A Case of Factitious Adrenal Insufficiency after Vascular Graft Surgery Caused by Spurious Immunometric Assays

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Abstract. A 70-year-old man with abdominal aortic aneurysm underwent surgical repair with Hemashield vascular graft. Postoperatively, he was found to have very low plasma cortisol levels, which failed to increase after stimulation with ACTH. A tentative diagnosis of adrenal insufficiency was made despite the lack of its clinical manifestations and a replacement therapy with hydrocortisone was started. He had also elevated plasma levels of TSH, thyroid hormones and estrogen without any clinical manifestations. Such abnormal hormone levels were spontaneously normalized three months after operation, which was later proven to be factitious by different immunometric assays (IMA). Since the vascular graft coated with bovine type I collagen has been reported to induce a transient immune response in some patients after surgery, we speculated that certain antibodies generated against heterologous collagen and/or yet-unknown components derived from the graft may have caused such factitious data; exogenous addition of bovine type I collagen and albumin to patient’s serum, however, failed to affect the assay results. Whatever the cause, caution must be paid that some patients with surgical repair using heterologous materials may have such factitious hormone data by IMAs.

Key words: Factitious hormone data, Immunometric assays (IMA), Collagen-coated vascular graft

IMMUNOMETRIC assays (IMA) are widely used to measure very low concentrations of circulating hormones and tumor markers because of their high sensitivity and specificity. However, some factitious results from IMAs using mouse monoclonal antibodies have been reported due to human anti-mouse immunoglobulin antibodies (HAMA) [1–3]. For example, it has been reported that a patient misdiagnosed as pregnancy underwent evacuation surgery based on the false positive result of β-human chorionic gonadotropin test due to the presence of HAMA [4]. Thus, IMAs are usually pretreated with mouse immunoglobulins to block the non-specific interference by HAMA. However, a few cases with factitious results have been reported even after such pretreatment [5].

Here, we report that a patient who underwent surgical repair of an abdominal aortic aneurysm with Hemashield vascular graft, was misdiagnosed as adrenal insufficiency and hyperthyroidism from the abnormal hormone data, and treated with glucocorticoid despite the lack of their clinical manifestations. These abnormal hormone levels gradually normalized over three months after surgery, which were later proven to be factitious by IMAs, possibly due to generation of autoantibodies against heterologous antigens and/or some yet-unknown components used for vascular graft coated with bovine collagen.
Methods and Materials

Hormone measurements

The commercial assay kits used earlier were as follows; enzyme immunoassays (EIA) for cortisol and E2 (ST AIA-PACK CORT and E2: TOSOH, Tokyo, Japan) and electrochemiluminescence assay (ECLIA) for TSH, FT3 and FT4: FUJIREBIO INC., Tokyo, Japan), respectively. The commercial assay kits used later were as follows: RIA for cortisol (CORTISOL RIA KIT: IMMUNOTECH, Marseille, France), RIA for E2 (Coat-A-Count Estradiol: Diagnostic Products Corporation, Los Angeles, CA) and ECLIA for TSH, FT3 and FT4 (Elecsys TSH, FT3 and FT4: Roche Diagnostics GmbH, Basel, Switzerland), respectively.

To precipitate immunoglobulins, polyethyleneglycol (PEG) was added to plasma sample and centrifuged [6]; the supernatants were then assayed by cortisol and E2 EIA kit. To eliminate the possible influence by HAMA, hormone levels were measured by the earlier assay kits after the addition of excess (500 µg/ml) mouse polyclonal immunoglobulins or goat polyclonal immunoglobulins. To absorb potential heterophilic antibodies against type I collagen and/or bovine serum albumin (BSA: Sigma, St. Louis), plasma samples were preincubated with excess concentrations of bovine type I collagen (1 mg/ml: Sigma) or BSA (1 mg/ml) for 1 h at room temperature and subjected to E2 EIA kit.

Case Report

A 70-year-old man with type 2 diabetes who had been treated with oral hypoglycemic agents during the past 30 years, was admitted to our hospital because of diabetic nephropathy in September, 2002. Abdominal echography incidentally revealed an abdominal aortic aneurysm, measured 5 cm in diameter. Surgical repair was performed with Hemashield vascular graft coated with bovine type I collagen (Knitted Microvel®, Boston Scientific, Boston, MA) in November, 2002. Postoperative course was uneventful. Postoperative laboratory data showed normochromic anemia with eosinophilia, impaired renal function, hyperglycemia and normal serum electrolytes levels (Table 1). To exclude the possible involvement of adrenal and thyroid disorders in the postoperative renal failure, plasma levels of cortisol and thyroid hormones were determined.

