Serum Pancreastatin

The Long Sought Universal, Sensitive, Specific Tumor Marker for Neuroendocrine Tumors?

Tetsuhide Ito, MD, PhD, Hisato Igarashi, MD, PhD,* and Robert T. Jensen, MD†*

One of the principal priorities identified at the National Cancer Institute summit for improving the management of neuroendocrine tumors ([NETs] pancreatic endocrine tumors and carcinoids)[1] was the need to identify tumor markers that could be used for early diagnosis and management. All clinicians who manage these patients would agree with this.[2] These are needed because most NETs (especially early in their course) are nonfunctional,[3] and they are usually discovered by endoscopic/imaging methods. Furthermore, their natural history is frequently long; they vary markedly in their aggressiveness and in their responses to various treatments, with the result that repeated imaging is required.[1,2] This is not only expensive, it is frequently inconvenient, and can, in many cases, result in late diagnosis.[1,2] The ideal would be to have a tumor marker that could be easily assessed (serum/plasma/urine), that is sensitive and specific for NETs, and whose magnitude correlates with the extent and rate of tumor growth, so that it could be used for both diagnosis and management. In this issue of *Pancreas*, the specificity of a newly described plasma assay for the CgA fragment pancreastatin is reported by Raines et al[4] that might be a useful candidate for fulfilling some of these requirements for the desired NET tumor marker. However, to understand why this could be an important step forward, it is important to summarize where we are at present.

Unfortunately, at present, no assay fulfills the above requirements for a generally useful tumor marker in patients with most of the NETs. For the functional pancreatic endocrine tumors ([PETs] gastrinomas, insulinomas, VIPomas, etc), assessment of the specific hormone or related fragments allows diagnosis when coupled with the appropriate assessment of hormone excess (acid secretion, hypoglycemia, etc) and, in some cases, their magnitude or changes in the magnitude of their serum levels correlate with tumor growth/extent.[5] Similarly, with carcinoid syndrome, associated in more than 95% of cases with metastatic disease in the liver, the assessment of blood serotonin/urinary breakdown products (5-hydroxyindoleacetic acid) allows diagnosis and has a prognostic significance. However, in various series, 50% to 75% of all PETs are not associated with a functional hormonal syndrome (ie, nonfunctional PETs), as is the case in all early carcinoids and most advanced carcinoid disease that is non-midgut in location. Therefore, for up to 60% to 95% of NET patients in various series, no hormonal marker exists for any disease phase.

Chromogranins (Cg), particularly chromogranin A (CgA), is found in the neurosecretory granules of almost all well-differentiated NETs, whether functional or nonfunctional; is commonly used immunocytochemically to establish their diagnosis; and circulates in nanomolar concentrations in the blood, thus was originally hoped would fulfill all the needed requirements for a universal blood NET tumor marker.[6–8] Unfortunately, it has failed to fulfill this promise for a number of reasons. There is no standardization of CgA assays resulting in divergent values in different assays, and many of the assays are not fully characterized, so it is unclear exactly what is being measured. Chromogranin A is a large peptide (MW-48,918) consisting of 439 amino acids and has an apparent molecular weight of 74,001 in tissues because of glycosylation and other modifications (Fig. 1).[9] It has 10 dibasic cleavage amino sites[9] (Fig. 1), which results in the generation of a number of different fragments, both in the NET and blood (Fig. 1).[6–10] A number of these fragments are reported to have biological activities, such as inhibitory effects on secretion of various hormones (insulin, parathyroid hormone, catecholamine secretion), effects on intermediary metabolism and carbohydrate and lipid metabolism, regulation of cardiovascular function, and regulation of inflammatory responses and reproduction.[6,7] If assay antibodies interact with varying degrees with the different CgA degradation products, then the assessment of blood concentrations between different assays becomes affected by many variables, including the fact that different tumors may degrade CgA differently and can contribute to nonuniformity of the results with different assays. Furthermore, it is further complicated by the fact that no

From the *Department of Medicine and Bioregulatory Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; and †Digestive Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD.
Reprints: Robert T. Jensen, MD, National Institutes of Health, Building 10, Room 9C-103, Bethesda, MD 20817 (e-mail: robertj@bdg10.niddk.nih.gov).
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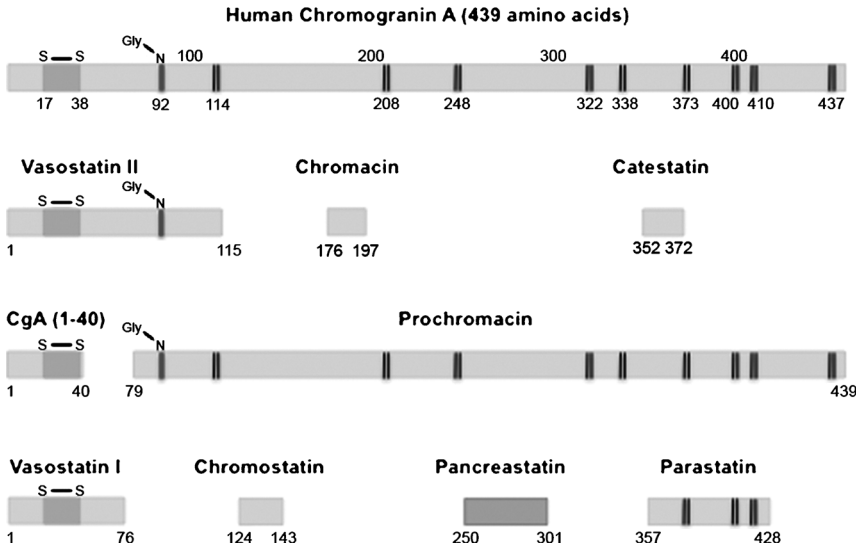

