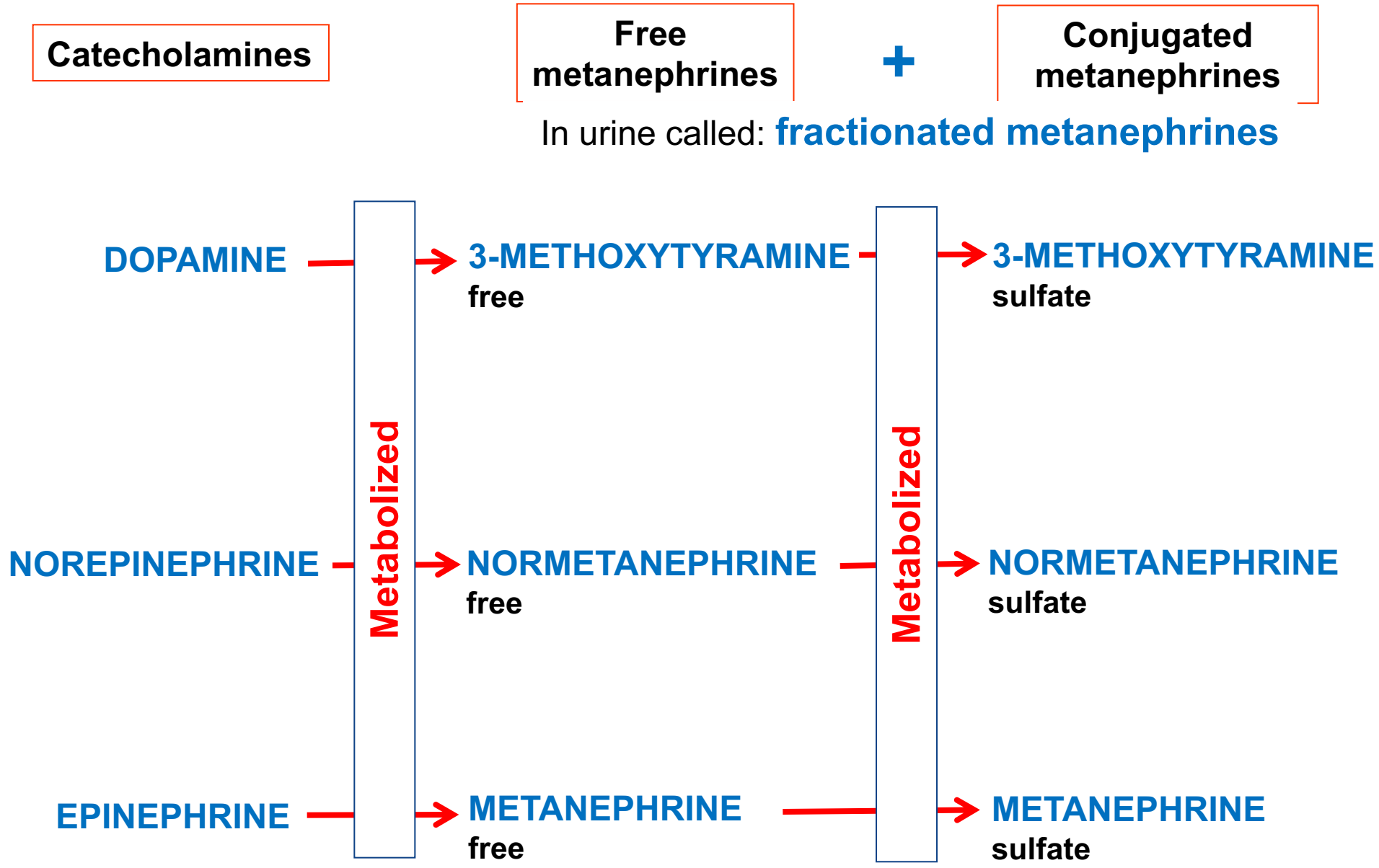
To discuss:

- What to measure when searching a PPGL: catecholamines or metanephrines?
- Should metanephrines be measured in blood or urine?
- How to collect blood and urine for metanephrines
  - seated or supine?
  - after rest?
  - vials: chilled?
  - food precautions?
- What about medication: how do they affect results?
- Reference values: what cut-off levels to be used? Age-adjustment?
- Test performance: how reliable are tests to exclude or confirm a PPGL?
- How follow-up testing in patients - with slightly elevated test results?
  - with previous adrenalectomy?


Catecholamines
Free metanephrines
+
Conjugated metanephrines
In urine called: fractionated metanephrines
DOPAMINE
NOREPINEPHRINE
EPINEPHRINE
Metabolized
3-METHOXYTYRAMINE
free
NORMETANEPHRINE
free
METANEPHRINE
free
Metabolized
3-METHOXYTYRAMINE
sulfate
NORMETANEPHRINE
sulfate
METANEPHRINE
sulfate

What to measure : catecholamines or metanephrines?

Metanephrines are preferred because the diagnostic accuracy of metanephrines is superior to that of catecholamines

In blood or urine sample?

