A 53-year-old man experienced periodic abdominal discomfort and a decreased capacity to work. His primary physician ordered a broad range of laboratory tests as part of the initial workup. The results revealed a greatly increased adrenocorticotropic hormone (ACTH) concentration of >1250 pg/mL (>278 pmol/L) [reference interval <46 pg/mL (<10.2 pmol/L)]. Cortisol was within the reference interval. Repeat measurements 4 weeks later confirmed the increased ACTH. Investigators rapidly excluded 2 well-known conditions associated with increased ACTH concentrations: Cushing disease (ACTH-producing pituitary tumor) and Addison disease (adrenal insufficiency) (1, 2). An investigation for an ectopic source of ACTH was begun (3).

Over the next 18 months, the patient underwent a plethora of imaging studies. A series of conventional studies failed to provide an explanation for the increased ACTH, and ultimately a positron emission tomography/computed tomography (PET/CT) scan using a relatively new radiotracer, 68Ga-labeled 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid-D-Phe1-Tyr3-octreotide (68Ga-DOTATOC), was performed (4). A 3.3-cm area in the head of the pancreas with an increased uptake of radiotracer was observed (Fig. 1). In light of the persistently increased ACTH concentration, this finding raised the suspicion of a pancreatic ACTH-secreting neuroendocrine tumor, a rare ectopic source of ACTH (3). Although MRI and conventional CT evaluations did not confirm the presence of a tumor, the patient was offered immediate surgical treatment. The patient declined the offer and subsequently sought second and third opinions at medical facilities in 2 different countries. In both facilities, a neuroendocrine tumor was deemed the likely cause of his problems, and surgery was again suggested. Wishing minimally invasive treatment, the patient contacted the Interventional Centre at our hospital, which offers laparoscopic resection of the pancreas.

Preoperative investigations with MRI, optimized multiphase CT, and 111In-labeled diethyleneetriamine pentaacetic acid octreotide (111In-DTPA-octreotide) single-photon emission computed tomography/CT (SPECT/CT), a well-established protocol for visualizing neuroendocrine tumors (4), failed to identify the supposed tumor. The data from the previously positive 68Ga-DOTATOC PET/CT evaluation were requested for reinvestigation, and surgery was postponed.

Laboratory results at our hospital were comparable with the earlier results. ACTH, measured in a morning sample on the Immulite 2000 platform (Siemens Healthcare Diagnostics), was highly increased at 923 pg/mL (203 pmol/L). Cortisol, measured concurrently on the Modular E platform (Roche Diagnostics), was normal at 16.9 μg/dL (467 nmol/L) [reference interval for morning samples, 8–25 μg/dL (220–690 nmol/L)]. Results for other hormones, electrolytes, and tumor markers (neuron-specific enolase, chromogranin A, serotonin metabolites) were unremarkable. An endocrinologist could not find convincing clinical evidence of pathology in the pituitary–adrenal axis (specifically, no hyperpigmentation of the skin) to support the laboratory findings. He suggested

CASE

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that the persistently increased ACTH could be a laboratory artifact.

Four laboratories in Norway currently offer analysis of ACTH; however, troubleshooting was complicated because all of the laboratories use the same commercial assay. To investigate potential heterophilic antibody interference, we routinely add aggregated murine monoclonal MAK33 (Roche Biochemicals) to samples and reassay. Unfortunately, the addition of aggregated MAK33, even in high concentrations, had no effect on the ACTH result.

**DISCUSSION**

Causes of an increased ACTH value can include (a) increased pituitary secretion of ACTH due to adrenal insufficiency (primary adrenal insufficiency), (b) increased secretion from a tumor in the pituitary (Cushing disease), or (c) ectopic secretion by neuroendocrine tumors outside the pituitary, ranging from small-cell lung cancers to carcinoids. Other causes are rare.

