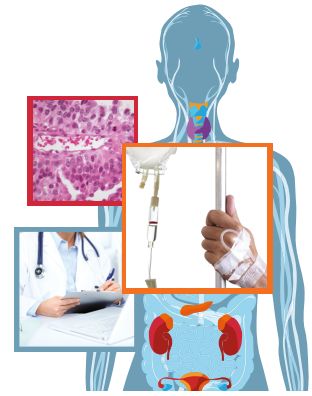
Review



International Journal of Endocrine Oncology

The role of 5-hydroxyindoleacetic acid in neuroendocrine tumors: the journey so far

Mfon Ewang-Emukowhate[1,2], Devaki Nair[2] & Martyn Caplin*[,1]
[1]Neuroendocrine Tumour Unit, Royal Free NHS Foundation Trust, London, NW3 2QG, UK
[2]Department of Clinical Biochemistry, Royal Free NHS Foundation Trust, London, UK, NW3 2QG, UK
*Author for correspondence: m.caplin@ucl.ac.uk

5-Hydroxyindole acetic acid (5-HIAA) is a surrogate marker for serotonin measurement and one of the first biochemical markers used in neuroendocrine tumors. In this review, we give a brief history of 5-HIAA and its precursor serotonin. We discuss its clinical utility and diagnostic performance in small intestinal neuroendocrine tumor and describe the challenges encountered during its analysis, historically performed in urine. The introduction of blood-based assays will help overcome some of the issues associated with its measurement in urine. The diagnostic performance of serum and plasma 5-HIAA has been shown to be comparable to that of urine 5-HIAA. Thus, analysis in either serum or plasma will provide a practical and convenient alternative to urine.

First draft submitted: 3 February 2019; Accepted for publication: 27 March 2019; Published online: 12 August 2019

Keywords: 5-hydroxyindole acetic acid (5-HIAA) • carcinoid syndrome • neuroendocrine tumours • serotonin • small intestinal neuroendocrine tumours

Practice points

- Neuroendocrine tumors (NETs) are a heterogeneous group of neoplasm that arise from cells within the neuroendocrine system.
- Patients with NETs can present with symptoms due to the increased production and secretion of biologically active hormones and biogenic amines.
- Small intestinal NETs are the most common cause of carcinoid syndrome, which presents clinically with diarrhea, flushing, wheezing and dyspnea as a result of the secretion of serotonin and other vasoactive substances.
- Over the years, biochemical markers have been used for the diagnosis and monitoring of patients with NETs.
- 5-Hydroxyindole acetic acid, a breakdown product of serotonin, was one of the first biochemical markers of NETs with specific utility in small intestinal NETs with carcinoid syndrome.
- Historically, 5-hydroxyindole acetic acid analysis has been performed in urine. However, measurement in blood, which shows a similar diagnostic and discriminatory performance, provides a practical and convenient alternative.

5-Hydroxyindole acetic acid (5-HIAA) is a metabolite of serotonin. The journey to the discovery of serotonin started as far back as the 19th century, when Ludwig and his colleague Schmidt in 1868 observed increased vascular resistance in the muscle of a dog perfused with defibrinated blood [1]. In 1912, O'Connor, following his investigation, deduced that the vasoconstrictor substance that exerts its effect in serum and not plasma is likely released during the clotting process by platelets [2]. Two different groups have been credited for the discovery of serotonin; Page, an American physiologist, and his colleagues Rapport, an organic chemist, and Green, a biochemist, isolated and identified this vasoconstrictor substance as 5-hydroxytryptamine (5-HT) also known as serotonin in 1948 [3,4]. An Italian pharmacologist and physiologist, Erspamer in the 1930s discovered a smooth muscle contracting substance found in the enterochromaffin cells of the GI tract, which he named 'enteramine.' This was later found to be the same substance as serotonin [5]. Not long afterward, Twarog and Page identified serotonin in brain extracts [6]. Much of what we know about the biochemistry of serotonin is credited to Udenfriend and colleagues, a biochemist who was involved in understanding the metabolic pathway for serotonin from tryptophan to 5-HIAA [7]. He and his team were the first to show 5-HIAA was a normal component of human urine and they went on to describe a method for its measurement [8].

Future Medicine

Table 1. Biochemical markers of neuroendocrine tumors.

NET markers	Study, year first isolated	NET type	Associated clinical syndrome
Specific markers			
Serotonin	Page *et al.* (1948)	SI-NET	Carcinoid
Urine 5-HIAA	Udenfriend *et al.* (1955)	SI-NET	Carcinoid
Gastrin	Edkins (1905)	Gastrinoma	Zollinger–Ellison
Insulin	Banting *et al.* (1922)	Insulinoma	Whipple's triad
Glucagon	Kimball *et al.* (1923)	Glucagonoma	None
Vasoactive intestinal peptide	Said *et al.* (1970)	VIPoma	WDHA
Somatostatin	Brazeau *et al.* (1973)	Somatostatinoma	None
Neurokinin A	Kimura *et al.* (1983)	SI-NET	None
Non-specific markers			
Chromogranin A	Blaschko *et al.* (1967)	Most NET	None
Pancreastatin	Tatemoto *et al.* (1986)	Most NET	None
Neurone-specific enolase	Marangos *et al.* (1974)	Poorly differentiated NET	None
Pancreatic polypeptide	Kimmel *et al.* (1971)	GEP-NET	None
NT-proBNP	Sudoh *et al.* (1987)	SI-NET (CHD)	None
Adrenaline	Abel & Takamine (1899)	Pheochromocytoma and paraganglioma	None
Noradrenaline	Euler (1946)	Pheochromocytoma and paraganglioma	None
Metanephrine	LaBrosse & Mann (1960)	Pheochromocytoma and paraganglioma	None
Normetanephrine	LaBrosse & Mann (1960)	Pheochromocytoma and paraganglioma	None