Metanephrines can be measured in a:

- blood sample: free plasma metanephrines
- 24-hour urine collection: urinary free or fractionated metanephrines
- overnight morning urine sample: urinary free or fractionated metanephrines

Most labs measure urinary fractionated metanephrines
Few labs measure urinary free metanephrines

How to collect blood for metanephrines?

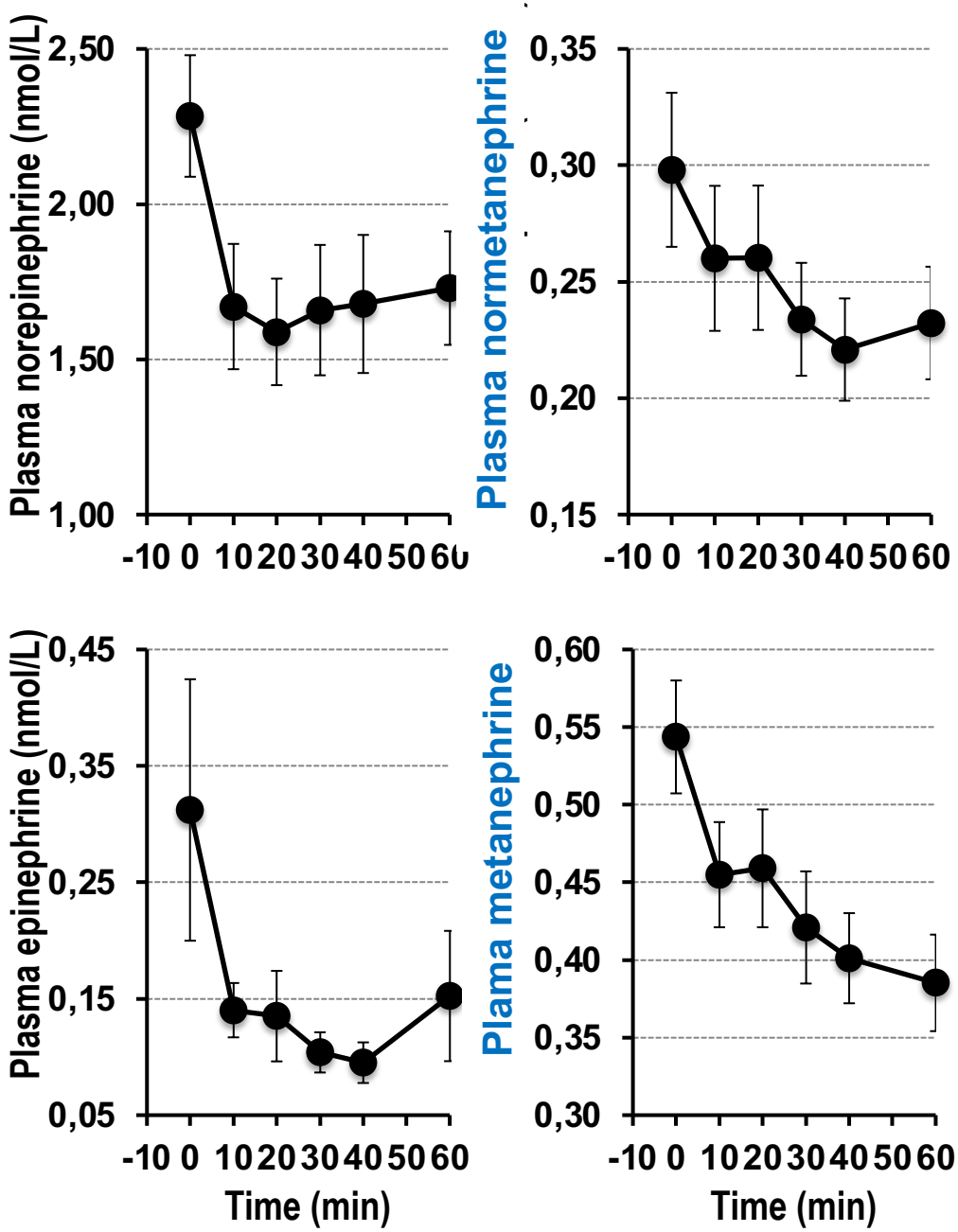
- seated or supine?
- after rest and if so, how long?
- vials: prechilled tubes and on ice?
- food precautions?

How to collect a blood sample for measurement of metanephrines?

- The patient gets an indwelling cannula in an antecubital vein
- The patient has supine rest for at least 20 minutes
- After these 20 minutes a blood sample is drawn in prechilled tubes and put immediately on ice to prevent degradation=breakdown
- Avoid any form of stress: no sampling in emergencies such as hypertensive crisis
- No need for specific diet or fasting except for 3-methoxytyramine

How to collect 24-hour urine?

