the LC-MS/MS method. Additional cost savings are possible with LC-MS/MS for combined immunosuppressive therapies where a calcineurin inhibitor is used together with a mTOR inhibitor because both drugs can be quantified with a single assay.

Because of its ease and rapidity, this method is ideally suited for the routine monitoring of these four major immunosuppressive drugs in clinical practice. Laboratories using such tests must, however, have sufficient expertise with operating LC-MS/MS systems. Furthermore, it should be considered that when switching from the commonly used immunoassays for CsA and tacrolimus to this LC-MS/MS method, the therapeutic ranges will need to be revised. As noted, a major disadvantage of the immunoassays is their cross-reactivity with variable concentrations of immunosuppressive drug metabolites present in the blood samples from transplant recipients. Because it has been shown that inactive metabolites make a major contribution to this bias (16–18), the clinical application of the LC-MS/MS method could, without appropriate adjustment of the therapeutic range, lead to a potential overdose. On the other hand, the LC-MS/MS method will provide the possibility for more accurate individualized patient dosing based on the parent drug. Rigorous reevaluation of the current therapeutic ranges for tacrolimus and CsA in cooperation with local transplant centers will therefore be necessary if a change to this highly specific method is planned.

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References


A Positive Drug Test in the Pain Management Patient: Deception or Herbal Cross-Reactivity? Kelly Hickey,1 Rania Seliem,1 James Shields,1 Alfred McKee,2 and James H. Nichols1 1 Department of Pathology and 2 Pain Management Center, Baystate Health System, Springfield, MA 01199; address correspondence to this author at: Clinical Chemistry, Department of Pathology, Baystate Medical Center, 759 Chestnut St., Springfield, MA 01199; fax 413-794-5893, e-mail james.nichols@bhs.org

Our laboratory was contacted by the Pain Management Center regarding a patient who tested positive for cocaine metabolite in urine, but denied abuse. Because this population is receiving prescriptions for controlled narcotics for pain, the physicians need to determine patient compliance and rule out abuse of street drugs for continued participation in the program. For this patient, the laboratory was consulted to distinguish whether the urine positivity was attributable to herbal medication cross-reactivity or whether the patient was deceiving the clinic physicians.

The patient was a 47-year-old female with a history of Wegener granulomatosis and vasculitis. She had undergone extensive surgery, including resection of the frontal and nasal sinus cavity and septum, and was receiving aggressive analgesic management, including opioid analgesics for head pain related to her condition. It is the policy of the Pain Management Center to test all patients on a random basis three to four times a year for medication compliance and to exclude abuse of street drugs. The patient tested positive once before this episode for urine cocaine metabolite.

On October 31, 2001, the patient’s urine tested positive for cocaine metabolite (qualitative, >300 µg/L) by fluo-
rescent polarization immunoassay (FPIA; Abbott Laboratories), but she denied abuse within the past several months. Instead, she claimed passive exposure to cocaine smoke from living in an apartment building where her upstairs neighbor was a “crack” addict. The patient submitted a second urine on November 2, 2001, which was also positive by immunoassay for cocaine metabolite (qualitative, >300 µg/L cutoff). At this particular time, the patient indicated use of an herbal product, mugwort.

Mugwort (Artemisia vulgaris) is a common herb used in alternative medicine. It is also known as common artemisia, felon herb, St. John’s herb, chrysanthemum weed, and sailor’s tobacco and is a close relative of wormwood (Artemisia absinthium L.). Mugwort has a long history of folk tradition and use. Anglo-Saxon tribes believed that the aromatic mugwort was one of the nine sacred herbs given to the world by the god Woden. It was also used as a flavor additive to beer before the introduction of hops. Mugwort is considered a magical herb, with special properties to protect road-wear travelers against exhaustion. The Romans planted mugwort by roadsides, where it would be available to passersby to put in their shoes to relieve aching feet. St. John the Baptist was said to have worn a girdle of mugwort when he set out into the wilderness. Some of the “magic” in mugwort is in its reputed ability to induce prophetic and vivid dreams when the herb is placed near the bed or under the sleeper’s pillow. Today, mugwort leaf and stem are used medicinally as a bitter digestive tonic, uterine stimulant, menstrual regulator, and antirheumatic. Infusions are made with 1 ounce (28 g) of fresh leaf in 1 pint (473 mL) of boiling water for 5–10 min. Alcoholic extracts can also be prepared by steeping the powdered dried plant for several days in a 50:50 mixture (by volume) of alcohol to water (1).

The patient’s physician contacted our laboratory questioning the possibility of immunoassay cross-reactivity with the herbal product and sent the patient’s mugwort to our laboratory for analysis. A tea was brewed from the leaves and analyzed by FPIA after cooling to room temperature. The tea was negative for amphetamine, phencyclidine, barbiturates, benzodiazepines, opiates, and cannabinoids at the standard cutoffs, but tested above linearity (>5000 µg/L) for cocaine metabolite. Both the tea and the patient’s urine (from October 31, 2001) were sent to a local reference laboratory for gas chromatography–mass spectrometry (GC/MS) analysis.

While we were waiting for confirmation results, we obtained mugwort from a local natural foods store managed by a certified herbalist. Visual comparison of the two mugwort specimens was significantly different (Fig. 1). The patient’s product was darker, more finely crushed, and coated with a white, granular powder, whereas the mugwort obtained from the herbalist was lighter in color, contained more whole leaves and flowers, and did not seem to have the same coating of white powder. Tea from the mugwort obtained from the herbalist, prepared in a manner identical to that of the patient’s mugwort, tested negative in all drug-of-abuse immunoassays, including the assay for cocaine metabolite. The patient produced a third urine, on November 13, 2001, that was also positive for cocaine metabolite (qualitative, >150 µg/L by FPIA).