Postoperative endocrine data showed very low plasma cortisol level which was normal before surgery (Table 1). Plasma cortisol levels did not rise after intravenous injection of ACTH (Cortrosyn® 250 µg).

Table 1. Postoperative Laboratory and Endocrine Data

<table>
<thead>
<tr>
<th>Peripheral blood</th>
<th>6500/µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cells</td>
<td></td>
</tr>
<tr>
<td>Stab</td>
<td>6.0%</td>
</tr>
<tr>
<td>Segment</td>
<td>55.0%</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>14.0%</td>
</tr>
<tr>
<td>Basophils</td>
<td>0.0%</td>
</tr>
<tr>
<td>Monocytes</td>
<td>6.0%</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>19.0%</td>
</tr>
<tr>
<td>Red Blood Cells</td>
<td>335 × 10^4/µl</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>10.2 g/dl</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>30.2%</td>
</tr>
<tr>
<td>Platelets</td>
<td>20.5 × 10^4/µl</td>
</tr>
</tbody>
</table>

Blood Chemistry

- Total protein: 7.5 g/dl
- Albumin: 3.9 g/dl
- Blood urea nitrogen: 47 mg/dl
- Creatinine: 4.47 mg/dl
- Sodium: 137 mEq/l
- Potassium: 4.5 mEq/l
- Chloride: 100 mEq/l
- Aspartate aminotransferase: 17 IU/l
- Alanine aminotransferase: 11 IU/l
- Lactate dehydrogenase: 194 IU/l
- Alkaline phosphatase: 249 IU/l
- Glucose: 190 mg/dl
- HbA1c: 7.2%
- Immunoglobulin G: 2017 mg/dl
- TSH receptor antibody: 6.1%
- thyroid stimulating antibody: 152%
- anti-thyroid peroxidase antibody: 0.4 IU/ml
- anti-thyroglobulin antibody: 0.9 IU/ml

Hormones

- ACTH: 59 (23) pg/ml
- Cortisol: 0.62 (11.42) µg/dl
- Plasma renin activity: 3.3 ng/ml/hr
- Plasma aldosterone concentration: 2.5 ng/dl
- LH: 40.5 (53.5) mIU/ml
- FSH: 103.6 mIU/ml
- GH: 0.11 ng/ml
- PRL: 14.4 (2.20) ng/ml
- Estradiol: 1044.8 pg/ml
- Human chorionic gonadotropin: 3.3 mIU/ml
- Dehydroepiandrosterone sulfate: 2.04 ng/ml
- TSH: 18.65 (0.566) µU/ml
- Free triiodothyronine: 5.42 (2.63) pg/ml
- Free thyroxine: 3.07 (1.15) ng/dl

( ): preoperative hormones data
FACTITIOUS ADRENAL INSUFFICIENCY AFTER VASCULAR GRAFT SURGERY

(Fig. 1A, open circle) or human CRH (100 µg), while plasma ACTH level in response to CRH was normal. These data were consistent with the diagnosis of adrenal insufficiency, although he had no evidence of stigmata of adrenal insufficiency, such as hypotension, emaciation, skin pigmentation, hypoglycemia, or hypornatremia. He had also increased plasma levels of TSH and thyroid hormones (Table 1), but low 99mTc uptake (0.26%) by thyroid scintigraphy. Magnetic resonance imaging (MRI) of the brain with Gd enhancement revealed a low intensity area in the pituitary, suggesting the presence of pituitary microadenoma. These data are compatible with the diagnosis of syndrome of inappropriate TSH secretion (SITSH), although no evidence of thyrotoxic symptoms, such as sweating, palpitation, goiter, and hand tremor. The presence of pituitary lesion associated with elevated plasma gonadotropin levels led us to measure his plasma estradiol (E2) level which was also increased without any evidence of feminization, such as gynecomastia. He was discharged with replacement therapy of hydrocortisone (20 mg) despite the unknown cause of adrenal insufficiency in December, 2002.