- Collect urine in containers without any preservative agent
- Take care for a complete 24-hour collection
- Alternative: overnight first morning urine sample
- Avoid vigorous exercise
- Food: amine-rich diet increases fractionated but not free metanephrines



Därr R et al.
Clin Endocrinol.
2014;80:478

Advantages / disadvantages of blood or urine testing

Blood sampling takes globally only 45 minutes

Blood sampling is more laborious for nursing staff than urine collections

Blood testing more reliable in patients with renal failure

Urine testing is alternative to blood testing in case of needle phobia

Urine collection over 24 hour is not convenient for the patient

Urine sampling: frequent incomplete collections

What about medications: how do they affect results?

HPLC-ECD /FD		
Labetalol	NMN & MN (U, P)	↑↑↑
Sotalol	NMN & MN (U, P)	↑↑↑
Buspirone	MN (U)	↑↑↑
Acetaminophen	NMN (U, P)	↑↑↑
Amoxicilline	NMN (U)	↑↑↑
Sulfasalazine[$$]	NMN (U)	↑↑↑
MHBA[#]	NMN, MN, MTY (U)	↓↓↓
Methenamine	NMN & MN (U)	↑↑↑
LC-MS/MS		
HMMA*	NMN (P)	↑↑↑
Midodrine**	MN (P)	↑↑↑

P: plasma. U: urine. NMN: normetanephrine, M: metanephrine, MTY: methoxytyramine

Drugs/agents that may cause false-positive results because of their pharmacological action: consider interruption or replacement

1. Sympathomimetics:
- amphetamine/metamphetamine
- ephedrine
- pseudoephedrine
- methylphenidate

2. Norepinephrine reuptake blockers:
- tricyclic antidepressants
- venlafaxine, duloxetine
- cocaine

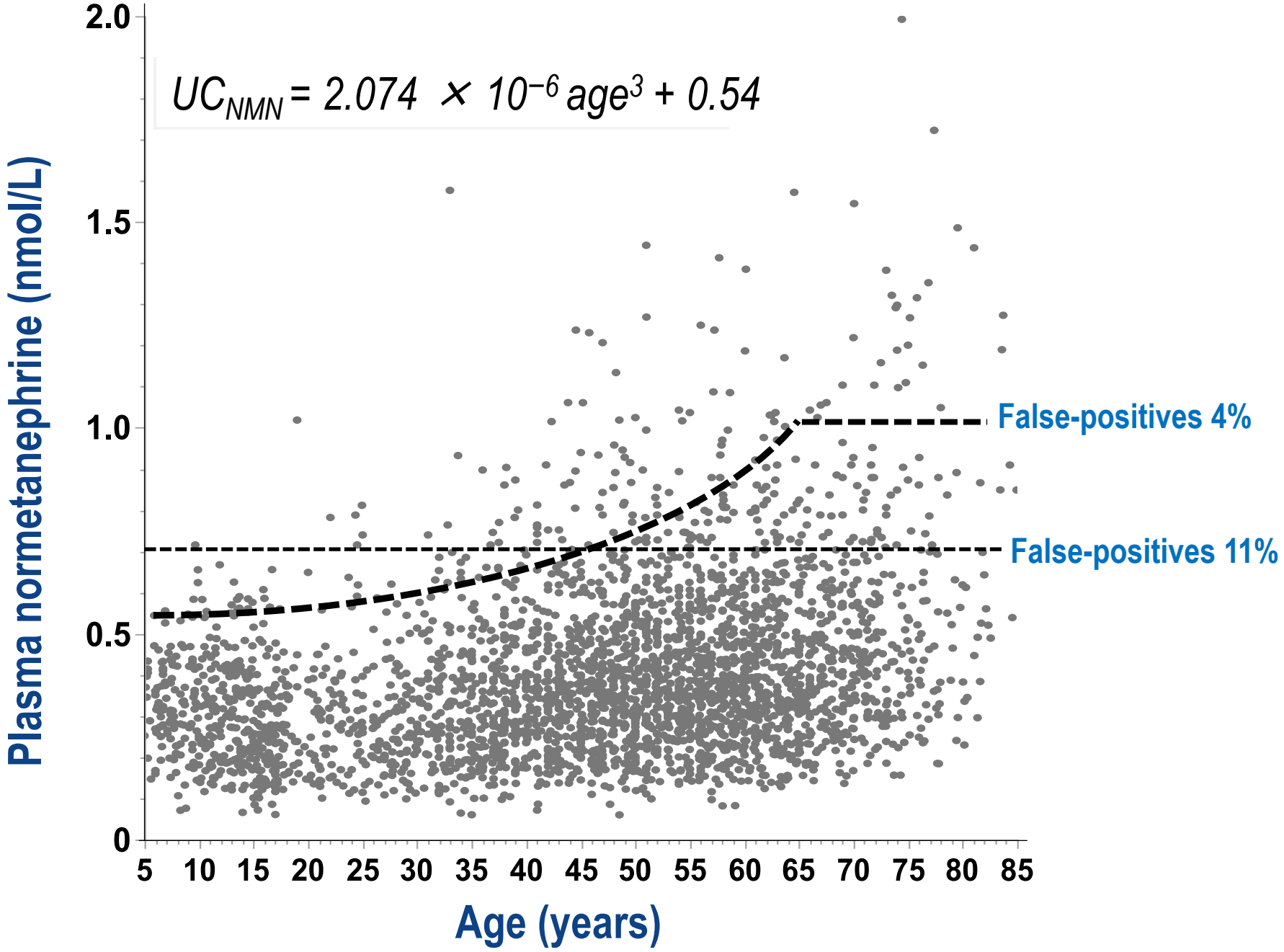
3. Alpha-2-adrenoceptor blockers:
- phenoxybenzamine
- mirtazapine