CHD: Carcinoid heart disease; GEP: Gastroenteropancreatic; NET: Neuroendocrine tumor; SI-NET: Small intestinal neuroendocrine tumor; WDHA: Watery diarrhea, hypokalemia and achlorhydria [30–41].

Interestingly, not long after the discovery of serotonin, it was implicated as the major substance secreted by carcinoid tumors. A lot of the knowledge that has been acquired over the years about the pathophysiology of serotonin can be traced back to studies carried out on patients with neuroendocrine tumors (NETs) [9,10].

Neuroendocrine tumors

In 1907, the German pathologist Oberndorfer first described NETs arising from the GI tract as a distinct entity with a more benign course. He gave the name Karzinoiden (carcinoid tumors) to differentiate these tumors from adenocarcinomas [11], they are now referred to as NETs. These diverse group of neoplasms originate from cells within the neuroendocrine system. They have the ability to secrete increased amounts of biologically active products, which may be associated with specific hypersecretory syndromes that determine their clinical presentation. These biologically active NETs are classified as functioning tumors. Non-functioning NETs, on the other hand, do not secrete excess bioactive substances but present with symptoms that are due to the compression or invasion of surrounding organs or tissues [12]. NETs are rare, but over the years there has been an increase in their incidence which may not be completely explained by earlier diagnosis or better classification. A US-based population study gives the annual incidence of NETs to be approximately 7 per 100,000 people [13,14].

Small intestinal NETs (SI-NETs) previously known as midgut NETs originate from serotonin-secreting enterochromaffin cells. These tumors are often slow growing with a low proliferation rate, and are often diagnosed at an advanced stage once metastasis has occurred [15]. They can cause functional symptoms due to carcinoid syndrome (CS) that presents clinically with diarrhea, flushing, wheezing and dyspnea as a result of the secretion of serotonin and other vasoactive substances. CS commonly occurs in SI-NETs when there is metastasis of the tumor to the liver, but it can also be seen in bronchial and, more rarely, pancreatic, ovarian and rectal NETs. In 20–30% of patients with a SI-NET and liver metastasis, CS is present. A large population-based study carried out by Halperin *et al.* revealed that CS is present in 19% of patients with a NET and of these, 32% had a SI-NET and 8% a bronchial NET [16,17].

Biochemical markers have played an important role in the diagnosis and management of NETs over the years (Table 1). Urine 5-HIAA, the metabolic product of serotonin, is the most commonly used biochemical marker in the diagnosis and monitoring of SI-NETs, particularly when CS is present. Chromogranin A is currently used

as a general marker for NETs but it is considered to have poor specificity (10–35%) [18]. A novel biomarker, the NETest™ is a multianalyte reverse transcription PCR (qRT-PCR) test involving the simultaneous measurement of 51 neuroendocrine-specific marker genes and is currently undergoing validation with preliminary studies, which are suggesting its higher specificity and sensitivity (>95%) [19–21].

CS is also associated with the development of carcinoid heart disease (CHD). The first case of CHD was described in 1954. 20% of patients with CS have CHD and it has been shown to occur more frequently in patients with SI-NET [22,23]. The pathogenesis of CHD, though not completely understood, is linked, based on several lines of evidence, to the secretion of serotonin by the metastatic tumor, leading to formation of fibrous plaques and thickening of the right heart valves with consequent regurgitation or stenosis of the affected valve [22,24,25] In patients with CHD, urine 5-HIAA levels are significantly raised (median urine 5-HIAA 576 μmol/24 h vs 233 μmol/24 h) compared with those without CHD [26]. Plasma 5-HIAA has been shown to correlate with the development of CHD [25]. A urine 5-HIAA level of 300 μmol/24 h or greater is regarded as an independent predictor for the development or progression of CHD [16].

Surgery is usually the initial treatment for NETs that have been diagnosed at an early stage of the disease. However, most patients with an SI-NETs present with metastases, commonly to the liver, mesentry and peritoneum, at the time of diagnosis. In distant metastatic disease, palliative surgery can still be offered [15,27]. Somatostatin analogs (SSA) are often used as first-line treatment in patients with metastatic NETs. They exert their anti-tumor effect by reducing the excessive secretion of hormones such as serotonin and by controlling tumor growth. Long-acting octreotide (intramuscular injection) and lanreotide (deep subcutaneous injection), both administered every 28 days, are the most commonly used SSA. Studies have shown that octreotide use in SI-NETs led to stabilization of tumor growth in approximately 50% of patients and regression of tumor in about 10% of patients. The majority of patients with CS experienced relief of their symptoms with SSA use [28].