If performed and interpreted adequately, standardized test protocols such as the dexamethasone suppression test or the ACTH stimulation test often reveal pathology in the pituitary–adrenal axis quite effectively (1, 2). The workup in such a case is rarely straightforward, however, and these tests are typically performed in parallel with clinical and radiologic investigations. According to the clinical notes presented by our patient, these tests had been performed early in the workup, but perhaps understandably, the pancreatic lesion and the persistently increased ACTH results complicated the interpretation.

While absent in our patient, hyperpigmentation and electrolyte disturbances can provide diagnostic clues in patients with increased ACTH. Several melanocyte-stimulating hormones are normally produced along with ACTH via cleavage of the common precursor protein, proopiomelanocortin. Importantly, hyperpigmentation is absent in some patients with ectopic ACTH production, either because ACTH is produced independently of the melanocyte-stimulating hormones or because the ACTH-producing tumor is so aggressive that hyperpigmentation does not have time to develop. The severity and pattern of associated electrolyte disturbances depends on the primary cause of the increased ACTH. Primary adrenal insufficiency typically causes hyponatremia and hyperkalemia, whereas ACTH-secreting tumors (pituitary or ectopic) are associated with treatment-resistant hypertension and hypokalemia.

It is challenging to measure and interpret the concentrations of pituitary and adrenal hormones. Their short plasma half-lives and their pulsatile and circadian secretion necessitate wide and time-dependent reference intervals. Single measurements usually give very limited information. Because of the instability of ACTH, sample processing must be undertaken rapidly, and if not assayed immediately, plasma samples must be flash-frozen and stored at −70 °C.

ACTH is usually quantified by immunometric assays. These methods use a solid-phase antibody to capture the analyte from the biological matrix. A second labeled tracer antibody then creates a “sandwich,” where the analyte is caught between the 2 antibodies. The apparent simplicity of this assay format belies its sensitivity to assay interference. Because ACTH-secreting pancreatic tumors are rare entities and because our patient had little clinical evidence to match the laboratory anomaly, our first priority was to validate the result for ACTH. Perhaps the most common and pernicious cause of anomalous immunoassay results is the presence of heterophilic antibodies in patient samples (5–7). These endogenous immunoglobulins can cross-link the assay antibodies to yield falsely positive results in the absence of analyte. Heterophilic antibodies are produced in response to no clear immunogen and are defined by their marked nonspecificity (8). Although they are found in individuals with autoimmune or inflammatory conditions, they also occur in healthy individuals. Human antianimal antibodies produced in response to therapeutic immunoglobulins are an additional source of assay interference.

**CASE RESOLUTION**

In addition to adding MAK33 antibodies, we also tested the patient’s sample in “nonsense” immunomet-
Table 1. Immulite ACTH measurements after addition of aggregated rabbit IgG to the patient sample.

<table>
<thead>
<tr>
<th>Sample</th>
<th>ACTH, pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native sample</td>
<td>923</td>
</tr>
<tr>
<td>Sample stored for 10 days at room temperature</td>
<td>859</td>
</tr>
<tr>
<td>Sample blocked with rabbit IgG, 180 μg/mL</td>
<td>673</td>
</tr>
<tr>
<td>Sample blocked with rabbit IgG, 330 μg/mL</td>
<td>255</td>
</tr>
<tr>
<td>Sample blocked with rabbit IgG, 570 μg/mL</td>
<td>73</td>
</tr>
<tr>
<td>Sample blocked with rabbit IgG, 1000 μg/mL</td>
<td>18</td>
</tr>
</tbody>
</table>

* Values corrected for dilution. Factor for conversion to SI units: 1 pg/mL = 0.22 pmol/L.