Results from the GC/MS analysis confirmed that the patient’s urine was positive for cocaine metabolite (qualitative, >150 µg/L), and the tea made from the patient’s sample of mugwort was positive for cocaine (qualita-
ative, cutoff >150 μg/L) and cocaine metabolite (qualitative, >150 μg/L). The patient was confronted with the results and continued to deny abuse. She did, however, submit three subsequent urines that were negative for cocaine metabolite; on November 28, 2001; December 12, 2001; and January 8, 2002 (qualitative, <300 μg/L). She reported that her urine tested negative because she had stopped using the tea. She was referred to addiction medicine for treatment.

With the increased prevalence of alternative medicine in America, clinicians are faced with the difficulty of determining whether a particular herbal product could be responsible for test positivity or whether the patient is truly positive. Although complete interference profiles have not been adequately defined for most immunoassays, the widespread use of herbs would argue against significant cross-reactivity in routinely used immunoassays. This case also emphasizes the need for GC/MS confirmation in some clinical situations where abuse is suspected. Only through GC/MS analysis were we able to definitively establish that the patient’s mugwort contained actual cocaine. Although there was no definitive proof that the patient actually contaminated the mugwort with cocaine, the sample she produced and claimed to be the source of her urine positivity was shown to contain both cocaine and cocaine metabolite. Tea brewed from mugwort obtained from an herbalist did not test positive. This case was clearly not an herbal cross-reactivity because the presence of drug was confirmed by GC/MS. Someone added the drug to the patient’s mugwort; whether it was a friend, family member, or the patient herself has not been established, but it is unlikely that she purchased this product from a legal distributor with cocaine on it. Clinicians thus should not underestimate the lengths that patients will take to evade detection.

**Reference**


**Comparative Evaluation of Serologic Tests for Celiac Disease Diagnosis and Follow-Up**, Silvia Martini,1 Giulio Mengozzi,2* Giuseppe Aimo,2 Laura Giorda,2 Roberto Pagni,2 and Carla Sategna Guidetti2 (1 Dipartimento di Medicina Interna, Università di Torino, 10126 Torino, Italy; 2 UOA Laboratorio Analisi Chimico-Cliniche, Azienda Ospedaliera San Giovanni Battista, 10126 Torino, Italy; * author for correspondence)

Anti-endomysium antibody (EmA) testing is used in the diagnosis of celiac disease (CD). The identification (1) of tissue transglutaminase (tTG) as the main antigen of EmA led to the development of commercial ELISAs for serum anti-tTG detection. At first, a guinea pig antigen (2–7) yielded both sensitivity and specificity lower than those of EmA; therefore, human recombinant tTG (8–11) was introduced to improve diagnostic accuracy and to overcome problems such as species specificity and cross-reactivity to contaminant proteins. However, the standardization of assays (12), the choice of cutoff value, the clinical relevance of these autoantibodies (13, 14), and the diagnostic accuracy of different commercial tests remain unresolved (15–18). This study aimed to assess the diagnostic accuracy of five commercially available IgA anti-tTG ELISA reagent sets (four using a human recombinant and one a guinea-pig tTG antigen) for pathologically confirmed CD and to evaluate the changes in anti-tTG autoantibody concentrations during treatment of CD with a gluten-free diet (GFD).

This prospective study included 101 consecutive untreated adults (79 women and 22 men; median age, 37 years; range, 21–72 years) referred to the University gastroenterologic outpatient clinic between January 2000 and May 2001 in whom CD was subsequently diagnosed by means of the typical appearance of small intestinal mucosa (19) (Marsh grade III in 95 patients and grade II in 6 patients) and by a positive clinical response to a GFD. We reported on 34 of these patients (all EmA-positive) previously (17). A duodenal biopsy was performed in all patients on the basis of clinical history and serologic assessment, including EmA testing and nutritional indexes. In all patients, a follow-up biopsy and serologic monitoring were repeated at 1 year ± 1 month after gluten withdrawal. For a control group, we studied 190 individuals (119 women and 71 men; median age, 38 years; range, 20–77 years). These included 89 healthy controls and 101 disease controls (56 with inflammatory bowel disease and 45 patients with other diseases: 12 with malignancies, 10 with autoimmune diseases, 9 with chronic liver disease, and 14 with heart failure). CD was excluded in all on the basis of clinical history, IgA-EmA negativity, or duodenal biopsy, the last having been performed on patients undergoing routine upper diagnostic endoscopy. The study was performed according to the principles of the Helsinki Declaration, and oral informed consent was obtained from each participant.

Serum EmAs were detected by immunofluorescence, using commercial slides of monkey esophagus (The Binding Site Ltd., distributed by Alfa Biotech) (20). Sera were tested, as indicated by the manufacturer, at a 1:10 initial dilution, with the inclusion of positive and negative controls in every batch of tests. CD EmA-negative sera were further tested at a 1:5 dilution, and no false-negative results were obtained.

Both qualitative and quantitative IgA anti-tTG antibody assessments were performed, as described previously (17), without the knowledge of the patients’ clinical diagnoses. We used four commercially available sandwich ELISAs that use human recombinant antigen (h-tTG): h-tTG 1 (DRG Diagnostics, distributed by Pantec S.r.l.; intra- and interassay CVs <10% and <15%, respectively); h-tTG 2 (EU-tTG® IgA; Eurospital S.p.A.; within- and between-assay CVs, 5.5% and 8.6%, respectively); h-tTG 3 (Immunodiagnostics, distributed by Li StarFISH s.a.s.; intra- and interassay CVs <11% and <15%, respec-