Telotristat ethyl is a tryptophan hydroxylase inhibitor, which decreases the production of serotonin. It has been approved in the USA and Europe in combination with a SSA for the treatment of refractory diarrhea in patients with CS [24,28]. In a post hoc analysis in the Phase III TELESTAR trial, 78 and 87% of patients on 250 and 500 mg dose of telotristat ethyl three-times daily achieved a ≥30% reduction in urine 5-HIAA compared with 10% in the placebo group [29].

Peptide receptor radionuclide therapy is recommended when other medical treatments have failed. Treatment is delivered using radiolabeled molecules that bind to specific peptide receptors expressed by the tumor. Integrating SSA in the radiolabeled molecule such as Lutetium (^{177}Lu) oxodotreotide ensures that the tumors that express somatostatin receptors are targeted. Quality of life analysis in the NETTER-1 trial suggests that the peptide receptor radionuclide therapy maintains and improves the quality of life in patents with SI-NETs [27,28].

Serotonin

Serotonin (5-HT) is a biogenic amine synthesized from the essential amino acid tryptophan. The majority of the body's 5-HT is produced in the enterochromaffin cells of the GI tract. A small proportion is synthesized in the serotonergic neurons of the CNS. Blood 5-HT is almost completely found in platelets where it is stored in dense granules. A small amount is present in plasma. Platelets do not synthesize 5-HT, their 5-HT content is predominantly from the eneterochromaffin cells of the GI tract following its release into the circulation. The metabolism of 5-HT is via oxidative deamination by monoamine oxidase to 5-hydroxyindoleacetaldehyde, which in turn is oxidized to 5-HIAA, the major metabolite or reduced to 5-hydroxytryptophol.

5-HT is an important signaling molecule involved in various physiological processes. It regulates gut motility and in the CNS is involved in temperature control, mood and sleep. It also plays a role in platelet aggregation, vascular tone and metabolic processes such as regulation of bone turnover, lipid metabolism and glucose homeostasis [42,43].

Serotonin analysis

Various methods have been employed in the analysis of 5-HT. Earlier methods such as paper chromatography and spectrofluorometry were superseded by more sensitive and specific techniques, including radioimmunoassays and enzyme-linked immunosorbent assays. The use of these assays was limited by cross-reactivity and interference by endogenous substances in the samples. HPLC and LC–MS methods are now commonly used as they provide better specificity and sensitivity, also allowing for the simultaneous measurement of 5-HT metabolites and other related compounds [43,44]. 5-HT has been measured in whole blood, platelet-rich plasma, platelet-poor plasma, serum and urine but they are challenges surrounding its analysis. Pre-analytically, there are precautions around sample

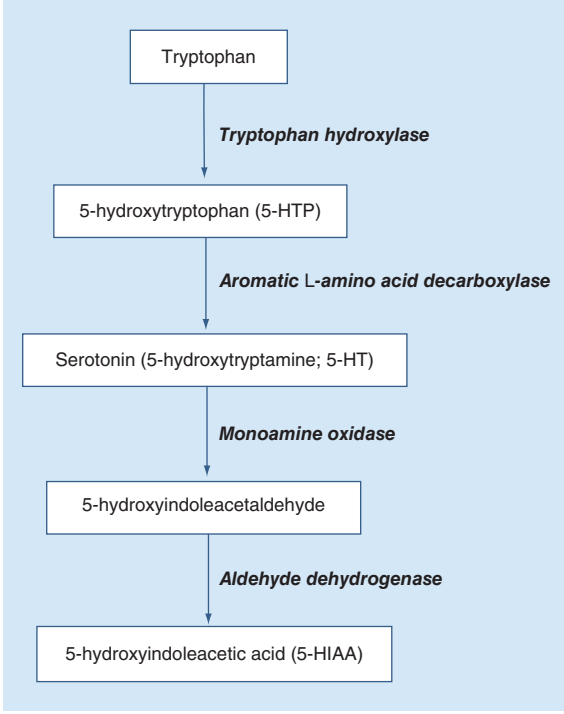


Figure 1. Pathway for 5-hydroxyindole acetic acid synthesis.

collection and preparation because 5-HT can easily be released from platelets, leading to a falsely elevated result. Also, 5-HT is readily oxidized and degraded enzymatically, which can give erroneously low results. The addition of antioxidants to specimen tubes and the immediate freezing of samples after collection are measures used to ensure the stability of 5-HT. The saturation of platelets at high 5-HT concentration and issues surrounding the reference range for measurement of 5-HT in platelet-poor plasma due to the huge variation in results reported by different studies limits the analysis of 5-HT in platelets [45,46]

5-Hydroxyindole acetic acid

5-HIAA is made up of an indole ring with two functional groups, a phenol and a carboxymethyl group. The production of 5-HIAA via oxidative deamination is the major metabolic fate of serotonin. It is synthesized mainly in the kidney and liver and excreted in the urine as it is water soluble [8,47]. Sjoerdsma, Udenfriend and their colleagues outlined the metabolic pathway for serotonin from tryptophan to 5-HIAA (Figure 1) [7,10]. In various neurological conditions, 5-HIAA is used as a surrogate for serotonin measurement [48,49].

Serotonin and 5-HIAA were the initial biochemistry markers used in NETs which had originally been identified as carcinoid tumors. This was due to earlier observations that include the discovery of high levels of serotonin in the blood and tissue of patients with metastatic carcinoid tumors, the demonstration of increased urinary 5-HIAA in this group of patients and the description and association of CS with 5-HT [10,50–52]. Over the years, further work and advances in the NET field corroborated these findings that serotonin is the main substance secreted by carcinoid tumors.

