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Non-invasive screening for neuroendocrine tumors—Biogenic amines as neoplasm biomarkers and the potential improvement of "gold standards"

Natalia Miękus [a], Tomasz Bączek [b,*]

[a] Department of Animal and Human Physiology, University of Gdańsk, Wita Stwosza 59, 80-308, Gdańsk, Poland
[b] Department of Pharmaceutical Chemistry, Medical University of Gdańsk, Hallera 107, 80-416, Gdańsk, Poland

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ABSTRACT

Early, noninvasive diagnosis of tumors is relevant, especially for rare, often asymptomatic, thus – hard to detect in curable stages of the disease – tumors, as neuroendocrine tumors (NET). To avoid or supplement the NET management *via* application of invasive biopsy or expensive imagining techniques, the biochemical evaluation of biomarkers from easy accessible body fluids could be the great, potential diagnostic or prognostic tool. Nevertheless, already existed biochemical diagnostic tools for NET must be improved. Biogenic amines' (BA) determination in biological samples is significant for the description of the most NET, such as pheochromocytoma, neuroblastoma or carcinoid tumor. The bioanalytical approaches applied for the analysis of BA concentration in patient's body fluids still are required to be improved. It is caused by the low BA levels in real samples, their distinct physiochemical properties, light sensitiveness and easy degradation in the presence of oxygen, among others. Moreover, the interpretation of single analyte result is not clinically sufficient recently and more precise biomarkers or – more ideally – panels of several biomarkers are considered to be simultaneously measured and analyzed. Therefore, the NET-management "gold standards" can be routinely modified. Accordingly, presented review will focus on the recent status of BA analysis treated as the potential biomarker in terms of analytical method development applied for the real patients' samples analysis. Furthermore, the main advantages of current dominance of panel of biomarkers analysis for the NET patients diagnosis, follow up and monitoring of the therapy, will be underlined.

1. Introduction

Neuroendocrine tumors (NET) are the group of rare tumors originating from diffused endocrine system. They can develop in almost all parts of the body, predominantly in the gastrointestinal track (GI) and pancreas [1,2]. Despite their different organ localization they all possess ability to secrete distinct bioactive compounds as chromogranin A (CgA), synaptophisin, and various biogenic amines (BA), as serotonin (5-HT), adrenaline (A), noradrenalin (NA) and dopamine (DA). This capacity is caused by their common origin from neural tube [3,4]. Due to the extensive excretion of bioactive compounds, NET can lead to numerous endocrine syndromes. The most well-known is "carcinoid syndrome" (it includes serious symptoms as flushing and diarrhea, among others), which is caused *inter alia* by the overproduction of 5-HT and occurs in few patients with GI NET. Also hypertension and tachycardia provoked by the overproduction of A and NA is much observed within the group of NET patients as pheochromocytoma. All NETs that pos-

Abbreviation: NET, neuroendocrine tumors; GI, gastrointestinal track; CgA, chromogranin A; BA, biogenic amines; 5-HT, 5-hydroxytryptamine (serotonin); A, adrenaline; NA, noradrenalin; DA, dopamine; NBL, neuroblastoma; 5-HIAA, 5-hydroxyindoleacetic acid; CAT, catecholamines; IND, indoleamines; 5-HTP, 5-hydroxytryptophan; HVA, homovanillic acid; VMA, vanillylmandelic acid; DOPAC, 3,4-dihydroxyphenylacetic acid; 3-MT, 3-methoxytyramine; L-DOPA, levodopa; RIA, radioenzymatic assay; GC, gas chromatography; HPLC–MS/MS, high-performance liquid chromatography-tandem mass spectrometry; CE, capillary electrophoresis; LOD, limit of detection; UV/VIS, ultraviolet-visible spectroscopy; LIF, laser induced fluorescent; MS, mass spectrometer; EIA, enzyme immunoassay; MEKC, Micellar electrokinetic chromatography; NME, normetanephrine; ME, metanephrine; ECD, electrochemical detection; ELISA, enzyme-linked immunosorbent assay; SALDI-TOF-MS, surface-assisted laser desorption/ionization time-of-flight mass spectrometry; SPE, solid phase extraction.

* Corresponding author.
E-mail address: tbaczek@gumed.edu.pl (T. Bączek).

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sess the ability to secrete higher amounts of bioactive compounds are called "functioning NET". Nevertheless, most of NET belong to the subgroup of non-functioning tumors and secrete only trace amounts of bioactive amines and peptides into the circulation [5]. In the second case, BAs' concentrations in the circulation are insufficient to cause easily detectable clinical symptoms making their noninvasive detection in early stage of the disease complicated [3,5]. Knowing that the early diagnosis of NET is up most important for the evaluation of the presence or level of a disease or assessment of the efficacy of various therapies [6,7], the biochemical analysis of tumor samples should be continuously improved. There is a huge interest in NET biomarkers evaluation from all the biomarkers categories (from 0 to II assigned by the National Institutes of Health) (Fig. 1) [7]. Due to the fact, that BA or their metabolites could be type as biomarkers at each level (as the measurement of 5-hydroxyindoleacetic acid (5-HIAA) – main serotonin metabolite – in the 24-h urine collection), their importance in NET management is clear [7]. Therefore, there is a need to optimize modern bioanalytical methods, which could be applied for the trace amounts of BA biomarkers analysis from easy accessible body fluids of patients suffering from NET. This review aims to present efforts of today's science to minimize the limitations of common analytical techniques for the analysis of low concentrations of biogenic amines, their precursors and main metabolites for the early detection, monitoring of progression and evaluation of the outcome of the therapy of some particular types of NET as carcinoid, pheochromocytoma and neuroblastoma (NBL). The review will focus mainly on the nowadays trends in the BAs' analysis from NET patients' samples and is organized by the subgroup of analytes: the L-tyrosine derivatives (catecholamines, CAT) or L-tryptophan derivatives (indoleamines, IND) (Fig. 2). Each paragraph will also pointed out the importance of panel of biomarkers analysis in opposition to analysis of each compound alone [8].

