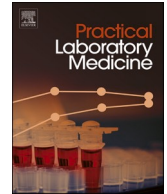




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Registered Report Stage II

Biogenic amine testing in the South African public health care system

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ABSTRACT

Background: Pheochromocytoma, paraganglioma and neuroblastoma are catecholamine secreting neuroendocrine tumours. Biochemical screening for suspected cases of these tumours involves the measurement of catecholamines and their metabolites in either urine or plasma. The South African National Health Laboratory service (NHLS) measures urine fractionated metanephrines (UMF) and normetanephrines (UNF), urine vanillylmandelic acid (UVMA) and urine homovanillic acid (UHVA).

Objectives: To analyse the demographic, biochemical and testing patterns of patients' UMF, UNF, UVMA and UHVA in the NHLS.

Methods: Data from January 2015 to December 2016 for all patients undergoing UMF, UNF, UVMA and UHVA testing was extracted from the NHLS central data warehouse. Neuroendocrine tumours were biochemically diagnosed when results were >2x multiples of the upper reference limits. Multiple testing was defined as ≥2 tests within a 14-day period. Ethnicity was determined through hot-deck imputation.

Results: Biochemically abnormal test results were identified by UMF/UNF measurements in 98.2 % of cases. In 1.8 % of cases, the addition of UVMA resulted in a previously unidentified biochemical positive. Adult white and coloured populations have significantly less biochemically positive UMF results compared to the African population. Multiple testing resulted in discordant results for 12.8 % of UMF and 13.1 % of UNF testing.

Conclusion: UVMA testing for pheochromocytoma and paraganglioma offers little benefit over testing with UMF alone. Requesting consecutive multiple samples is preferred, however, a single 24-h fractionated UMF/UNF is efficient and cost-effective for pheochromocytoma and paraganglioma screening, with further testing recommended when clinically indicated. African individuals are more likely to have raised catecholamines and requires further investigation.

1. Background

Pheochromocytomas (PCC), neuroblastomas (NB) and paragangliomas (PGL) are relatively rare neuroendocrine tumours. Paragangliomas are derived from cells of the neural crest and are located in both the sympathetic and parasympathetic paraganglia [1].

About 80 % of PCCs, which are catecholamine-producing tumours, originate from the adrenal medulla, whereas PGLs are mainly seen in extra-adrenal chromaffin tissue [1,2]. Catecholamines include epinephrine, norepinephrine and dopamine, which are metabolised via the catechol-O-methyltransferase (COMT) pathway into vanillylmandelic acid (VMA), homovanillic acid (HVA), metanephrine and normetanephrine, respectively [3]. These tumours secrete catecholamines causing elevated blood pressure, palpitations, hyperhidrosis and headaches [3]. NBs are tumours of the sympathetic nervous system, commonly found in childhood; they are responsible for an estimated 15 % of all childhood cancer deaths [4]. These tumours are often painless, until they metastasise, however, children may present with weight loss, complaints of feeling full and not wanting to eat, breathing problems, bruising around the eyes, and palpable masses [5,6]. While NBs typically have elevated VMA and HVA, PCCs and PGLs are mostly associated with elevated fractionated metanephrine and normetanephrine (MNF) (7–9). Biochemical analysis is the first line approach for screening of

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<https://doi.org/10.1016/j.plabm.2025.e00457>

Received 18 December 2024; Received in revised form 22 January 2025; Accepted 27 January 2025

Available online 28 January 2025

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these tumours because of the resulting aberrant catecholamine profiles [2].