5-HIAA analysis

Historically, urine 5-HIAA has been the preferred biochemical marker for carcinoid tumors. Udenfriend *et al.* described a colorimetric method for its measurement [8]; a problem with the colorimetric method was its poor specificity, which required several modifications. Fluorimetric assays were also used in urine 5-HIAA analysis. Although a sensitive method, its use was limited by interference from other urine constituents. Other methods such as immunoassays, thin layer chromatography, gas chromatography, HPLC and MS/MS have been described. HPLC assays are now commonly used, with the advantage of measuring other compounds such as metanephrines simultaneously [47,53–57].

A 24-h urine collection is required for the analysis of 5-HIAA as random urine produces varying concentrations. The specimen is collected in an acidified container because 5-HIAA, like other 5-hydroxyindoles, easily undergoes

Table 2. Interfering factors in urine 5-hydroxyindole acetic acid analysis.

Interfering factors	Urine 5-HIAA concentration	Mechanism of effect
Foods		
Bananas, plantain, plums, pineapples, kiwi fruit, figs, dates, cantaloupe melon, honeydew melon, grapefruit, walnuts, pecan, macadamia, and Brazil nuts, aubergine, olives, broccoli, spinach, cauliflower	Increased	Rich in serotonin or tryptophan
Medications		
Glyceryl guaiacolate present in cough remedies, naproxen, paracetamol	Increased	Analytical interference
Cisplatin, fluorouracil, melphalan	Increased	Increased 5-HIAA excretion
Imipramine	Decreased	Blocks serotonin re-uptake
Isoniazid and methyldopa	Decreased	Inhibits 5-HT synthesis
Isocarboxazid and moclobemide	Decreased	Inhibits conversion of 5-HT to 5-HIAA
Levodopa and ethanol	Decreased	Diverts tryptophan and 5-HT to alternative pathways
Chlorpromazine	Decreased	Analytical interference

Data taken from [47,53,59–61].

oxidation at an alkaline pH. Addition of a weak acid such as acetic acid was recommended but it was found to interfere with a colorimetric method. Commonly, hydrochloric acid is used to lower the pH to 3 [47,53].

Urine 5-HIAA excretion is increased by tryptophan- or serotonin-rich foods (Table 2). A study on the influence of diet on urine 5-HIAA excretion suggests that 5-H1AA levels return to baseline by the second day after a serotonin-rich diet is stopped [58]. Thus, prior to urine 5-HIAA collection, tryptophan- and serotonin-containing foods should be stopped for about 48 h. Certain medications can affect urine 5-HIAA concentration (Table 2). Chemotherapy drugs such as cisplatin cause an increase in urine 5-HIAA excretion, presumably due to the release of a large amount of 5-HT by the cancer cells. Other medications such as monoamine oxidase inhibitors exert their effect by acting on the metabolic pathway of serotonin, which can alter 5-HIAA excretion. Interference with analytical methods for urine 5-HIAA is another way in which medications have been found to either falsely increase or decrease 5-HIAA concentration. This is not an issue with the modern 5-HIAA assays, which predominantly employ the HPLC method [47,53,59].

5-HIAA: diagnostic performance & utility

Several studies have shown the sensitivity of urine 5-HIAA to be between 35 and 73%, and the specificity 89–100% depending on the cutoff used [62–64]. In patients with CS, a urine 5-HIAA level greater than 300 µmol/24 h is associated with an increased risk of developing CHD [65]. The prognostic role of urine 5-HIAA was demonstrated in a study of patients with SI-NETs [66]. However, other studies have shown that in multivariate analysis, urine 5-HIAA had no prognostic benefit [67].

The biochemical marker CgA, whose diagnostic specificity is dependent on the type of NET and the tumor burden [68], has been shown to be a prognostic marker of NET. Studies have shown its sensitivity to be between 43 and 100% and its specificity 10–35% [62,63,69–71]. The experience of a single center has shown that in SI-NETs, the sensitivities of both urine 5-HIAA and CgA was similar (69 vs 68%) and in patients with liver metastases, the sensitivity of urine 5-HIAA was greater (86 vs 77%) [72]; both markers have demonstrated good sensitivities in patients with CS [62].

Measurement of 5-HIAA in plasma or serum addresses the inconvenience and issues surrounding urine collection, commonly the stress associated with the timing and collection of the urine and the exposure to a hazardous substance used as a preservative in the sample container. Several methods have been described in the analysis of plasma or serum 5-HIAA, they include HPLC, gas chromatography MS and LC–MS/MS [71,73–75].

