

COMMUNIQUÉ

IMPROVING PATIENT CARE THROUGH ESOTERIC LABORATORY TESTING

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Glutamic Acid Decarboxylase Autoimmunity With Brainstem, Extrapyramidal, and Spinal Cord Dysfunction

The 65-kd enzyme glutamic acid decarboxylase (GAD65) catalyses conversion of glutamic acid to γ -aminobutyric acid (GABA), which is a major inhibitory neurotransmitter in the central nervous system (CNS). This enzyme is expressed selectively in GABAergic neurons and endocrine tissues, such as the pancreatic β -cell, testis, and fallopian tubal epithelium. Eight percent of white North Americans are seropositive for GAD65 autoantibody, but values are generally less than 2.00 nmol/L (reference range, ≤ 0.02 nmol/L). The GAD65 antibody is detected in 80% of patients with newly diagnosed type 1 diabetes mellitus. The serum level of GAD65 antibody in patients who lack a polyendocrine or a pertinent neurological accompaniment is generally lower (0.03–20 nmol/L) than in patients with polyendocrine autoimmunity, stiff-man syndrome (SMS), or autoimmune cerebellar ataxia (≥ 20 nmol/L).

The GAD65 antibody was first described in a single patient with Moersch-Woltman SMS, who had coexisting type 1 diabetes mellitus and epilepsy.

Subsequently, it was recognized that up to 90% of patients with SMS have GAD65 antibody, usually at very high titer, as do patients with variants of SMS, including stiff-limb syndrome, jerking SMS, and progressive encephalomyelitis with rigidity, and a subset of patients with idiopathic cerebellar ataxia and idiopathic epilepsy. In rare single case reports, other neurological manifestations have been associated with GAD65 antibody, including ocular movement disorders suggestive of brainstem dysfunction. A study of trigeminal brainstem reflexes in patients with SMS suggested primary dysfunction of the inhibitory interneurons at the brainstem level. In contemporary practice, neurologists generally reserve GAD65 antibody testing for patients who present with SMS, idiopathic cerebellar ataxia, or juvenile epilepsy. The fact that GAD65 antibody testing was not requested by the clinician for any patient in our study supports this practice. Our study was performed to identify novel neurological manifestations associated with GAD65 autoimmunity.

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- #88809 *Antimicrobial Susceptibility, Mycobacterium tuberculosis Complex, Rapid Broth Method*
- #88897 *B-Cell Lymphoma, FISH, Blood and Bone Marrow*
- #88874 *Bullous Pemphigoid, BP180 & BP230, IgG Antibodies, Serum*

Patients and Methods

Between January 1, 1987, and July 1, 2003, Mayo Clinic's Neuroimmunology Laboratory performed prospective paraneoplastic autoantibody screening on serum samples from approximately 60,000 patients with neurological syndromes, which included indirect immunofluorescence testing with a composite of mouse tissues as substrate. During this service, we incidentally identified 62 patients whose serum IgG bound selectively to CNS tissues in a pattern consistent with GAD65-specific IgG and for whom clinical information was available retrospectively. Positive serum samples were titrated in doubling dilutions to ascertain the farthest dilution that was positive by immunofluorescence. In all cases, GAD65 specificity was confirmed by radioimmunoprecipitation assay (RIA). In no case had the treating physicians entertained the diagnosis of a GAD65 autoimmune disorder, and neither GAD65 nor other islet cell antibody testing had been requested.

In searching for paraneoplastic neuronal nuclear or cytoplasmic IgGs (antineuronal nuclear antibody types 1, 2, and 3; collapsin response-mediator protein-5; Purkinje cell cytoplasmic antibody types 1 and 2; amphiphysin), the initial immunofluorescence screening assay comprised a substrate of mouse cerebellum, stomach, and kidney cut in 4- μ m-thick sections and postfixed with 10% formalin. This revealed gastric parietal cell antibody in many cases. We additionally analyzed all these incidentally identified GAD65-positive serum samples for other coexisting autoantibodies by Western blot with native and recombinant neuronal antigens and also by RIAs, seeking antibodies reactive with cation channels (neuronal voltage-gated calcium channels [P/Q-type and N-type], voltage-gated potassium channels [α -dendrotoxin sensitive] extracted from human brain, and nicotinic acetylcholine receptors extracted from a human ganglionic neuronal cell line and skeletal muscle, respectively) and neuroendocrine antibodies (using 125 I-recombinant human GAD65 or an islet cell tyrosine phosphatase-like protein [IA-2]). We used enzyme-linked immunosorbent assay to detect skeletal muscle striational antibodies and agglutination assays to detect thyroid antibodies (peroxidase and thyroglobulin).