4. Monoamine oxidase inhibitors:
- tranylcypromine

5. Atypical antipsychotics:
- quetiapine
- clozapine
- risperidone

6. Caffeine: increases in **NMN** and less in **MN**

Reference values: what cut-off levels to be used? Age-adjustment?



Eisenhofer et al. Endocr Rev. 2023 Mar 30:bnad011. doi: 10.1210/endrev/bnad011. Online ahead of print

Upper Reference Limits for plasma metabolites (after supine rest)

Age (yr)	Plasma NMN	MN	3MT (nmol/L)
5 - 17	0.55		
18 - 29	0.58		
30 - 39	0.70	0.45	0.11
40 - 49	0.79		
50 - 59	0.87		
> 65	1.09		

NMN: Age-dependent curvilinear model:
$2.07 \times 10^{-6}.age^{3}+0.545$

Eisenhofer et al, Clin Chim Acta 2019 490;46

Upper Reference Limits for urinary metanephrines

Free	**NMN** *(nmol/day)*	*.ol/day)*
Males	**289**	**299**
Females	**242**	**248**
Fractionated	**N** *.l/day)*	**MN** *(nmol/day)*
Males	**.08**	**2051**
Female.	**2313**	**1408**

Eisenhofer et al. Clin Chim Acta. 2019;490:46.

Test performance:
how reliable are tests to exclude or confirm a PPGL?

False-negative test result

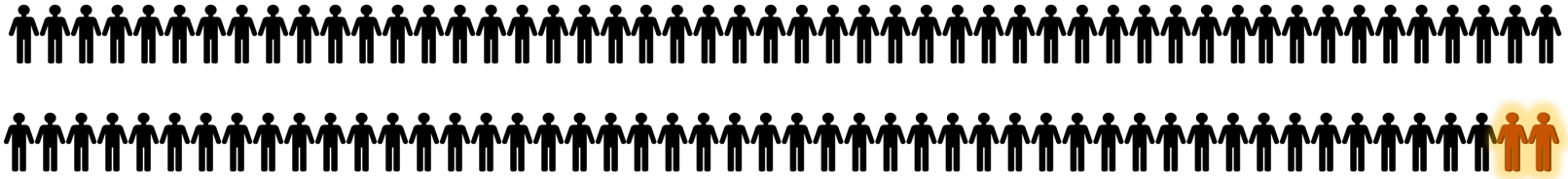
This is a normal test result in a patient **with** a pheochromocytoma

False-positive test result

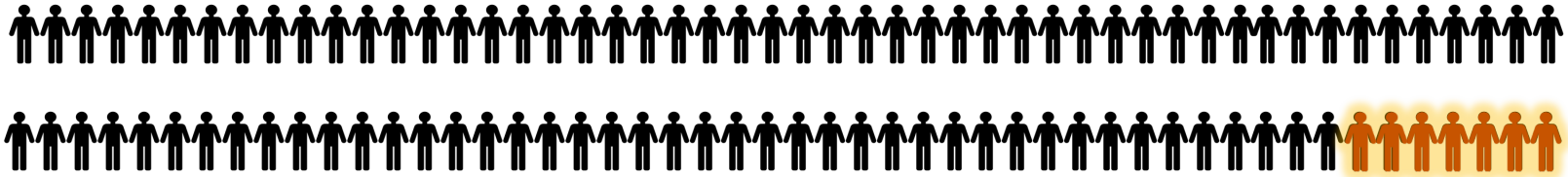
This is an abnormal test result in a patient **without** a pheochromocytoma