Studies comparing urine and plasma 5-HIAA have shown good agreement and statistically significant correlation between both sample types [75,76]. The diagnostic performance of these tests depends on the chosen cutoff. Urine 5-HIAA at a cutoff of 40–56 µmol/24 h showed sensitivities between 74 and 85% compared with 79.6 and 89% in plasma 5-HIAA at a cutoff of 118 nmol/l. Specificities were between 90–97% in urine and 74–100% in plasma. Concordance was also shown in their discriminating capacity. The receiver-operating characteristic (ROC) curve obtained by Adaway *et al.* in their comparison of urine and plasma 5-HIAA showed the area under the curve (AUC)

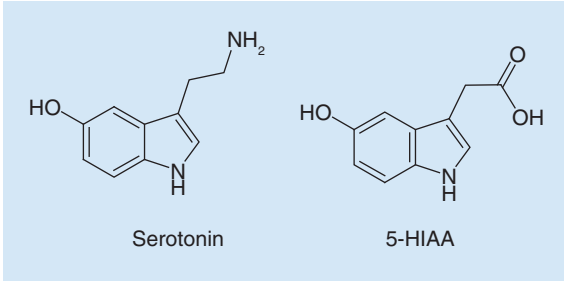


Figure 2. The chemical structure of serotonin and 5-hydroxyindole acetic acid.

to be 0.920 for urine 5-HIAA and 0.917 for plasma 5-HIAA. AUC for the ROC curve in the study by Carling *et al.* was 0.895 and 0.902 for urine and plasma 5-HIAA, respectively [64,71,76].

Adaway *et al.* also compared urine and serum 5-HIAA in 68 patients and reported a good agreement between urine and serum 5-HIAA in more than 90% of these patients. Another study comparing urine and serum 5-HIAA showed the sensitivity of urine 5-HIAA at a cutoff of 40 µmol/24 h was 67% with a specificity of 81% compared with serum 5-HIAA with a sensitivity of 57% and specificity of 95% at a cutoff of 123 nmol/l. ROC analysis showed similarities in their ability to detect NETs, with AUC for urine 5-HIAA 0.83 and 0.81 for serum 5-HIAA [71,76].

Comparison of serum and plasma 5-HIAA revealed higher 5-HIAA concentration in the serum, which may have been caused by the release of 5-HIAA from cells during clotting [76]. A study looking at the association between biomarkers and CHD showed NT-proBNP and plasma 5-HIAA had similar discriminatory ability in the diagnosis of CHD; ROC curve analysis showed the AUC for NT-proBNP was 0.82 and plasma 5-HIAA was 0.85 [25].

Conclusion & future perspective

NETs are a heterogenous group of neoplasms. Although 5-HIAA has its limitations as a biomarker for NETs, its utility as a specific marker of SI-NETs, which commonly presents with CS, cannot be overlooked. Studies performed to date have shown that serum and plasma 5-HIAA have similar diagnostic performance and discriminatory capacity as the urine 5-HIAA assay. Avoidance of serotonin-rich foods may only be required 8–12 h before blood sample collection [64,71]. Therefore, the way forward will be measurement of 5-HIAA in serum or plasma, which will offer a more practical and convenient alternative to its historic measurement in urine (Figure 2).

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Author contributions

M Ewang-Emukowhate wrote the first draft of the article. All the authors agreed on the original concept and design of the article, reviewed and revised the draft, and contributed to the final version of the article.

References

Papers of special note have been highlighted as: • of interest; •• of considerable interest

1 Ludwig C, Schmidt A. Das Verhalten der Gase, welche mit dem Blut durch den reizbaren Säugethiermuskel strömen, Arbeiten aus der physiologischen Anstalt zu Leipzig. *Arb Physiol Ans Leipzig* 3, 1–61 (1868).

2 O'Connor J. Über den Adrenalingehalt des Blutes. *Arch. Exp. Pathol. Pharmakol.* 67, 195–232 (1912).

3 Rapport MM, Green AA, Page IH. Serum vasoconstrictor, serotonin; isolation and characterization. *J. Biol. Chem.* 176(3), 1243–1251 (1948).

4 Rapport MM, Green AA, Page IH. Crystalline serotonin. *Science* 108(2804), 329–330 (1948).

5 Erspamer V, Asero B. Identification of enteramine, the specific hormone of the enterochromaffin cell system, as 5-hydroxytryptamine. *Nature* 169(4306), 800–801 (1952).

6 Twarog BM, Page IH. Serotonin content of some mammalian tissues and urine and a method for its determination. *Am. J. Physiol.* 175(1), 157–161 (1953).

7 Sjoerdsma A, Smith TE, Stevenson TD, Udenfriend S. Metabolism of 5-hydroxytryptamine (serotonin) by monoamine oxidase. *Proc. Soc. Exp. Biol. Med.* 89(1), 36–38 (1955).

8 Udenfriend S, Titus E, Weissbach H. The identification of 5-hydroxy-3-indoleacetic acid in normal urine and a method for its assay. *J. Biol. Chem.* 216(2), 499–505 (1955).

9 Sjoerdsma A, Palfreyman MG. History of serotonin and serotinin disorders. *Ann. NY Acad. Sci.* 600(1), 1–8 (1990).

10 Thorson AH. Studies on carcinoid disease. *Acta Med. Scand. Suppl.* 334, 1–132 (1958).

11 Oberndorfer S. Karzinoide tumoren des dünndarms. *Frankf. Z. Pathol.* 1, 426–432 (1907).

12 Kanakis G, Kaltsas G. Biochemical markers for gastroenteropancreatic neuroendocrine tumours (GEP-NETs). *Best Pract. Res. Clin. Gastroenterol.* 26(6), 791–802 (2012).

13 Huguet I, Grossman AB, O'Toole D. Changes in the epidemiology of neuroendocrine tumours. *Neuroendocrinology* 104(2), 105–111 (2017).