Our standard method uses a murine anti–carcinoembryonic antigen monoclonal antibody (T84.66, IgG1) as the solid phase and a europium-labeled murine anti–α-fetoprotein monoclonal antibody (K57, IgG1) as the tracer. Because this assay uses a noncomplementary antibody pair it is unable to detect either carcinoembryonic antigen or α-fetoprotein. A positive signal therefore indicates the cross-linking of the reagent antibodies and hence presumptive detection of heterophilic antibody. In this assay, the patient’s plasma gave a strong signal. In contrast, nonsense assays constructed with recombinant antibodies or antibody fragments lacking the Fc region did not give any signals. These observations strongly suggested that Fc-reactive heterophilic antibodies were present in the sample. Because the Immulite assay for ACTH is designed with a monoclonal murine antibody in combination with a polyclonal rabbit antibody, we also established a nonsense assay with a polyclonal rabbit immunoglobulin as the solid phase with a mouse monoclonal tracer. A positive signal was also observed in this assay, suggesting that the heterophilic antibodies present in the patient’s plasma were able to cross-link antibodies from the same species as those used in the Immulite assay. These results did not prove that the result for ACTH was false, however.

In marked contrast to the lack of effect by the aggregated MAK33, sample pretreatment with heat-aggregated (74 °C for 5 min) rabbit immunoglobulin (Dako) produced dramatic reductions in the apparent ACTH concentration as measured with the Immulite assay (Table 1). This finding strongly implicated heterophilic antibody interference as the cause of the increased ACTH result. Reassay of the native sample after storage for 10 days at room temperature produced a result similar to that for the freshly thawed sample, highly uncharacteristic given the instability of ACTH.

The pancreatic tumor identified by the 68Ga-DOTATOC PET/CT evaluation warranted further scrutiny. Because of its enhanced affinity for somatostatin receptors and improved spatial resolution, 68Ga-DOTATOC PET/CT is reportedly superior to conventional imaging with 111In-DTPA-octreotide SPECT in detecting neuroendocrine tumors (9). The latter examination did show a slightly increased signal in the pancreatic head consistent with physiological uptake in cells expressing somatostatin receptors. This uptake, however, was more pronounced in the reevaluated 68Ga-DOTATOC PET/CT examination, most likely reflecting this radiotracer’s higher affinity for the type 2 somatostatin receptors often overexpressed in the uncinate process (10). Ultimately, an endoscopic ultrasound evaluation was performed. No tumor was found, but a poorly demarcated lesion in the pancreatic head was observed, possibly indicating a low-grade focal pancreatitis. Fine-needle biopsies did not reveal tumor cells. The surgery was cancelled, and our patient was informed about the presence of heterophilic antibodies in his blood samples. He had a completely normal control CT scan 6 months later.

This case illustrates the need for vigilance among clinicians and laboratory scientists for the dangers of heterophilic antibody interference. We hope that the approach we have described may prove helpful to others when laboratory results are discordant with the clinical findings; however, the most important tool in the resolution of these cases is successful communication between clinicians and the laboratory, which thankfully requires very little technology.

**POINTS TO REMEMBER**

- When investigating potential antibody interference, neutralizing (blocking) antibodies should ideally mirror assay antibodies with respect to species and subclass.
- A lack of effect with blocking antibodies does not exclude interference, and multiple methods, such as sample dilutions, nonsense immunoassays, polyethylene glycol precipitation, and repeat assay with a different immunoassay can be useful tools in detecting assay interference.
- Modern PET protocols can be sensitive diagnostic tools, but the physiological accumulation of radiotracer in nonmalignant tissue can complicate interpretation. If a tumor with a diameter >3 cm is present, it would normally be visualized by optimized CT and MRI.
- Interdisciplinary communication between clinicians and the laboratory is vital when laboratory results and clinical findings are discordant.
Clinical Case Study

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures or Potential Conflicts of Interest: No authors declared any potential conflicts of interest.

Acknowledgment: We obtained written, informed consent from the patient before writing this manuscript.