False-negative tests in 100 patients with pheochromocytoma

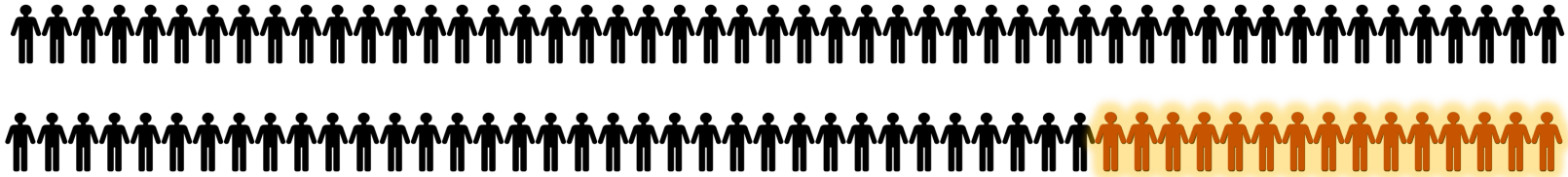
Plasma metanephrines



Urinary free or fractionated metanephrines

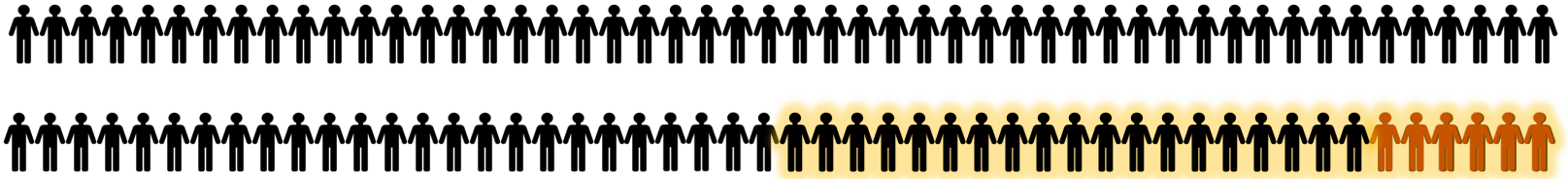


Plasma or urinary catecholamines



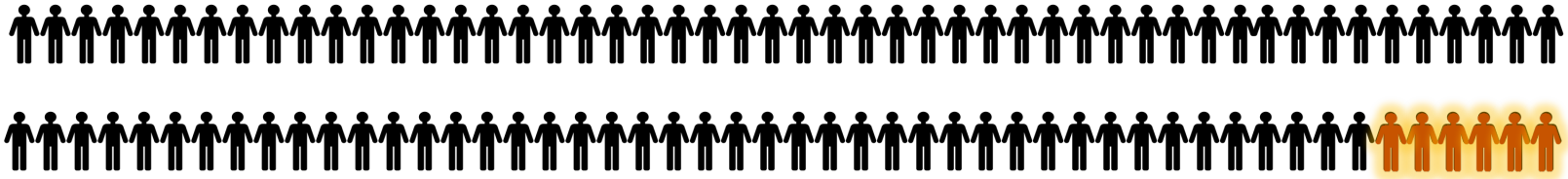
False-positive tests in 100 patients without pheochromocytoma

Plasma metanephrines

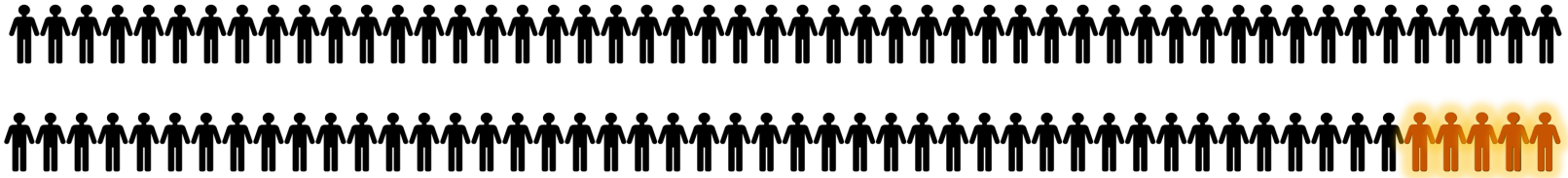


↑↑ Sympathetic activity

Urinary free metanephrines



Urinary catecholamines



Diagnostic performance of plasma versus urinary tests of normetanephrine, metanephrine and methoxytyramine

	Plasma Free	Urinary Free	Urinary fractionated
Sensitivity	97.9% * (231/236)	93.4% (211/226)	92.9% (210/226)
Specificity	94.2% † (1714/1820)	94.2% † (1655/1756)	92.1% (1619/1757)