FIGURE 1. Schematic representation of chromogranin A and its postulated biologically active sequences with the sites of proteolytic cleavage at dibasic amino acids indicated (vertical lines). Gly-N indicates putative N-linked glycosylation site; S-S, sites of a disulfide linkage. The numbers below each sequence refer to the location in chromogranin A.

one completely agrees on which of these products best reflects the needed diagnostic and management features of a good blood tumor marker. The full characterization of the antibody's specificity, even if the exact molecule needed to be measured is agreed on, is not a trivial consideration. This point is clearly demonstrated in a recent article on the assessment of serum gastrin concentration, which is a widely used radioimmunoassay in medicine. Numerous NH_2 and COOH terminal extended gastrins circulate that are sulfated and nonsulfated. Whereas there is general agreement that assessment of the biologically active amidated COOH terminus is clinically important, a recent study demonstrates[11] that 7 of 12 commercially available assays do not accurately measure the clinically important gastrin because they are using not fully characterized antibodies. Therefore, even if agreement is reached on the CgA molecule to be measured, good characterization and standardization are needed.

There is disagreement in the literature whether blood CgA levels correlate with the extent of tumor or growth of the tumor and are useful for the management of NETs.[5,6,12,13] Furthermore, blood CgA levels are frequently decreased by the use of somatostatin analogs that are commonly used in the treatment of functional and nonfunctional NETs either alone or in combination with other drugs, and it is unclear whether their effect is variable, making it difficult to perform assessments when these commonly used drugs are administered. Assessment of CgA levels has also failed as a tumor marker for the early detection of NETs. This has primarily occurred because CgA levels are affected not only by the NET, but numerous other processes including inflammatory disorders, other endocrine diseases, gastrointestinal disorders, cardiovascular diseases, renal function, and, particularly important, the use of potent acid suppressant drugs such as proton pump inhibitors (PPIs).[6,14] The PPIs are a particular problem because they increase blood CgA in 90% to 100% of patients with protracted use; they are widely used and available now over the counter so their use is often not even mentioned in the medical history; they increase blood CgA after only 5 days of use and they can lead to CgA levels 5- to 10-fold normal, which overlap with that seen in many patients with early NETs.[15]

It is in this latter context that the study by Raines et al,[4] reporting results of a recently described specific radioimmunoassay for pancreastatin[12] in patients taking PPIs, is of particular interest. Human pancreastatin is a 52–amino acid peptide corresponding to CgA (250–301)[9] (Fig. 1), and its generation from CgA depends to a large part on the activity of prohormone convertase-1.[16] Pancreastatin is present in NETs,[17,18] is present in picomolar concentrations in normal plasma [12,19] primarily as the CgA (250–301) form and in higher molecular weight forms.[19] The results of the study by Raines et al[4] are particularly important because they show no increase in plasma pancreastatin levels in patients chronically taking PPIs, whereas serum gastrin is increased in all and CgA levels in almost 70%.[4] These results are of particular interest because they raise the possibility that plasma pancreastatin could be a more sensitive and specific assay than CgA in detecting early NETs as well as nonfunctional NETs and possibly useful for management. Is there any evidence to support these speculations? The principal finding of this study is supported by results of an early study by Syversen et al[20] using a specific pancreastatin assay in patients with gastrinomas, where they found plasma pancreastatin levels to correlate closely with gastrin levels, but not with CgA levels, leading them to propose the there is little or no processing of CgA to pancreastatin in ECL cells; whereas in gastrinoma cells, it is extensive. Will this assay be sensitive enough to be useful? That remains to be proven. In one study[20] using a different antibody from that used in the study by Raines et al,[4] plasma pancreastatin levels were normal in 44% of patients with gastrinomas[20] and in another study in 27% of patients with NETs and 100% of patients with NETs with only lymph node metastases.[21] Will it be specific enough for NETs? At present, this is also not proven.

Hopefully, lessons will be learned from the experience with CgA, and these questions will be answered rapidly. Particularly important will be, in all assays for plasma pancreastatin, the complete characterization of all antibodies generated for cross-reactivity with the various CgA products that could react within the assay (Fig. 1). Next, the inclusion of patients with a wide variety of conditions that lead to false-positives, as seen in the

CgA assay, needs to be specifically done, as well as a clear determination of sensitivity. Last, correlations with important clinical changes in tumor growth/size will need to be done. The present study by Raines et al[4] is important because the data suggest that the new assay could be a significant advance.

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