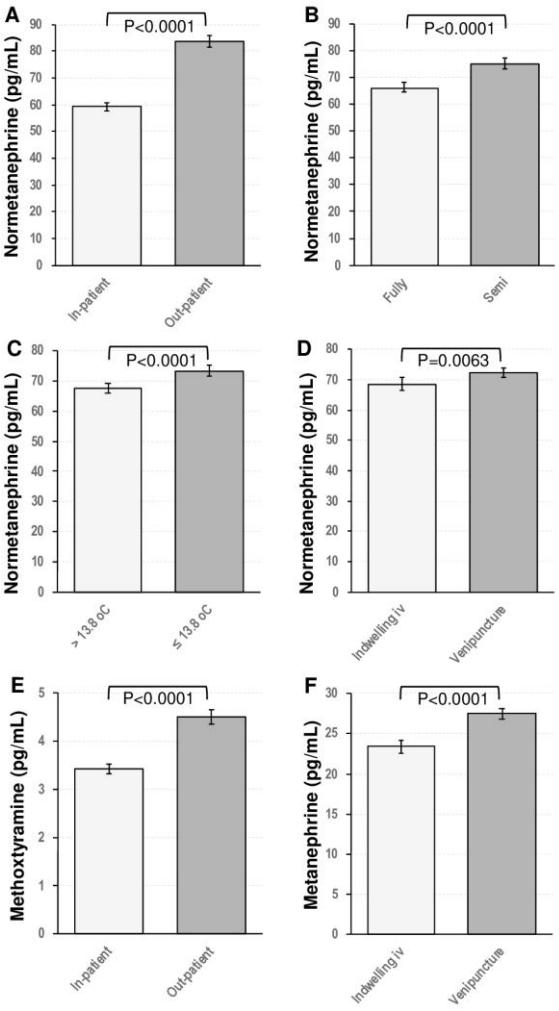

Figure 2