* *P<0.05 higher than both urinary panels*

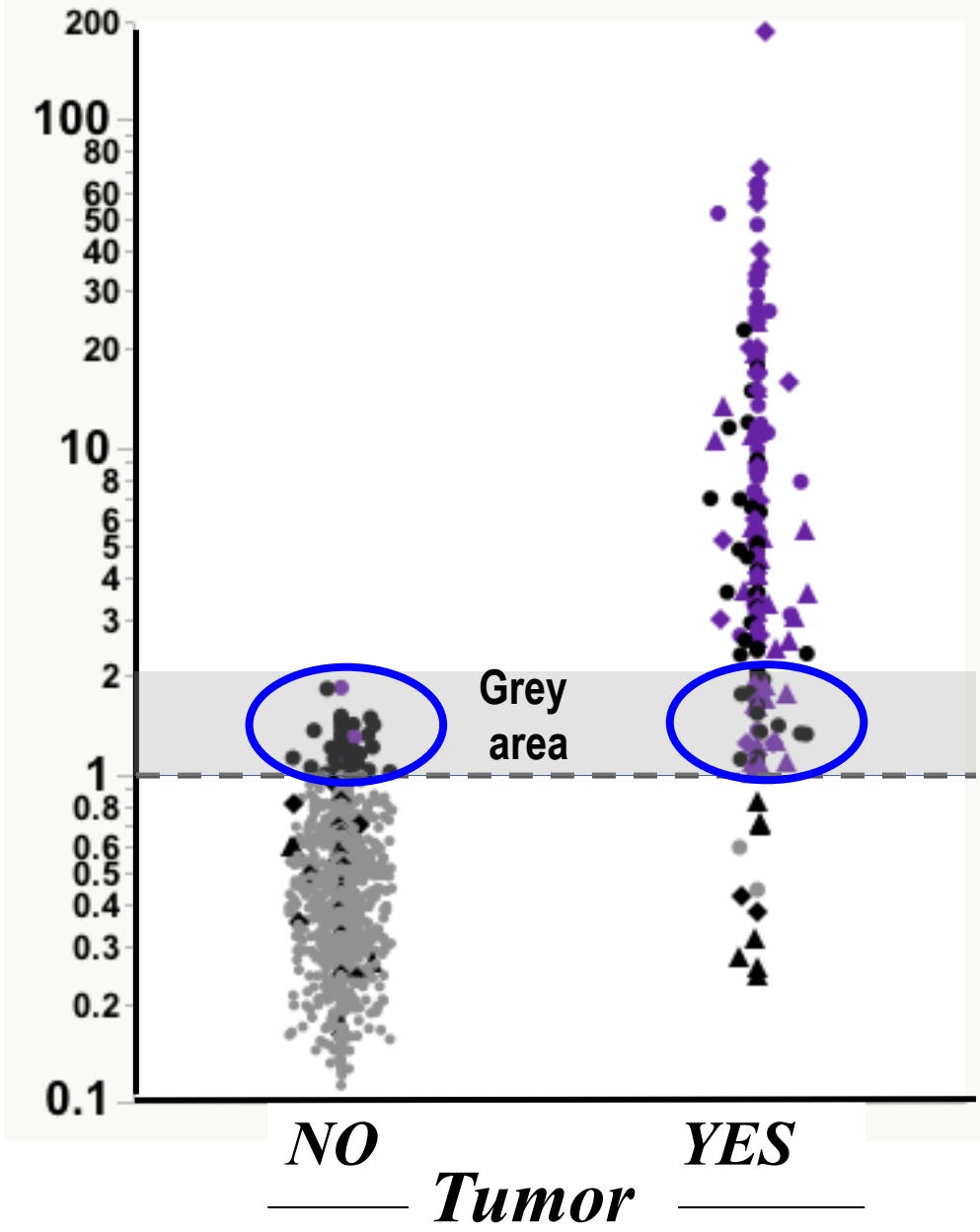
† *P<0.02 higher specificity that urinary fractionated*

Reasons for **false-negative** test results of plasma or urinary metanephrines

- incorrect blood/urine sampling
- very small tumors
- rarely some tumors do not produce catecholamines

Reasons for **false-positive** test results of plasma or urinary metanephrines

- incorrect blood sampling
- stressful conditions / acute illness
- interference of medication