14 Dasari A, Shen C, Halperin D *et al.* Trends in the incidence, prevalence, and survival outcomes in patients with neuroendocrine tumors in the United States. *JAMA Oncol.* 3(10), 1335–1342 (2017).

•• **Shows the rise in the incidence and prevalence of neuroendocrine tumor (NET). It also shows improved survival over time, which can be attributed to the advances in the management of NET.**

15 Niederle B, Pape UF, Costa F *et al.* ENETS consensus guidelines update for neuroendocrine neoplasms of the jejunum and ileum. *Neuroendocrinology* 103(2), 125–138 (2016).

•• **This guideline covers in detail the identification, investigation and management of small intestinal NET.**

16 Kaltsas G, Caplin M, Davies P *et al.* ENETS consensus guidelines for the standards of care in neuroendocrine tumors: pre- and perioperative therapy in patients with neuroendocrine tumors. *Neuroendocrinology* 105(3), 245–254 (2017).

17 Halperin DM, Shen C, Dasari A *et al.* Frequency of carcinoid syndrome at neuroendocrine tumour diagnosis: a population-based study. *Lancet Oncol.* 18(4), 525–534 (2017).

18 Modlin IM, Bodei L, Kidd M. Neuroendocrine tumor biomarkers: from monoanalytes to transcripts and algorithms. *Best Pract. Res. Clin. Endocrinol. Metab.* 30(1), 59–77 (2016).

19 Modlin IM, Frilling A, Salem RR *et al.* Blood measurement of neuroendocrine gene transcripts defines the effectiveness of operative resection and ablation strategies. *Surgery* 159(1), 336–347 (2016).

20 Bodei L, Kidd M, Modlin IM *et al.* Measurement of circulating transcripts and gene cluster analysis predicts and defines therapeutic efficacy of peptide receptor radionuclide therapy (PRRT) in neuroendocrine tumors. *Eur. J. Nucl. Med. Mol. Imaging* 43(5), 839–851 (2016).

21 Cwikla JB, Bodei L, Kolasinska-Cwikla A, Sankowski A, Modlin IM, Kidd M. Circulating transcript analysis (NETest) in GEP-NETs treated with somatostatin analogs defines therapy. *J. Clin. Endocrinol. Metab.* 100(11), E1437–E1445 (2015).

22 Bhattacharyya S, Davar J, Dreyfus G, Caplin ME. Carcinoid heart disease. *Circulation* 116(24), 2860–2865 (2007).

23 Pellikka PA, Tajik AJ, Khandheria BK *et al.* Carcinoid heart disease. Clinical and echocardiographic spectrum in 74 patients. *Circulation* 87(4), 1188–1196 (1993).

24 Hayes AR, Davar J, Caplin ME. Carcinoid heart disease: a review. *Endocrinol. Metab. Clin. North Am.* 47(3), 671–682 (2018).

25 Dobson R, Burgess MI, Banks M *et al.* The association of a panel of biomarkers with the presence and severity of carcinoid heart disease: a cross-sectional study. *PLoS ONE* 8(9), e73679 (2013).

•• **5-Hydroxyindole acetic acid (5-HIAA) is shown to be a sensitive and specific marker for carcinoid heart disease in this study of biomarkers.**

26 Zuetenhorst JM, Bonfrer JM, Korse CM, Bakker R, van Tinteren H, Taal BG. Carcinoid heart disease: the role of urinary 5-hydroxyindoleacetic acid excretion and plasma levels of atrial natriuretic peptide, transforming growth factor-beta and fibroblast growth factor. *Cancer* 97(7), 1609–1615 (2003).

27 Caplin M. The recent European approval of lutetium (^{177}Lu) oxodotreotide increases treatment options for gastroenteropancreatic neuroendocrine tumors. *Int. J. Endocr. Oncol.* 5(2), IJE09 (2018).

28 Oberg K. Medical therapy of gastrointestinal neuroendocrine tumors. *Visc. Med.* 33(5), 352–356 (2017).

29 Kulke MH, Horsch D, Caplin ME *et al.* Telotristat ethyl, a tryptophan hydroxylase inhibitor for the treatment of carcinoid syndrome. *J. Clin. Oncol.* 35(1), 14–23 (2017).

•• **This placebo-controlled Phase III study shows two different doses of telotristat ethyl (250 and 500 mg) versus placebo produced a significant reduction ($p < 0.001$) in mean urine 5-HIAA levels and improvement in symptoms in patients with carcinoid syndrome and refractory diarrhea not adequately controlled by somatostatin analogs.**

30 Winkler H, Fischer-Colbrie R. The chromogranins A and B: the first 25 years and future perspectives. *Neuroscience* 49(3), 497–528 (1992).

31 Marangos PJ, Zomzely-Neurath C, Luk DC, York C. Isolation and characterization of the nervous system-specific protein 14–3-2 from rat brain. Purification, subunit composition, and comparison to the beef brain protein. *J. Biol. Chem.* 250(5), 1884–1891 (1975).

32 Marangos PJ, Zomzely-Neurath C, York C. Determination and characterization of neuron specific protein (NSP) associated enolase activity. *Biochem. Biophys. Res. Commun.* 68(4), 1309–1316 (1976).

