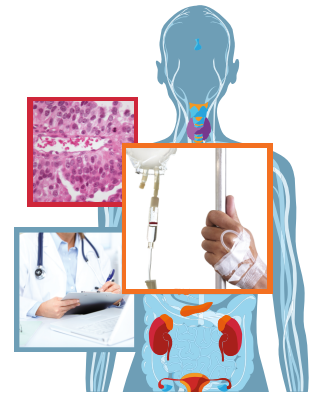
Editorial



International Journal of Endocrine Oncology

Bad kits in the diagnosis of endocrine tumors

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First draft submitted: 30 January 2020; Accepted for publication: 6 February 2020; Published online: 6 March 2020

Keywords: diagnostics • endocrine tumors • hormone chaperones (granins) • immunoassays • peptide-hormone biosynthesis • peptide-producing tumors • plasma measurements • post-translational processing

Bad kits are like bad kids. Both behave badly. But bad kits can be pretty harmful to innocent people, including patients with serious malignant diseases. Several factors are necessary to explain the occurrence of bad behaving kids and kits. The following, however, will focus on some commercial, immunochemical kits used diagnostically in endocrine oncology.

The correct diagnosis of hormone-producing tumors requires measurements of the true biomarker concentrations in circulation. Often the biomarker is a peptide hormone or neuroendocrine chaperone protein, whose concentration is to be measured immunochemically, using assay technologies such as a radioimmunoassay (RIA) or enzyme-linked immunosorbent assay. It seems simple and straightforward, which it is when you are dealing with simple systems. For instance, the measurement of insulin in patients suspected for harboring an insulin-producing tumor, an insulinoma. The simplicity of insulin measurement is, however, atypical and primarily due to the fact that there is only one insulin molecule (except in rats). Moreover, the concentration levels of insulin in plasma are in a relatively high picomolar range. In addition, it is easy to raise specific high-titer and high-affinity antibodies to insulin for immunoassay purposes.

In contrast to insulin, however, most other peptide hormones and endocrine chaperone proteins – like the granins – are complex, heterogeneous systems, which are extensively processed during the post-translational phase of their biogenesis, both in normal endocrine cells and in tumor cells [1,2]. Consequently, these cells release not a single hormonal molecule to blood, but a mixture of peptides and protein fragments with varying size, bio- and immuno-reactivity. The cellular post-translational processing of prohormones and granins is extensive and includes three sorts of processes: endoproteolytic cleavages, primarily at mono- and di-basic sites (i.e., at C-termini of arginyl- and lysyl-residues); exoproteolytic trimmings of N- and C-termini of terminal amino acid residues (in order to protect against amino- and carboxy-peptidase degradations) and amino acid derivatizations, of which there are many different types. The modifications are well-described for some peptide–hormone systems, but far from fully elucidated for all [3,4]. For instance, the types and extension of glycosylations of peptide hormones have only just come into recognition in recent years [5]. The degree and chemical nature of the molecular heterogeneity has to be considered when the hormones and their chaperones are going to be measured for diagnostic purposes. Not least because the molecular pattern of heterogeneity in plasma may vary between tumor patients and healthy persons [6,7]. Thus, the molecular heterogeneity of the tumor marker is one side of the coin.

The other side is the immunochemistry of the assay used for the tumor marker. For the immunoassay, it is important to realize that the binding site of an antibody has a size corresponding to a peptide sequence of four to seven amino acid residues [8]. Exactly how many residues depends on the size of each residue. Consequently, the antibody will recognize only a small part of a protein or peptide tumor marker. If the epitope is present in other proteins or peptides, the immunoassay will be unspecific and often useless for diagnostic purposes. But even if the

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epitope is specific, the hormone heterogeneity may cause decisive problems for the antibody binding, if the epitope is cleaved, trimmed and/or derivatized.

One way out of these problems is to use an analysis that is independent of post-translational modifications, in other words, the molecular heterogeneity. Such 'processing-independent analysis' (PIA) can be established by careful examination of the prohormone structure and finding an epitope sequence, which is known neither to be cleaved nor derivatized; but it has at its N-terminus to neighbor a trypsin-sensitive cleavage site. If you then pre-analytically trypsinize the plasma sample, the selected epitope will be exposed and can be quantitated with the immunoassay specific for the N-terminal sequence of the epitope. We have developed such PIA-assays for a number of peptide hormones and for a general neuroendocrine tumor marker (chromogranin A) [9–12]. The PIA-assays seem to fulfill their promises, not only in terms of diagnosis, but also in prognosis, because they provide a measure of the tumor burden.