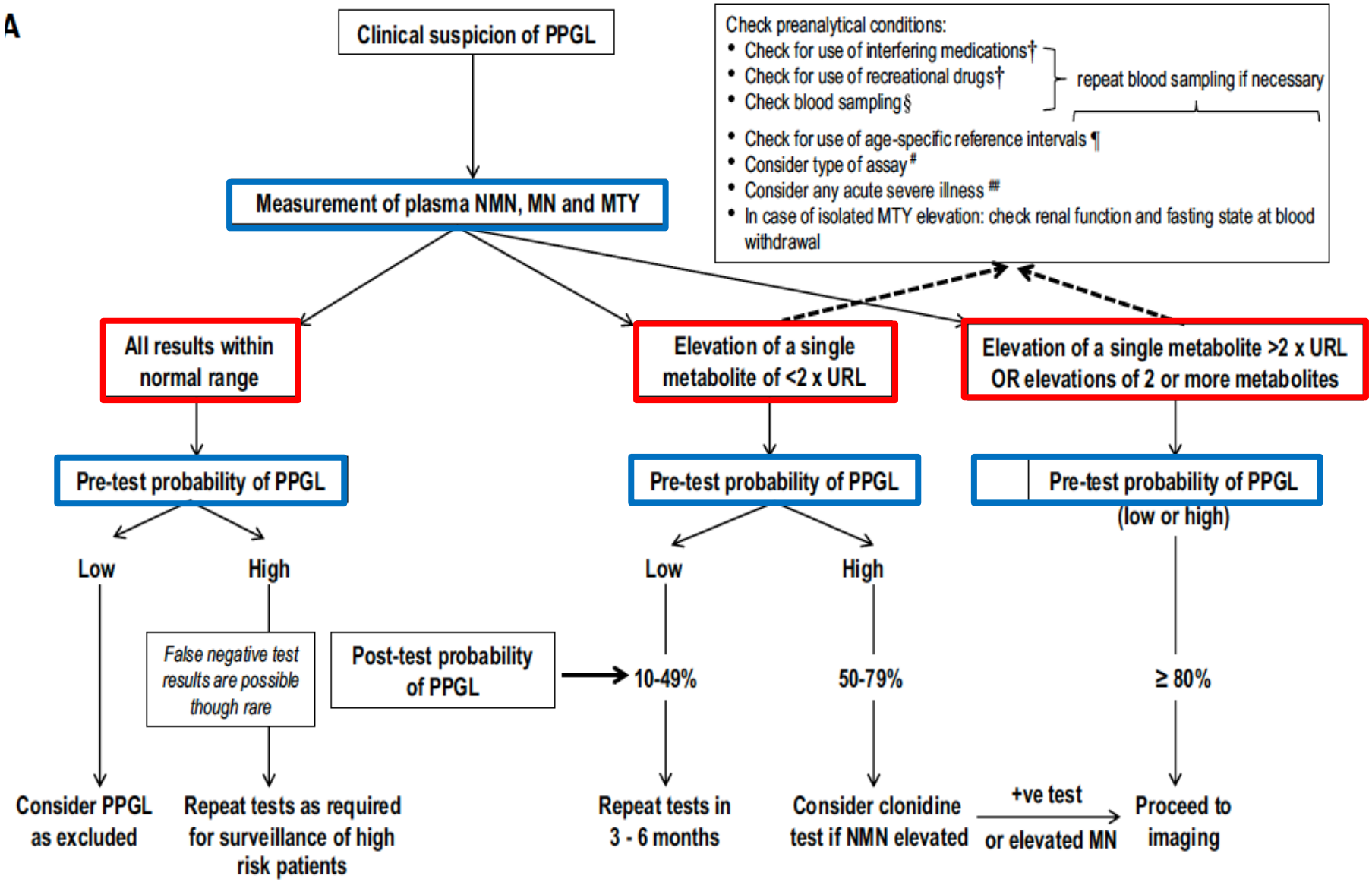


	Tumor NO	Tumor YES
NMN+MN+MTY	0.06%	23.0%
NMN+ MN	0.11%	26.3%
NMN+MTY	0.23%	21.6%
NMN	3.4%	22.1%
MN	0.23%	4.2%
MTY	0.76%	1.4%
Negative	95.1%	1.4%

Upper reference limits in patient after unilateral adrenalectomy

- *Plasma and urinary* **levels** of metanephrines*: about 20-25% lower*
- *No specific reference limits available for patients after adrenalectomy!!!*
- ***Advice:*** *follow-up in time will disclose any relevant increase but this requires follow-up!!!*

FOLLOW-UP based on plasma metanephrines



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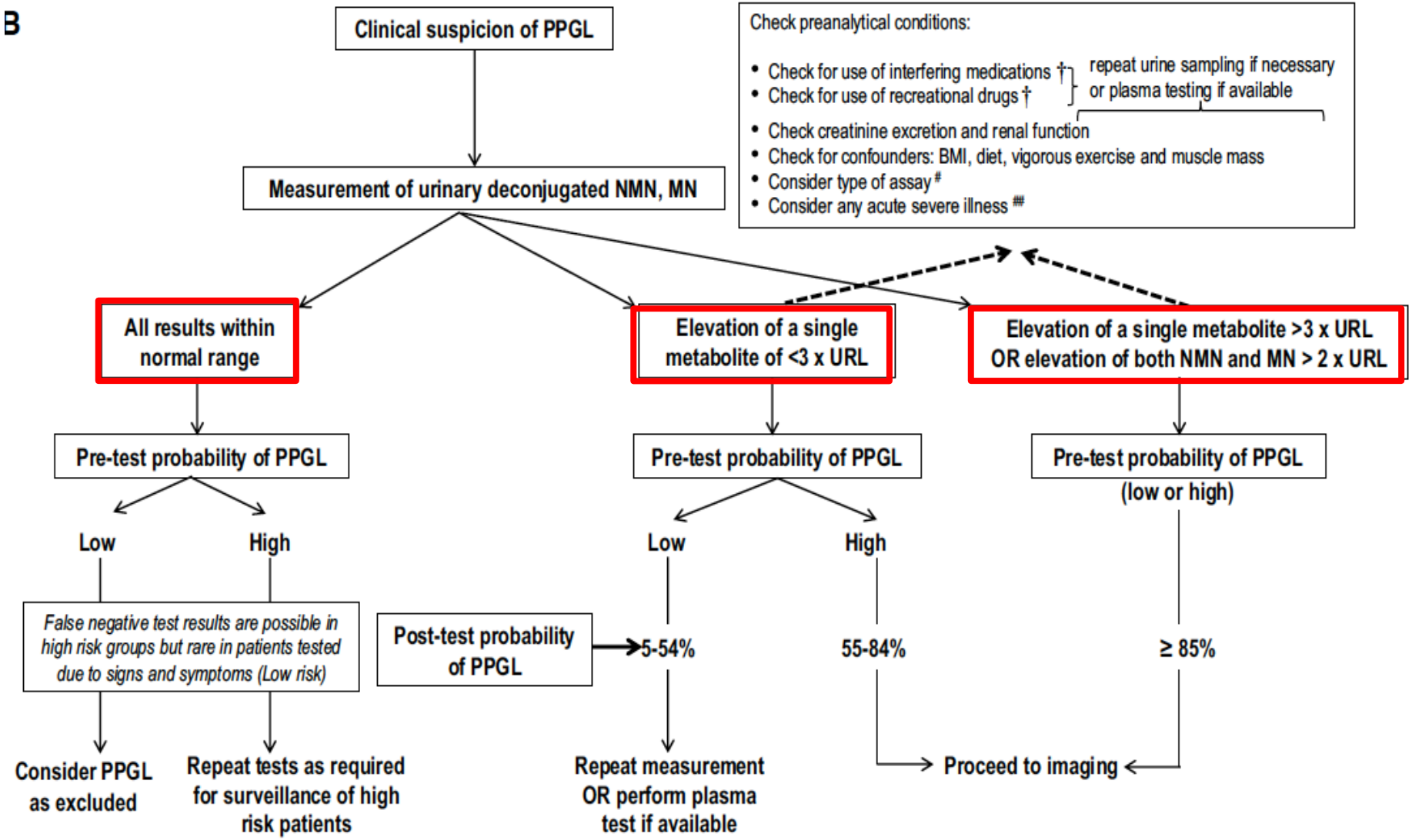
FOLLOW-UP based on fractionated urinary metanephrines