33 Heitz P, Polak JM, Bloom SR, Adrian TE, Pearse AG. Cellular origin of human pancreatic polypeptide (HPP) in endocrine tumours of the pancreas. *Virchows Arch., B, Cell Pathol.* 21(3), 259–265 (1976).

34 de Herder WW, Rehfeld JF, Kidd M, Modlin IM. A short history of neuroendocrine tumours and their peptide hormones. *Best Pract. Res. Clin. Endocrinol. Metab.* 30(1), 3–17 (2016).

35 Said SI, Mutt V. Potent peripheral and splanchnic vasodilator peptide from normal gut. *Nature* 225(5235), 863–864 (1970).

36 Brazeau P, Vale W, Burgus R *et al.* Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. *Science* 179(4068), 77–79 (1973).

37 Kimura S, Okada M, Sugita Y, Kanazawa I, Munekata E. Novel neuropeptides, neurokinin α and β, isolated from porcine spinal cord. *Proc. Jpn. Acad. Ser. B. Phys. Biol. Sci.* 59(4), 101–104 (1983).

38 Sudoh T, Kangawa K, Minamino N, Matsuo H. A new natriuretic peptide in porcine brain. *Nature* 332(6159), 78–81 (1988).

39 Bennett MR. One hundred years of adrenaline: the discovery of autoreceptors. *Clin. Auton. Res.* 9(3), 145–159 (1999).

40 Tatemoto K, Efendic S, Mutt V, Makk G, Feistner GJ, Barchas JD. Pancreastatin, a novel pancreatic peptide that inhibits insulin secretion. *Nature* 324(6096), 476–478 (1986).

41 Labrosse EH, Mann JD. Presence of metanephrine and normetanephrine in normal human urine. *Nature* 185, 40 (1960).

42 Martin AM, Young RL, Leong L *et al.* The diverse metabolic roles of peripheral serotonin. *Endocrinology* 158(5), 1049–1063 (2017).

43 Szeitz A, Bandiera SM. Analysis and measurement of serotonin. *Biomed. Chromatogr.* 32(1), e4135 (2018).

44 Kema IP, de Vries EG, Muskiet FA. Clinical chemistry of serotonin and metabolites. *J. Chromatogr., B, Biomed. Sci. Appl.* 747(1-2), 33–48 (2000).

45 Lindstrom M, Tohmola N, Renkonen R, Hamalainen E, Schalin-Jantti C, Itkonen O. Comparison of serum serotonin and serum 5-HIAA LC-MS/MS assays in the diagnosis of serotonin producing neuroendocrine neoplasms: a pilot study. *Clin. Chim. Acta.* 482, 78–83 (2018).

46 Brand T, Anderson GM. The measurement of platelet-poor plasma serotonin: a systematic review of prior reports and recommendations for improved analysis. *Clin. Chem.* 57(10), 1376–1386 (2011).

47 Deacon AC. The measurement of 5-hydroxyindoleacetic acid in urine. *Ann. Clin. Biochem.* 31(3), 215–232 (1994).

48 Rodan LH, Gibson KM, Pearl PL. Clinical use of CSF neurotransmitters. *Pediatr. Neurol.* 53(4), 277–286 (2015).

49 Gasparini CF, Smith RA, Griffiths LR. Genetic and biochemical changes of the serotonergic system in migraine pathobiology. *J. Headache Pain.* 18(1), 20 (2017).

50 Thorson A, Biorck G, Bjorkman G, Waldenstrom J. Malignant carcinoid of the small intestine with metastases to the liver, valvular disease of the right side of the heart (pulmonary stenosis and tricuspid regurgitation without septal defects), peripheral vasomotor symptoms, bronchoconstriction, and an unusual type of cyanosis; a clinical and pathologic syndrome. *Am. Heart J.* 47(5), 795–817 (1954).

51 Page IH, Corcoran AC, Udenfriend S, Szoedsma A, Weissbach H. Argentaffinoma as endocrine tumour. *Lancet* 268(6856), 198–199 (1955).

52 Hanson A, Serin F. Determination of 5-hydroxy-indole-acetic acid in urine; and its excretion in patients with malignant carcinoids. *Lancet* 269(6905), 1359–1361 (1955).

53 Corcuff JB, Chardon L, El Hajji Ridah I, Brossaud J. Urinary sampling for 5HIAA and metanephrines determination: revisiting the recommendations. *Endocr. Connect.* 6(6), R87–R98 (2017).

54 Korf J, Valkenburgh-Sikkema T. Fluorimetric determination of 5-hydroxyindoleacetic acid in human urine and cerebrospinal fluid. *Clin. Chim. Acta.* 26(2), 301–306 (1969).

55 Mukerjee H, Pincus MR. A colorimetric determination of 5-hydroxyindole acetic acid in urine. *Clin. Chim. Acta.* 209(1–2), 105–106 (1992).

56 Mulder EJ, Oosterloo-Duinkerken A, Anderson GM, De Vries EG, Minderaa RB, Kema IP. Automated on-line solid-phase extraction coupled with HPLC for measurement of 5-hydroxyindole-3-acetic acid in urine. *Clin. Chem.* 51(9), 1698–1703 (2005).

57 de Jong WH, Graham KS, de Vries EG, Kema IP. Urinary 5-HIAA measurement using automated on-line solid-phase extraction-high-performance liquid chromatography-tandem mass spectrometry. *J. Chromatogr., B, Analyt. Technol. Biomed. Life Sci.* 868(1-2), 28–33 (2008).