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10. Boy C, Heusner TA, Popeppel TD, Redmann-Bischofs A, Unger N, Jentzen W, et al. ⁶⁸Ga-DOTATOC PET/CT and somatostatin receptor (sst1-sst5) expres-

Commentary

Jonathan McConathy*

This case illustrates a false-positive PET study with the radio-
labeled somatostatin receptor (SSR) ligand, ⁶⁸Ga-
DOTATOC, presumably due to physiological tracer binding to SSRs in the uncinate process of the pancreas. Radiophar-
maceuticals that target SSRs are used clinically for the detection, staging, and treatment of neuroendocrine tumors, in-
cluding carcinoid tumors, gastroenteropancreatic tumors, and pheochromocytomas/paragangliomas. The octreotide analog ¹¹¹In-pentetreotide has been used for 2 decades for planar and SPECT imaging of SSR-positive tumors. More recently, other analogs, including ⁶⁸Ga-DOTATOC, have been developed for PET imaging for this indication. There are several important points to consider from an imaging perspective:

1. Clinical parameters can influence image interpre-
tation, especially for a subtle or equivocal finding. Many imaging specialists would be more likely to bring attention to subtle areas of increased tracer uptake when there is clinical and/or laboratory evidence of a neuroendocrine tumor, as in this case.

2. SSR imaging combined with anatomic and morphologic imaging with CT and/or MRI often improves overall diagnostic accuracy. In some cases, SSR imaging may demonstrate pancreatic neuroendocrine tumors not seen prospectively or poorly visualized by CT alone (1); however, the lack of CT or MRI results that correlated with the relatively large 3.3-cm “lesion” in the ⁶⁸Ga-DOTATOC PET evaluation in this case was discordant and warranted a less aggressive approach.

3. The higher spatial resolution of PET and differences in affinities for SSR subtypes likely contribute to better visual-
ization of both physiological and pathologic SSR expression with ⁶⁸Ga-DOTATOC compared with ¹¹¹In-
pentetreotide. A recent publication demonstrated higher uptake of ⁶⁸Ga-DOTATOC in the uncinate process of the pancreas than in the remainder of the pancreas, a finding that corresponds to higher tissue concentrations of SSR type 2 mRNA (2). Both referring and imaging physicians should be aware of this potential source of false positives in SSR imaging with ⁶⁸Ga-DOTATOC and other newer agents.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

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Commentary

David E. Bruns*

The authors faced a very challenging patient, whose imaging and laboratory results suggested the presence of an ACTH-secreting tumor. Several laboratory findings could have been erroneously attributed to 2 phenomena, “big ACTH” and complexes of ACTH with immunoglobulin, that occur in patients with ACTH-secreting tumors and affect measurement of ACTH.

In the normal pituitary, ACTH is made by proteolytic processing of the precursor molecule proopiomelanocortin. When processing is faulty, as occurs in some cases of ectopic production of ACTH by tumors, a big form of ACTH is formed. This form is often inactive, and thus the presence of inactive, tumor-associated ACTH could easily have been postulated to explain the discrepancy between the high ACTH result and the normal electrolyte results and normal skin pigmentation seen in this patient. Moreover, “big ACTH” is measured by some assays (notably RIAs) and not by others (1). This latter fact, too, could have been wrongly used to explain the discrepant results of the 2 assays in this case, further leading the diagnostician toward an erroneous conclusion.

ACTH–immunoglobulin complexes occur in patients with malignancies. In one study, such complexes led to increased circulating concentrations of ACTH, as measured by RIA, in 4 of 7 patients with small-cell carcinoma of the lung (2). Only 1 patient had evidence of ACTH excess (Cushing syndrome). Thus, ACTH–immunoglobulin complexes offer another explanation for the discrepant clinical and laboratory findings in the patient and would have reinforced the conclusion that the patient had an ACTH-secreting tumor. Moreover, the stability of the immunoreactive ACTH could have been attributed to formation of the immune complexes, which often stabilize the structure of the complexed antigen.

The authors are to be congratulated for carrying out the extensive testing that was required to document the presence of the heterophile antibodies that explained the findings in this patient.

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