The most commonly used and recommended tests for biochemical detection of PCCs and PGLs are urine fractionated MNF (UMNF) and plasma free MNF, analysed using liquid chromatography with either mass spectrometric or electrochemical detection methods [2]. VMA and HVA are generally measured in urine using high performance liquid chromatography (HPLC) with electrochemical detection or liquid chromatography and tandem mass spectrometry (LC-MS/MS). The Endocrine Society clinical practice guidelines [2] for PCC and PGL recommend measurement of either urine or plasma MNF for biochemical testing for PCC and PGL. While plasma free MNFs have been suggested as a superior testing method to UMNF [7–9], this has not been conclusively proven, and both methods of screening provide similar levels of specificity and sensitivity [2]. An earlier study carried out by Lenders et al. indicated that plasma metanephrine had a negative predictive value of 100 % in normal patients, however, sampling position needs to be noted and appropriate reference ranges need to be selected [10,11]. Consequently, the use of plasma free MNF results in a high number of false positives [12]. For UMNFs a 24 h collection is recommended, and a creatinine measurement is required to verify completeness of the collection [2].

The sensitivity and specificity of different methods for the biochemical diagnosis of PCCs and PGLs have been extensively investigated [2]. Urine VMAs (UVMA) have been found to be highly specific but not particularly sensitive, whereas UMNF are highly sensitive but poorly specific [7]. It has been noted, however, that the specificity of MNF measurements can be increased by utilising multiples of the upper limit of the reference interval as a diagnostic cut off. MNF results that are greater than two to three times the upper reference limit are unlikely to be false positives [2,13]. It is also less likely to be a false positive result where both metanephrine and normetanephrine are above the reference interval [2]. The appropriate cut offs for diagnosis, however, are not standardised [2,3].

Biochemical testing for neuroblastoma most commonly consists of the measurement of the end products of catecholamine metabolism, namely, HVA and VMA [14,15]. HVA has a sensitivity of approximately 81 % whereas VMA has a reported sensitivity of approximately 82 % and specificities of 96–100 % [14,15]. However, in metastatic disease the sensitivity increases to 99.7 % for HVA and 100 % for VMA [15]. The diagnostic sensitivity has been reported to be similar for both spot and 24-h urine collections [16]. Due to the relatively low sensitivity of urinary HVA and VMA in non-metastatic disease, it is currently being debated whether both analytes should be included in neuroblastoma diagnostic workups [16]. It has been suggested that an extended panel consisting of eight catecholamine metabolites (HVA, VMA, dopamine, 3-methoxytyramine, norepinephrine, epinephrine and MNF) should be used for suspected neuroblastoma diagnosis as the panel has higher accuracy than HVA and VMA alone [16].

Currently, to our knowledge, two studies have looked at the clinical characteristics of PCC in the South African population [17,18]. Of the PCC cases, sporadic tumours accounted for 91 % and 82.9 % in Johannesburg and Durban, respectively. In addition adrenal gland tumours were the most prevalent in both the Johannesburg (61 %) and Durban (69 %) studies [17,18]. Huddle reported that at Chris Hani Baragwanath hospital, Johannesburg, only 54 cases of PCC were documented between 1980 and 2009 [17] and Zorgani and colleagues described 35 cases over a 14 year period (2002–2016) at Inkosi Albert Luthuli Central hospital, Durban [18].

Thirty-four patients from South Africa and 14 from Namibia, diagnosed with NB between 1983 and 1997, showed a patient median age of 18 months irrespective of ethnicity [19]. Interestingly, urinary VMA was elevated in 63 % of children tested [19]. In a study on 52 patients with PGL attending the Tygerberg hospital, it was found that 36 % were found to be associated with a germline mutation in the succinate dehydrogenase complex [20]. In addition, in the South African Afrikaaner population, a Dutch founder mutation consisting of a deletion in exon 3 of the succinate dehydrogenase subunit B, was found to predispose to pheochromocytoma-paraganglioma [21].

Thus, a descriptive retrospective study was undertaken to determine the trends in requests for urine metanephrine (UMF) and normetanephrine (UNF), urine vanillylmandelic acid (VMA) and urine homovanillic acid (UHVA) within the South African parastatal laboratory testing facility, the National Health Laboratory Service (NHLS).

2. Methodology

2.1. Data mining

Data requested from the NHLS data warehouse included the results for all UMF, UNF, UVMA and UHVA tests run by NHLS laboratories over the period January 1, 2015 to December 31, 2016. All data linked with these test results, including patients' folder number, sex, date of birth/age, race, requesting location, creatinine, urine volume, collection period and urine pH was collected. Linked histopathology tests results were also requested.