During the last 3 to 4 decades of the previous century, the assays for endocrine tumor markers were developed in research laboratories at universities and university hospitals, often in association with clinical projects to improve the diagnosis and therapy for patients with these relatively rare, but often serious neuroendocrine tumors. The academic, scientific background of the laboratories that developed the original immunoassays ensured that the available basic biochemical, pathobiochemical and oncologic knowledge about the endocrine tumor markers and the tumors was taken into account in the design of the assays. Consequently, the reliability of these assays was quite high regarding accuracy, quality of the plasma measurements and the diagnostic sensitivity and specificity. But this picture has now changed. Hence, commercial diagnostic companies have, during the last 2 or 3 decades, now taken a dominant position in the production of diagnostic kits for endocrine tumors (often in association with new automatized analytical platforms), for which the proper diagnosis in the majority of patients suspected to have endocrine tumors today is now dependent on the quality of commercial immunoassay kits. Among these, there are indeed some bad kits as described in studies from the last decade.

One study was regarding gastrin kits [7]: 12 years ago, some gastroenterologists began to note that occasionally patients with symptoms of fulminant Zollinger–Ellison syndrome (which is caused by hypersecretion from gastrin-producing tumors [gastrinomas]) were denied the expected gastrinoma diagnosis. The reason was that their gastrin concentrations in plasma were measured to be normal. These Zollinger–Ellison patients, however, were in hospitals whose diagnostic laboratory used commercial kits for gastrin measurements. Therefore, in order to examine the situation, we bought available commercial kits on the market (n = 12) and compared their results with those of a thoroughly validated in-house gastrin radioimmunoassay, on plasma samples from 40 patients with well-characterized Zollinger–Ellison syndromes. The results indicated that more than half (seven) of the kits were misleading. In other words, they were bad. The false low results caused months of delays in the diagnosis, which resulted in severe complications of nearly fatal character. According to the guiding kit instructions from the commercial manufacturers, the mismeasuring kits had been insufficiently validated.

CgA is a widely used general marker for neuroendocrine tumors. Like most prohormones, however, the CgA protein undergoes extensive cellular processing and therefore also circulates as a mixture of modified peptide fragments [13]. Consequently, the results of different CgA-assays vary and so does the diagnostic sensitivity of the measurements in patient plasma. Two recent studies have examined commercial CgA kits and again compared the kit results with those of carefully validated in-house CgA RIAs (including a CgA-PIA assay) [14]. In the largest study, the material comprised plasma samples from 130 well-characterized patients with small intestinal neuroendocrine tumors [UNPUBLISHED DATA]. The results demonstrated that three commercial CgA kits displayed an unacceptably low diagnostic sensitivity. They were bad. But even the most sensitive commercial kits missed the diagnosis in some patients with metastatic disease. The best results were obtained with the in-house CgA-PIA assay [12].

Although glucagonomas and cholecystokininomas are rare tumors [15,16], patients with the specific syndromes caused by glucagonomas and cholecystokininomas also deserve the correct diagnosis. But again – as detailed above for CgA in neuroendocrine tumors and progastrin in gastrinomas, proglucagon and procholecystokinin are also heavily processed during the post-translational maturation to several different peptides. Consequently, the concentrations measured by the vast number of commercial kits on the market vary so widely that their use in tumor diagnosis is questionable [17–19]. Again, a majority of these kits need careful reliability assessments for measurements.

A last, but different problem with commercial kits, even with those of acceptable diagnostic quality, is a business-attitude among kit-producers, which copes poorly with the ethics of medical diagnostics. Hence, some diagnostic companies suddenly and without warning discontinue their production of selected kits. Such discontinuation may

jeopardize a timely diagnosis in tumor patients and endanger costly oncological projects at hospitals. This behavior is bad, calling for a regulation of the market for diagnostic kits in a manner similar to that of the pharmaceutical drug market.

Acknowledgments

The skillful secretarial assistance of C Bundgaard (MA) is gratefully acknowledged.

Financial & competing interests disclosure

The author has 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.

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