2. Tyrosine derivatives – catecholamines and their metabolites

Among NET there are catecholamine-producing tumors. They can develop within the adrenal gland and are named pheochromocytomas. They could also develop beyond the adrenal gland and then they are called paragangliomas. Mostly they develop sporadically but also could be connected to familial syndromes as multiple endocrine neoplasia type 2 or von Hippel Lindau disease [9]. Interestingly, despite oversecretion of the catecholamines, some patients with catecholamine-producing tumors do not demonstrate any significant symptoms since the amount of CAT produced into circulation is in the nanomolar range. That fact makes the diagnosis of those tumors even more complicated [8,10–12]. Moreover, for the diagnosis of neuroblastoma the determination of CAT is advantageous, as well [13,14]. Neuroblastoma is the most common extracranial solid tumor of childhood [9,15]. Therefore, there is an urgency to determine CAT from easily accessible body fluids for the detection of catecholamine-producing NET tumors [16]. The development of the modern bioanalytical methods significantly improved the determination of CAT from biological matrices for NET tumor detection but still there is a need to replace the previously applied methods (based on the radioenzymatic (RIA) and immunological assays, gas chromatography (GC) or fluorometry techniques) by more sophisticated and advanced methods based on techniques such as high-performance liquid chromatography with tandem mass spectrometry (HPLC–MS/MS) [17–19] or capillary electrophoresis (CE) [20–23]. Those modern analytical platforms allowed not only the high-throughput of the analysis and obtainment of lower limits of detection (LODs) for all analytes of interest but also the simultaneous analysis of several compounds.

Among CAT, adrenaline and noradrenalin are routinely determined for the NET biochemical diagnosis or monitoring of the progression of the tumor. The A, NA measurement was considered as the gold standard for catecholamine-producing NET biochemical diagnosis, for a decade. As an example, Goldenberg et al. demonstrated the usefulness of photofluorometric evaluation of urinary A and NA level for the diagnosis of pheochromocytoma. The isolation of CAT from the hydrolyzed urine was carried out by the use of aluminum oxide column and for a long time this method has been applied for CAT isolation [24–27]. The proper sample preparation is almost as relevant as the selection of the most compatible, sensitive and specific method of separation and detection. Nevertheless its application for the long time, the aluminum oxide-based methods suffer from interference caused by several drugs and are time-consuming, among others [28]. Kushnir et al. presented the predominance of the use of diphenylboronic acid 2-aminoethyl ester-based extraction to be more effective than cation-exchange and aluminum-based solid phase extraction for the CAT isolation from biological samples [28] and the sample preparation methods were further improved and achieved. Even the most analytically-advanced methods for sample preparation as magnetic molecularly imprinted polymer were applied for A and NA determination in urine [23]. In terms of the method of detection improvement, Westermann et al. proposed the non-competitive enzyme immunoassay for the determination of A and NE in urine samples. The newly proposed immunoassay was compared to HPLC electrochemical detection with extraction by aluminum oxide and was found to be a complementary method for A and NA analysis for the pheochromocytoma diagnosis [29]. Nevertheless, the high accuracy and reliability of HPLC-based methods are the main features of HPLC which made this technique frequently applied for the CAT analysis in NET biological samples nowaday [29,30]. Chadaram *et al.* noted that previously the flurometric method of detection has been applied for A and NA analysis but due to its irreproducibility, he dedicated his research for the HPLC with ultraviolet–visible spectroscopy (UV/VIS) detection [31]. Interestingly, the use of UV/VIS was demonstrated as convenient for CAT analysis and the usefulness of UV/VIS detector was discussed. UV/VIS had at least one clear advantage over the other types of detectors (such as laser induced fluorescent detector (LIF) or mass spectrometer (MS)): it is accessible in almost all diagnostic laboratories. Moreover, UV/VIS is versatile, fast and accurate for the analysis of various compounds as CAT not only while is coupled to HPLC but also when is coupled to CE [21,23].

Further studies of pheochromocytoma indicated the necessity for determination likewise DA in the patients' body fluids, since some tumors can secrete to body fluids not only A and NA but also there are some pheochromocytomas with registered higher concentrations of DA and normal A and NA levels. Dubois et al. stressed the necessity to determine A, NA together with DA from the biological fluids of the patients for the unbiased diagnosis of those tumors [8,32]. As well, for neuroblastoma there is a need to screen for the urine levels of DA and even levodopa (L-DOPA) [8,33]. Simultaneous determination of DA, A and NA could be assessed using a specific RIA or HPLC methods [34]. However, while applying above-mentioned approaches, the issue of false-negative results for the NET diagnosis remained even while those three CAT were measured at the same time applying the most sophisticated bioanalytical methods. Therefore, nowadays there is a trend to develop new methods to clear out the false-positive results for the diagnosis of catecholamine-secreting tumors. Moreover, the measurement of not only the catecholamines but also their precursors and metabolites are deeply studied.

The CAT metabolites' analysis as the importance of the main CAT metabolites: homovanillic acid (HVA) and vanillylmandelic acid (VMA) determination in the urine were pointed out time ago.

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