2.2. Data processing

Raw data was received in a Microsoft Excel 2010 spreadsheet format containing 250 000 entries. Each line of data was reviewed and duplicate results removed, until a final database of approximately 5500 entries was generated.

2.2.1. Requests database

Where the original database included creatinine results with no urine biogenic amine results, the entry was excluded. In addition, external proficiency testing results were excluded thus generating the requests database used for this study. The requests database was used to determine the number of test requests received, requesting institutions and their associated province.

This database was also used to determine the number of multiple requests. A multiple request refers to one or more of the same test authorised within a two week period. Multiple tests were considered discordant if at least one result was greater than 2x the upper

reference limit and at least one result was less than 2x the upper reference limit.

2.2.2. Diagnostic database

To generate the diagnostic database, all data entries for each patient were consolidated into a single entry. Where individual patients had more than one test result for a particular test, the highest result was used to determine whether the patient was biochemically positive for a tumour. The number of repeat and multiple tests performed was recorded prior to consolidating patient data.

As reference ranges used for biochemical diagnosis are age and gender specific and are expressed as a creatinine to biogenic amine ratio, the diagnostic database was further refined to exclude samples with no age, sex or creatinine value.

2.3. Biochemical diagnosis

The ratio of each of the analytes of interest (UMF, UNF, UHVA, UVMA) to creatinine were calculated and compared to the laboratory specific reference ranges. For the purpose of our study, UMF/UNF samples were categorised as biochemically positive for PCC/PGL where the result exceeded 2x the upper reference limit [13]. For UVMA and UHVA the samples were categorised as biochemically positive for NBs if levels exceeded 2x the upper reference limit [7].

All biogenic amine tests were run at either the NHLS laboratory based at Charlotte Maxeke Academic Hospital, the Red Cross Children's Hospital or Nkosi Albert Luthuli Hospital laboratories.

The method used for quantification of UMF and UNF was solid phase extraction followed by HPLC with electrochemical detection. The method used for the quantitation of UHVA and UVMA at the NHLS laboratory based at Charlotte Maxeke Academic Hospital and Inkosi Albert Luthuli hospital include anion exchange chromatography followed by catecholamine separation via HPLC with electrochemical detection. The Red Cross Hospital laboratories prepare UHVA samples using solvent based liquid-liquid extractions followed by derivatisation and subsequent analysis by gas chromatography – mass spectrometry (GC-MS) in single ion monitoring mode.

2.4. Population group imputation

Population group data (ethnicity) was captured in only 3.5 % of the initial data set. Thus, a “Hot Deck Imputation” technique, developed by the National Cancer Registry was used to impute the ethnicities of the patients. This technique compares the surname of an individual of unknown ethnicity to a database of known cases, and then assigns the individual to a population group [22]. This approach correctly classifies 90 % of cases [22].

2.5. Statistical analysis

All statistical analysis were performed on Statistica software (StatSoft, TIBCO Software, California, USA). Continuous data was considered normal when it had a skewness value of between +1 and –1. Gaussian data are presented as means and standard deviation and non-Gaussian data as median and interquartile ranges. To compare the differences between categorical data, a Chi squared test was performed. Gaussian data were compared between groups using either a *t*-test or an ANOVA.

Table 1

Characteristics of adult (≥ 18 years) and paediatric (< 18 years) patients tested for catecholamines in the National Health Laboratory Services between January 1, 2015 and December 31, 2016.