He was readmitted to our hospital because of pneumonia in November, 2003. Due to his tentative diagnosis of adrenal insufficiency, hydrocortisone (100 mg) was immediately administered intravenously in case of adrenal crisis. Plasma cortisol response after the second ACTH stimulation test was poor (Fig. 1B, open circle). However, judging from the discrepancy between the lack of clinical manifestations of adrenal insufficiency and very low plasma cortisol levels, we reasoned that this may be due to factitious data, and decided to withdraw hydrocortisone replacement therapy. He was completely free from any withdrawal syndrome even after cessation of hydrocortisone. All the abnormal hormone data were gradually normalized over three months after surgery. Stimulation with GRH, CRH, TRH and LHRH caused normal responses of GH, ACTH, TSH, PRL, and gonadotropins secretion, suggesting his pituitary lesion as a nonfunctioning tumor.

Results

Using two different assay kits, cortisol levels were simultaneously determined in the same patient’s plasma samples obtained just after the surgery. In contrast to low cortisol level measured by the earlier EIA kit, the later RIA kit yielded almost normal value (Table 2). We then re-evaluated the plasma cortisol levels in response to ACTH after surgery; the later RIA kit yielded a normal increment of plasma cortisol level after

![Graph A](image1.png)

**Fig. 1.** Plasma cortisol responses after ACTH stimulation test. ACTH stimulation tests were performed (A) 10 days after the vascular graft surgery and (B) on the second admission; plasma cortisol levels were measured by two different IMAs using RIA kit (●) and EIA kit (○), respectively.

![Graph B](image2.png)

<table>
<thead>
<tr>
<th>Hormones</th>
<th>Values</th>
<th>Assays</th>
<th>Antibodies (Species)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol (µg/dl)</td>
<td>0.36</td>
<td>EIA</td>
<td>Rabbit</td>
</tr>
<tr>
<td></td>
<td>20.8</td>
<td>RIA</td>
<td>Mouse</td>
</tr>
<tr>
<td>E2 (pg/ml)</td>
<td>614</td>
<td>EIA</td>
<td>Rabbit</td>
</tr>
<tr>
<td></td>
<td>10.6</td>
<td>RIA</td>
<td>Rabbit</td>
</tr>
<tr>
<td>TSH (µIU/ml)</td>
<td>21.04</td>
<td>ECLIA (sandwich)</td>
<td>Mouse</td>
</tr>
<tr>
<td></td>
<td>0.739</td>
<td>ECLIA (sandwich)</td>
<td>Mouse</td>
</tr>
<tr>
<td>FT3 (ng/dl)</td>
<td>5.42</td>
<td>ECLIA (competitive)</td>
<td>Goat and mouse</td>
</tr>
<tr>
<td></td>
<td>2.18</td>
<td>ECLIA (competitive)</td>
<td>Goat</td>
</tr>
<tr>
<td>FT4 (ng/dl)</td>
<td>3.07</td>
<td>ECLIA (competitive)</td>
<td>Mouse</td>
</tr>
<tr>
<td></td>
<td>1.52</td>
<td>ECLIA (competitive)</td>
<td>Goat</td>
</tr>
</tbody>
</table>

EIA: enzyme immunoassay; RIA: radioimmunoassay; ECLIA: electrochemiluminescence assay.
ACTH stimulation (Fig. 1A, closed circle), whereas no increment was observed when measured by the earlier EIA kit (Fig. 1A, open circle). These unexpected results led us to re-examine TSH, FT3, FT4, and E2 levels by the different assay kits as listed in Table 2. We found that the spuriously elevated TSH, FT3 and FT4 levels measured by the earlier IMA kits and E2 EIA kit turned out to be almost within normal ranges when measured by the later IMA kits and E2 RIA, respectively.

To confirm that the abnormal hormonal data were spurious in the earlier IMAs, the serial dilution of patient’s plasma was compared with that of standard in TSH assay kit. Dilution curve of patient’s plasma could not construct typical dilution curve parallel to human TSH standard (data not shown). To determine whether any antibodies in the patient’s plasma may have interfered with earlier IMAs, the effect of PEG was examined in the earlier cortisol and E2 EIA kits. After precipitation of patient’s γ-globulins by PEG, plasma cortisol levels increased from 4.8 to 12.8 µg/dl and plasma E2 level decreased from 607 to 25 µg/dl, respectively.