Eisenhofer et al. Endocr Rev. 2023 Mar 30:bnad011. doi: 10.1210/endrev/bnad011. Online ahead of print

Endocrine Reviews, 2023, **00**, 1–48
https://doi.org/10.1210/endrev/bnad011
Advance access publication 30 March 2023
Review

Biochemical Assessment of Pheochromocytoma and Paraganglioma

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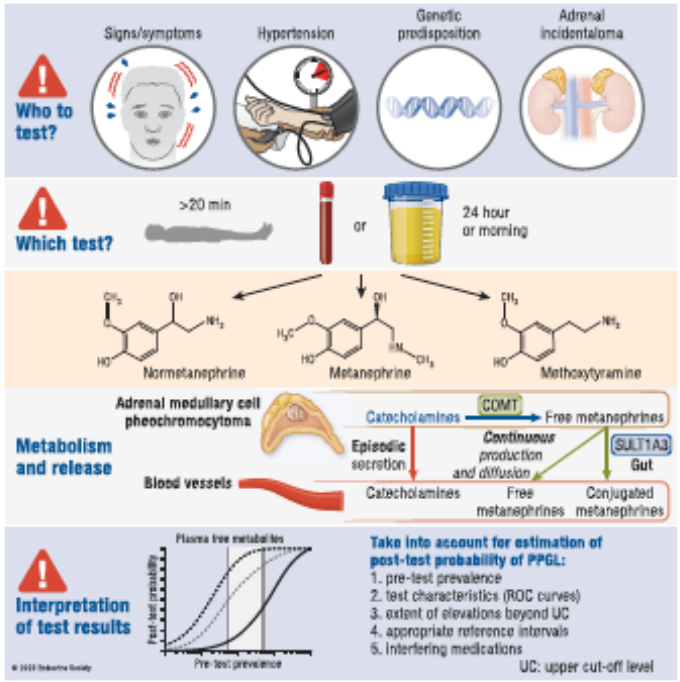
Abstract

Pheochromocytoma and paraganglioma (PPGL) require prompt consideration and efficient diagnosis and treatment to minimize associated morbidity and mortality. Once considered, appropriate biochemical testing is key to diagnosis. Advances in understanding catecholamine metabolism have clarified why measurements of the O-methylated catecholamine metabolites rather than the catecholamines themselves are important for effective diagnosis. These metabolites, normetanephrine and metanephrine, produced respectively from norepinephrine and epinephrine, can be measured in plasma or urine, with choice according to available methods or presentation of patients. For patients with signs and symptoms of catecholamine excess, either test will invariably establish the diagnosis, whereas the plasma test provides higher sensitivity than urinary metanephrines for patients screened due to an incidentaloma or genetic predisposition, particularly for small tumors or in patients with an asymptomatic presentation. Additional measurements of plasma methoxytyramine can be important for some tumors, such as paragangliomas, and for surveillance of patients at risk of metastatic disease. Avoidance of false-positive test results is best achieved by plasma measurements with appropriate reference intervals and preanalytical precautions, including sampling blood in the fully supine position. Follow-up of positive results, including optimization of preanalytics for repeat tests or whether to proceed directly to anatomic imaging or confirmatory clonidine tests, depends on the test results, which can also suggest likely size, adrenal vs extra-adrenal location, underlying biology, or even metastatic involvement of a suspected tumor. Modern biochemical testing now makes diagnosis of PPGL relatively simple. Integration of artificial intelligence into the process should make it possible to fine-tune these advances.

Graphical Abstract



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