Adult Patients					
	African (n = 2395)	Asian (n = 67)	Coloured (n = 441)	White (n = 520)	p-value
Age (years)	36.0 \pm 12.3	42.9 \pm 16.1	40.2 \pm 15.5	44.4 \pm 16.7	<0.0001
Sex: Female (n (%))	1293 (54.0)	33 (49.3)	254 (57.6)	299 (44.0)	0.223
Male (n (%))	1102 (46.0)	34 (50.7)	187 (42.4)	221 (56.0)	
Biochemically positive UMF (%)	13.3 (184/1387)	7.1 (3/42)	8.1 (26/321)	8.2 (28/343)	0.006
Biochemically positive UNF (%)	18.5 (257/1390)	23.8 (10/42)	17.4 (56/321)	18.4 (63/343)	0.796
Biochemically positive UVMA (%)	3.1 (41/1317)	2.8 (1/36)	2.8 (6/215)	4.8 (12/252)	0.678
Biochemically positive UHVA (%)	1.5 (14/922)	12.0 (3/25)	2.9 (5/172)	4.2 (9/212)	<0.001
Paediatric Patients					
	African (n = 316)	Asian (n = 8)	Coloured (n = 48)	White (n = 51)	p-value
Age (years)	9.2 \pm 6.1	8.5 \pm 5.5	9.5 \pm 6.0	10.6 \pm 6.1	0.494
Sex: Female (n (%))	160 (50.6)	3 (37.5)	24 (50.0)	26 (51.0)	0.908
Male (n (%))	156 (49.4)	5 (62.5)	24 (50.0)	25 (49.0)	
Biochemically positive UMF (%)	4.8 (8/167)	0.0 (0/3)	10.0 (3/30)	9.7 (3/31)	0.941
Biochemically positive UNF (%)	19.8 (33/167)	0.0 (0/3)	26.7 (8/30)	25.8 (8/31)	0.584
Biochemically positive UVMA (%)	13.4 (26/194)	20.0 (1/5)	6.5 (2/31)	6.9 (2/29)	0.515
Biochemically positive UHVA (%)	7.2 (10/138)	50.0 (1/2)	15.8 (3/19)	13.3 (2/15)	0.116

UMF = urine metanephrines; UNF = urine normetanephrines UVMA = Urine vanillylmandelic acid; UHVA = homovanillic acid.

2.6. Ethics clearance

Ethics clearance for this study was received from the Human Research Ethics Committee of the University of the Witwatersrand (clearance certificate: M170218).

3. Results

The NHLS received 3296 test requests for 2409 individuals for UMF/UNF testing for the two-year duration of this study. This averaged to approximately 32 tests per week. In addition, 2546 tests for 2006 individuals for UVMA and 1981 tests for 1563 individuals for UHVA were requested. Results for 82 UMF, 71 UVMA and 52 UHVA were excluded due to missing data.

The province with the largest percentage of test requests was Gauteng (46 %), followed by the Western Cape (16 %) and the Free State (15 %). Only 3 % of requests came from Kwa-Zulu Natal. Multiple UMF/UNF results ($n = 337/336$) were discordant in 12.8 and 13.1 % of cases, respectively (see supplementary information).

In total, 655 individuals out of 3712 (17.9 %) had biochemically positive results based on either UMF, UNF, UVMA, UHVA levels or a combination thereof. The number of biochemical positive results for UMF/UNF, UHVA and UVMA for each ethnic group (imputed data) are reflected in Table 1. The black South African population had a significantly higher percentage of positives for UMF requests as compared to both the white and coloured populations in adults ($p = 0.021$ and $p = 0.023$, respectively), whereas there were no significant differences between ethnic groups for children.

For black South Africans, there were a significantly higher number of biochemically positive results in children (<18 years) compared to adults (Table 1) for UVMA (13.4 vs. 3.1 %; $p < 0.001$) and UHVA (7.2 vs. 1.5 %; $p < 0.001$). Whereas black South African adults had a higher percentage of biochemically positive UMF compared to children (13.3 vs. 4.8 %; $p = 0.002$). For the coloured population, there were significantly more children with positive UHVA compared to adults (15.8 vs. 2.9 %; $p = 0.008$). There were no significant differences in positivity for any of the analytes studied in the white and Asian population between adults and children. Black participants who were biochemically positive for UMF were found to have a significantly younger age than white participants who were biochemically positive (39.5 ± 13.5 vs. 47.2 ± 15.8 years; $p = 0.005$). Similarly, white participants who were biochemically positive for UNF, were significantly older than their black (46.5 ± 14.7 vs. 38.9 ± 13.8 ; $p < 0.001$) and coloured (46.5 ± 14.7 vs. 37.8 ± 13.8 ; $p < 0.001$) counterparts. No significant difference in age between races was found for participants biochemically positive for UHVA or UVMA.