To exclude the possible involvement of HAMA in the earlier IMAs, the effect of mouse IgGs was examined by blocking the interference from HAMA. The addition of excess (500 µg/ml) mouse polyclonal IgG to TSH and FT4 assay kits and goat polyclonal IgG for FT3 assay kit failed to correct his factitious results. Finally, we determined whether heterophilic antibodies against bovine type I collagen (coated on the vascular graft) and/or BSA (used in all assay kits) interfered with IMAs. The addition of excess (1 mg/dl) bovine type I collagen and BSA to patient’s serum failed to correct his factitious results.

Discussion

After vascular graft surgery for abdominal aortic aneurysm, the diagnosis of adrenal insufficiency was tentatively made due to the very low plasma cortisol levels, and the patient was discharged with replacement therapy of hydrocortisone. In addition, the patient had elevated plasma levels of TSH, thyroid hormones and E2, although he had no signs and symptoms of adrenal insufficiency, thyrotoxicosis or feminization. However, judging from the discrepancy between many abnormal hormone data and the complete lack of clinical manifestations, we reasoned that these abnormal hormone levels may be spurious by IMA kits used upon the second hospitalization. Using two different IMA kits, we found the discrepant hormonal data for cortisol, TSH, thyroid hormones and E2. In fact, the non-parallelism of dilution curve by patient’s plasma to standard TSH in TSH assay kit is consistent with factitious data. The correction of spurious data by the addition of PEG to plasma samples strongly suggests that certain circulating macromolecular substances, especially immunoglobulins, interfered with IMA to cause such factitious data.

Currently, IMAs are widely used for measurements of hormones due to their high sensitivity and specificity. However, it has been reported that the heterophilic antibodies present in patient’s plasma by their binding to the antibodies used in IMAs can lead to factitious data. Some patients who had previously received monoclonal antibody therapies or injection of animal products such as vaccines, could have HAMA in their serum to interfere with IMAs [7, 8]. However, the present case had never received such treatments. Since the monoclonal antibodies used for IMAs are usually prepared from various species, non-specific immunoglobulins of any given species had been added to the assay kits to eliminate HAMA’s interference. Nevertheless, it has been reported that the amount of the immunoglobulins added to a given assay system is too small to block HAMA’s interference [5]. In the present case, however, the addition of excess amount of mouse polyclonal IgG to patient’s plasma did not affect the factitious results. Thus it is suggested that HAMA is not responsible for the factitious data in this case.

Collagen-sealed (Hemashield) knitted Darcon grafts without the necessity for preclotting have been developed and widely used for vascular repair surgery without major clinical consequences [9]. However, it has been reported that some patients are allergic to Hemashield vascular graft [10, 11]; they showed a transient inflammatory and immune response after vascular graft surgery, such as fever, leukocytosis with eosinophilia, increased erythrocyte sedimentation rate, CRP levels, and anti-bovine collagen antibodies which resolved spontaneously. In the present case, a transient eosinophilia (14%) with elevated IgE level (2115 IU/ml) was observed after the graft surgery, but spontaneously resolved within a few weeks. The vascular graft is coated with specially-processed bovine type I collagen, which usually disappears from the circulation.
during approximately three months [12, 13]. The 3-month-period exactly corresponds to the time for factitious hormonal data to be normalized in the present case.

It is therefore possible to speculate that generation of certain autoantibodies to the heterologous collagen and/or its metabolites may be responsible for an interference with the IMAs. However, the addition of excess bovine type I collagen to absorb such antibodies if any, in patient’s plasma, failed to correct the factitious data. However, it remains possible to assume that specially-processed bovine type I collagen may have generated such antibodies that could not recognize intact molecule of bovine type I collagen. Alternatively, autoantibodies against other yet-unknown components derived from the graft may react with these materials used for IMA kits, BSA for example. However, the failure of exogenous BSA added to the patient’s plasma to correct factitious data excludes at least BSA as a causative candidate.

Although the cause of the factitious hormonal data in the present case remains unknown, we should keep it in mind that some patients who underwent surgical repair using heterologous materials may lead to falsely positive or negative hormonal data, when determined by IMAs. Therefore, caution must be paid whenever there exists dissociation between clinical manifestations and hormonal data in such postoperative patients.

Acknowledgements

This study was supported in part by Grants-in-Aids from the Ministry of Health, Labor and Welfare and the Ministry of Education, Culture, Sports, Science and Technology. We thank TOSOH and FUJIREBIO INC. for their cooperation in this study.

References