58 Mashige F, Matsushima Y, Kanazawa H *et al.* Acidic catecholamine metabolites and 5-hydroxyindoleacetic acid in urine: the influence of diet. *Ann. Clin. Biochem.* 33(1), 43–49 (1996).

59 O'Toole D, Grossman A, Gross D *et al.* ENETS consensus guidelines for the standards of care in neuroendocrine tumors: biochemical markers. *Neuroendocrinology* 90(2), 194–202 (2009).

60 Feldman JM, Lee EM. Serotonin content of foods: effect on urinary excretion of 5-hydroxyindoleacetic acid. *Am. J. Clin. Nutr.* 42(4), 639–643 (1985).

61 Ross G, Weinstein IB, Kabakow B. The influence of phenothiazine and some of its derivatives on the determination of 5-hydroxyindoleacetic acid in urine. *Clin. Chem.* 4(1), 66–76 (1958).

62 Bajetta E, Ferrari L, Martinetti A *et al.* Chromogranin A, neuron specific enolase, carcinoembryonic antigen, and hydroxyindole acetic acid evaluation in patients with neuroendocrine tumors. *Cancer* 86(5), 858–865 (1999).

63 Seregni E, Ferrari L, Bajetta E, Martinetti A, Bombardieri E. Clinical significance of blood chromogranin A measurement in neuroendocrine tumours. *Ann. Oncol.* 12(Suppl. 2), S69–S72 (2001).

64 Carling RS, Degg TJ, Allen KR, Bax ND, Barth JH. Evaluation of whole blood serotonin and plasma and urine 5-hydroxyindole acetic acid in diagnosis of carcinoid disease. *Ann. Clin. Biochem.* 39(6), 577–582 (2002).

65 Bhattacharyya S, Toumpanakis C, Chilkunda D, Caplin ME, Davar J. Risk factors for the development and progression of carcinoid heart disease. *Am. J. Cardiol.* 107(8), 1221–1226 (2011).

66 van der Horst-Schrivers AN, Post WJ, Kema IP *et al.* Persistent low urinary excretion of 5-HIAA is a marker for favourable survival during follow-up in patients with disseminated midgut carcinoid tumours. *Eur. J. Cancer.* 43(18), 2651–2657 (2007).

67 Zandee WT, Kamp K, van Adrichem RC, Feelders RA, de Herder WW. Limited value for urinary 5-HIAA excretion as prognostic marker in gastrointestinal neuroendocrine tumours. *Eur. J. Endocrinol.* 175(5), 361–366 (2016).

68 Oberg K, Couvelard A, Delle Fave G *et al.* ENETS consensus guidelines for standard of care in neuroendocrine tumours: biochemical markers. *Neuroendocrinology* 105(3), 201–211 (2017).

69 Oberg K. Circulating biomarkers in gastroenteropancreatic neuroendocrine tumours. *Endocr. Relat. Cancer.* 18(Suppl. 1), S17–S25 (2011).

70 Modlin IM, Oberg K, Taylor A, Drozdov I, Bodei L, Kidd M. Neuroendocrine tumor biomarkers: current status and perspectives. *Neuroendocrinology* 100(4), 265–277 (2014).

71 Tohmola N, Itkonen O, Sane T *et al.* Analytical and preanalytical validation of a new mass spectrometric serum 5-hydroxyindoleacetic acid assay as neuroendocrine tumor marker. *Clin. Chim. Acta.* 428, 38–43 (2014).

72 Nolting S, Kuttner A, Lauseker M *et al.* Chromogranin a as serum marker for gastroenteropancreatic neuroendocrine tumors: a single center experience and literature review. *Cancers (Basel)* 4(1), 141–55 (2012).

• **Showed the diagnostic sensitivity of 5-HIAA was similar to chromogranin A (69 and 68%) in patients with small intestinal NET. In those with metastatic liver disease, the sensitivity of 5-HIAA was higher than chromogranin A (86 vs 77%).**

73 Degg TJ, Allen KR, Barth JH. Measurement of plasma 5-hydroxyindoleacetic acid in carcinoid disease: an alternative to 24-h urine collections? *Ann. Clin. Biochem.* 37(5), 724–726 (2000).

74 Miller AG, Brown H, Degg T, Allen K, Keevil BG. Measurement of plasma 5-hydroxyindole acetic acid by liquid chromatography tandem mass spectrometry-comparison with HPLC methodology. *J. Chromatogr. B, Analyt. Technol. Biomed. Life Sci.* 878(7–8), 695–699 (2010).

75 Tellez MR, Mamikunian G, O'Dorisio TM, Vinik AI, Woltering EA. A single fasting plasma 5-HIAA value correlates with 24-hour urinary 5-HIAA values and other biomarkers in midgut neuroendocrine tumors (NETs). *Pancreas* 42(3), 405–410 (2013).

76 Adaway JE, Dobson R, Walsh J *et al.* Serum and plasma 5-hydroxyindoleacetic acid as an alternative to 24-h urine 5-hydroxyindoleacetic acid measurement. *Ann. Clin. Biochem.* 53(5), 554–560 (2016).

•• **Similar diagnostic performance was shown for urine and plasma 5-HIAA in this study.**