The prevalence of males vs. females who tested positive according to our cut-offs for UMF/UNF, UVMA and UHVA were determined for each ethnic group for both adults and children (Table 2). In the black South African population, there were significantly more adult females than males who were biochemically positive for UVMA (4.8 vs. 2.5 %, respectively; $p = 0.033$). In the adult coloured population there were significantly more males than females who had a positive UNF result (24.6 vs. 12.3 %, respectively; $p = 0.004$). Furthermore, in the black, white, Asian and coloured adult populations, there were no females with a biochemically positive UHVA result (males vs. female: 3.4 vs. 0.0 %; $p < 0.001$; 11.8 vs. 0.0 %; $p < 0.001$, 30.0 vs. 0.0 %; $p = 0.024$ and 7.9 vs. 0.0 %; $p = 0.011$, respectively). In children, no significant differences were found for biochemical positivity rates between sexes for any of the analytes studied, with the exception of UMF in the coloured population where there were significantly more males (23.1 %) with a biochemical positive than females (0.0 %; $p = 0.037$). There were too few Asian children sent for biochemical testing to allow for statistical analysis.

UMF/UNF was requested together with UVMA 723 times. Concordant results were found in 549 (75.9 %) cases. Of the discordant results ($n = 174$; 24.1 %), UVMA alone was positive in 13 cases, whereas in 161 cases either a UMF, UNF or a combination thereof, were positive and the UVMA was negative. If no UVMA was requested, 13 positives (1.8 %) would have been missed.

3.1. Histopathology confirmation

Relevant histopathology was available for only 23 patients. The mean age of participants with histologically confirmed NB ($n = 8$) was 6.1 years compared to 51.0 and 50.7 years for PCC ($n = 4$) and PGL ($n = 11$), respectively ($p < 0.001$).

The histological diagnosis was collated with biochemical test results. The eight individuals diagnosed by histopathology for NB had been tested for either UVMA, UMF, UNF and/or UHVA. Of all histopathology confirmed NBs, only two (25 %) were ever tested for UMF/UNF, both of which had UNF results greater than 6x the upper reference limit. Only one of the histopathology confirmed NB cases was biochemically positive for UVMA (12.5 %; 75 % tested). Of histopathology positive results, 75.0 % were tested for UHVA with 12.5 % of those tested reaching biochemically positive levels. Of all histopathology confirmed NBs, 62.5 % did not show significantly elevated results on any test and were thus biochemically negative at the time of testing.

The histopathology confirmed PGL (11 cases) were screened using UMF/UNF in 81.8 % of cases and of these 55.6 % were biochemically positive. 36.4 % of cases were screened using UVMA, and 36.4 % using UHVA. Both UVMA and UHVA tests were used together to screen 27.3 % of cases. None of the participants (0.0 %) had a biochemically positive UVMA result, whereas one participant (25 %) had a biochemically positive UHVA result.

All participants with histopathology confirmed PCC ($n = 4$) were screened for UMF/UNF. All participants had biochemically positive UNF results, in agreement with the histopathology. Biochemically positive UMF results were in agreement with histopathology results 75.0 % of the time. UVMA and UHVA were screened in 50.0 % of cases ($n = 2$). One hundred percent of UVMA tests and 50 % of UHVA tests were biochemically positive.

Table 2Sex comparison of adult (≥ 18 years) and paediatric (< 18 years) patients tested for catecholamines in the National Health laboratory services between January 1, 2015 and December 31, 2016.

Adult Patients												
	African males (n = 1102)	African females (n = 1293)	P value	Asian males (n = 34)	Asian females (n = 33)	P value	Coloured Males (n = 187)	Coloured females (n = 254)	P value	White males (n = 221)	White females (n = 299)	P value
Age (years)	36.0 \pm 11.8	35.9 \pm 12.8	0.880	40.5 \pm 16.5	45.3 \pm 15.5	0.228	38.6 \pm 14.3	41.4 \pm 16.2	0.063	41.6 \pm 15.4	46.5 \pm 17.3	<0.001
Biochemically positive												
UMF (%)	13.4 _(89/666)	13.2 _(95/721)	0.918	4.3 _(1/23)	10.5 _(2/19)	0.439	11.2 _(15/134)	5.9 _(11/187)	0.085	5.6 _(8/142)	10.0 _(20/201)	0.150
UNF (%)	16.7 _(111/666)	20.2 _(146/724)	0.093	34.8 _(8/23)	10.5 _(2/19)	0.066	24.6 _(33/134)	12.3 _(23/187)	0.004	16.9 _(24/142)	19.4 _(39/201)	0.556
UVMA (%)	4.8 _(24/500)	2.5 _(17/680)	0.033	7.1 _(1/14)	0.0 _(0/22)	0.204	4.2 _(4/95)	1.7 _(2/120)	0.261	5.8 _(5/86)	4.2 _(7/166)	0.572
UHVA (%)	3.4 _(14/406)	0.0 _(0/516)	<0.001	30.0 _(3/10)	0.0 _(0/15)	0.024	7.9 _(6/76)	0.0 _(0/96)	0.011	11.8 _(9/76)	0.0 _(0/136)	<0.001
Paediatric Patients												
	African males (n = 156)	African females (n = 160)	P value	Asian males (n = 5)	Asian females (n = 3)	P value	Coloured Males (n = 24)	Coloured females (n = 24)	P value	White males (n = 25)	White females (n = 26)	P value
Age (years)	9.1 \pm 6.2	9.3 \pm 6.1	0.791	9.8 \pm 5.2	6.2 \pm 6.1	0.400	8.9 \pm 6.5	10.1 \pm 5.6	0.518	8.9 \pm 6.2	12.2 \pm 5.6	0.056
Biochemically positive												
UMF (%)	9.2 _(8/87)	12.5 _(10/80)	0.491	0.0 _(0/1)	0.0 _(0/2)	–	23.1 _(3/13)	0.0 _(0/17)	0.037	0.0 _(0/13)	16.7 _(3/18)	0.211
UNF (%)	17.2 _(15/87)	22.5 _(18/80)	0.394	0.0 _(0/1)	0.0 _(0/2)	–	30.8 _(4/13)	23.5 _(4/17)	0.657	7.7 _(1/13)	38.9 _(7/18)	0.050
UVMA (%)	10.0 _(9/90)	16.3 _(17/104)	0.196	0.0 _(0/4)	100.0 _(1/1)	–	0.0 _(0/17)	14.2 _(2/14)	0.107	13.3 _(2/15)	0.0 _(0/14)	0.157
UHVA (%)	9.7 _(7/72)	4.5 _(3/66)	0.241	0.0 _(0/1)	100.0 _(1/1)	–	11.1 _(1/9)	20.0 _(2/10)	0.596	0.0 _(0/8)	28.5 _(2/7)	0.104

UMF = urine metanephrines; UNF = urine normetanephrines UVMA = Urine vanillylmandelic acid; UHVA = homovanillic acid.

4. Discussion

In this study on biogenic amine testing in the South African public health care system, we found that UMF/UNF measurements were able to identify 98.2 % of biochemically abnormal test results. The addition of a UVMA test was consequently only useful in 1.8 % of cases. The adult African population have significantly more biochemically positive UMF results compared to the white and coloured populations. In addition, we found that when multiple tests were requested for UMF and UNF, 12.8 and 13.1 % of results were discordant.

Our data shows that numerous clinicians request simultaneous measurement of UMF/UNF and UVMA, however in the majority of cases, running only the UMF/UNF would have detected the abnormal urine catecholamine. Thus, it is probable that there is no diagnostic advantage to requesting both tests simultaneously. The Endocrine Society clinical practice guidelines [2] for PCC and PGL recommend measurement of either urine or plasma fractionated metanephrines for biochemical testing for PCC and PGL, but do not advocate the use of UVMA. UVMA is no longer routinely recommended as it has poor sensitivity, despite being highly specific [23]. In 1.8 % of cases, if the UVMA had not been requested, an abnormal result would have been missed as the UMF/UNF was negative. It would be interesting to know if these are PCC/PGLs that would potentially have been missed, had UVMA not been run together with the UMF/UNF. Unfortunately, we do not have the histological diagnosis for these cases to confirm.

The mean age of adult black participants biochemically positive for UMF and UNF was significantly younger than that seen in the white and coloured populations. This needs further investigation, however, it may indicate that black South African participants develop PCC/PGL at an earlier age compared to their white and coloured counterparts. The differences in UMF between Africans compared to the white and coloured populations is difficult to explain. There is a paucity of literature with regards to ethnic differences in both the incidence of neuroendocrine tumours and the levels of catecholamine excretion between different ethnic groups. American data suggests that PCC is rarer in the African population [24]. However, in our data, there was an increased proportion of biochemically positive UMF results in the black population, suggesting a higher incidence of PCC in this group compared to other ethnicities. There may be ethnic differences in catecholamine secretion that could account for the increased levels of metanephrine within the African population. In a South African study, no significant difference in epinephrine and norepinephrine levels between white and African obese women was found [25]. A further explanation may be differences in catechol o-methyl transferase (COMT) levels or activity between different ethnic groups. Such differences have been described by McLeod [26], who found that the high activity variant of COMT is more common in African individuals. However, as both UNF and UMF are generated through COMT activity, and as there was no significant increase in UNF biochemical positives in the black population compared to other ethnic groups, it is unlikely to be the explanation for the increases in UMF positives in the black South African population.

The motivation for three consecutive 24-h urine collections is that PCCs secrete catecholamines episodically [12]. Bettacchioli and colleagues performed a study in order to determine the value of this practice [27]. Out of 182 patients, of which seven had a PCC, six would have been detected using a single urine collection, while all seven were detected using three consecutive 24-h collections. They thus concluded that there is value in the use of three consecutive 24-h collections. The Endocrine Society clinical practice guidelines for PCC and PGL recommend follow up testing in the case of biochemically positive results, but do not mention a recommended number of 24-h collections that are required [2].

It was noted that several institutions requested multiple 24-h collections. All multiple collections were further examined and it was found that 12.8 % of UMF and 13.1 % of UNF multiple tests had discrepant results. We therefore concur with Bettacchioli et al. [27] that multiple collections may offer superior sensitivity. However, in the context of our public health system the benefits of increased diagnostic performance based on testing of multiple samples across the board needs to be carefully weighed up against the increased costs.

Neuroblastoma accounts for approximately 5.7 % of childhood cancers in South Africa [28,29]. Neuroblastoma was also found to be the fifth most prevalent childhood cancer in a study in the rural Eastern Cape [30]. Urine VMA and HVA are recommended for the biochemical screening and diagnosis of NBs [31,32]. This may account for the higher proportion of positive UVMA and UHVA results in our black paediatric population, and the higher number of UHVA in the coloured paediatric population. In the black population, a higher number of biochemical positives for UMF was observed in the adult compared to the paediatric population. It has been reported that the majority of PCC/PGL are diagnosed in adulthood [33,34], therefore this finding is not unexpected.

The M:F ratio in our patients for biochemically positive UHVA/UVMA was 1:1.8 and 1:2, respectively, contradicting a previous epidemiological study of neuroblastoma in Southern Africa which found a M:F ratio of 0.9 [19]. It is possible that our results differed from the study by Hesseling and colleagues, due to different criteria for inclusion. All participants in the Hesseling study had neuroblastoma confirmed using histopathology [19], whereas in our study the ratio is based on biochemical data. The M:F ratio in our patients with biochemical positive results for UMF and UNF was 1: 0.95 and 1: 1.17, respectively. These differ from the ratio found by Zorgani and colleagues (1: 1.5) and Huddle (1:3.2) in South African populations [17,18]. While all of these results show that more females than males have been diagnosed with PCC/PGL, the differences in the ratios may reflect ethnic difference in the populations studied. The patients described by Huddle [17] are all African, and drawn from the areas surrounding Johannesburg, whereas Zorgani [18] reports on patients of various ethnicities from Durban and surrounding areas. Our patients are of various ethnicities and were drawn from the entire country.

Recent guidelines recommend the use of both urine and plasma-free metanephrine measurements [2,35]. There are situations in which plasma-free metanephrines may be preferred, however, the decision should be made based on the availability of the test, ability to meet pre-analytical requirements and clinician experience [35]. Studies have indicated that plasma shows a slight improvement in sensitivity over urine, with both possessing similar specificities [36,37]. However, currently, plasma-free metanephrines are unlikely to be considered as a routine test in the South African public healthcare sector. The superiority of plasma-free metanephrines over

urine metanephrines is not conclusive and when described requires strict adherence to pre-analytical requirements such as resting 30 min prior to sampling and avoiding sympathoadrenal activation [2,10,11,35]. The pre-analytical requirements are difficult to control especially at distal clinics and rural health centres. Thus, plasma metanephrine testing may be used on occasion in the public health care sector i.e. for dynamic function tests and in specified patient populations.

Where possible international guidelines are followed for the testing and diagnosis of PCC, PGL and NBs and as such no changes to policies will be drawn from this subset of data. However, a larger study with good clinical histological correlation would help to identify if international guidelines are suitable in the South African setting.

The main limitation of this study was that we had no histological confirmation for the majority of individuals whose biogenic amines were evaluated. Additionally, the reference ranges were not based on data specific to our population, therefore, no conclusions as to the efficacy of the current reference ranges can be made. Determination of the appropriate cut-offs would require further study. Furthermore, we are unable to determine appropriate, sensitive and specific diagnostic cut offs for PCC, PGL and NB. In addition, race data had to be imputed, and it is therefore possible that some participants were misclassified.

5. Conclusion

In our data set, UVMA and UMF/UNF results concur in over 90 % of samples, which indicates that it may not be necessary to run both tests simultaneously when screening for PCC and PGL. Requesting consecutive multiple samples, whilst preferred, may not be financially feasible in the public health care system. In our setting, a single 24-h fractionated UMF/UNF seems to be the most efficient and cost-effective approach for PCC and PGL screening, with further testing recommended if the results are ambiguous and discordant with the clinical picture.

In addition, these data show an ethnic difference in UMF positivity, indicating that African individuals may be more likely to have raised catecholamine levels. We are unable to confirm if this trend is indicative of PCC due to the lack of histological diagnoses and thus further research is needed.

CRedit authorship contribution statement

D. Legg-E-Silva: Writing – original draft, Formal analysis, Data curation, Conceptualization. **E.M. Cave:** Writing – original draft, Formal analysis, Data curation. **T. Snyman:** Writing – review & editing, Conceptualization. **S. Currin:** Writing – review & editing. **N. Kone:** Writing – review & editing. **K.L. Prigge:** Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We would like to acknowledge Patricia Kellett of the National Cancer Registry (NCR) for race imputation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.plabm.2025.e00457>.

Data availability

The data that has been used is confidential.

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Glossary

COMT: Catechol-O-methyltransferase

MNF: Fractionated metanephrine and normetanephrine

HVA: Homovanillic acid

NB: Neuroblastoma

PGL: Paraganglioma

PCC: Pheochromocytoma

UMNF: Urine fractionated metanephrine and normetanephrine

UNF: Urine fractionated normetanephrines

UHVA: Urine homovanillic acid

UVMA: Urine vanillylmandelic acid