This study was approved by the Mayo Foundation Institutional Review Board. We reviewed the medical records of 44 Mayo Clinic patients (71% of the study

population) and all available clinical information for the 18 non-Mayo patients (29% of the study population). Clinical status at last follow-up was scored according to the modified Rankin scale (MRS). The MRS defines 6 levels of disability: 0, no symptoms at all; 1, no significant disability despite symptoms, able to carry out all usual duties and activities; 2, slight disability, unable to carry out all previous activities but able to look after own affairs without assistance; 3, moderate disability, requires some help but able to walk without assistance; 4, moderately severe disability, unable to walk without assistance and unable to attend to own bodily needs without assistance; and 5, severe disability, bedridden, incontinent, and requiring constant nursing care and attention.

Descriptive statistics are reported as median (interquartile range [IQR]) for continuous variables and frequencies and proportions for categorical variables. The association between RIA values for GAD65 antibody and immunofluorescence titers was assessed with the Spearman rank correlation coefficient. For each type of immunotherapy, the time from symptom onset to treatment was compared between responders and nonresponders (physician reported) using the Wilcoxon rank sum test. The relationship between IA-2 antibody status and development of diabetes was tested with a χ^2 analysis. All 2-sided $P < .05$ values were considered statistically significant. Analyses were performed using JMP statistical software, version 5.1 (SAS Institute Inc, Cary, NC).

Results

The immunofluorescence staining pattern from the serum of 62 patients in routine screening was characteristic of GAD65 immunoreactivity (Figure 1). By GAD65-specific RIA, all samples were confirmed to have antibody values that exceeded 20 nmol/L (range, 69–13,900 nmol/L). Forty-eight patients (77%) were women, with a median age of neurological symptom onset at 51 years (IQR, 41.0–66.7 years). Fourteen patients (23%) were men, with a median age of neurological symptom onset at 43.5 years (IQR, 34.2–58.5 years). The median follow-up period for the entire cohort was 24 months (IQR, 8–60 months). No malignancy was identified at follow-up.

Forty-four patients (71% of the study population) were evaluated at the Mayo Clinic. Of these 44 Mayo

system are not stained. In 40% of cases, gastric mucosal cells were stained by serum IgG attributable to coexisting parietal cell antibody. The RIA values for GAD65 antibody (median, 1429 nmol/L; IQR, 643–3078 nmol/L; reference range, ≤ 0.02 nmol/L) correlated positively with immunofluorescence titers (median, 3840; IQR, 1920–15,360; $r=0.81$; $P<.001$; reference range, <120 ; Figure 1, top).

Neurological Associations and Disability

The neurological symptom onset was subacute in 37 patients (60%), acute in 2 (3%), insidious in 22 (35%), and relapsing in 1 (2%). In 41 patients (66%), neurological manifestations were multifocal and included, in decreasing order of frequency, the following: cerebellar ataxia, 39 (62%); brainstem manifestations, 18 (29%); seizures, 17 (27%); stiff-man phenomena, 16 (26%; SMS in 2 patients); extrapyramidal signs, 10 (16%); and myelopathy, 5 (8%). Eleven (55%) of 20 African American patients had brainstem findings.

Scores of disability by MRS at last follow-up (median, 24 months) were as follows: MRS score 1, 9 patients (median, 37 months; IQR, 8–108 months); MRS score 2, 16 patients (median, 21 months; IQR, 6–69 months); MRS score 3, 21 patients (median, 24 months; IQR, 11–72 months); MRS score 4, 15 patients (median, 30 months; IQR, 14–47 months); and MRS score 5, 1 patient (6 months).

Cerebrospinal Fluid and Radiological Findings

Results of cerebrospinal fluid analysis were available for 23 patients (37%). Twelve results (52%) were abnormal. Seven patients had elevated protein levels, although modest in most (median, 55 mg/dL; IQR, 52–81 mg/dL; reference range, 14–45 mg/dL); 1 had an elevated IgG index (1.06; reference range, ≤ 0.85); 9 had supernumerary oligoclonal IgG bands; and 1 had an elevated white blood cell count ($0.043 \times 10^9/L$; 3 had a white blood cell count of $0.005 \times 10^9/L$; reference range, $0-0.005 \times 10^9/L$).

Brain magnetic resonance imaging reports were available for 45 patients (73%). Twenty-six (58%) of these reports had abnormal results, although some of the imaging findings may have been incidental. Cerebellar atrophy was present in 12 (often vermal), 5 exhibited mesial temporal or hippocampal lesions (abnormal T2 signal in hippocampi in 2 [1 enhancing];

mesial temporal sclerosis was noted in 2 [1 enhancing]; right hippocampal atrophy in 1), 5 exhibited nonspecific changes consistent with small-vessel ischemic disease, 3 had infarcts (1 bilateral lacunar infarcts, 1 cerebellar infarct, 1 left temporoparietal infarct), and 3 exhibited generalized cerebral atrophy.

Serological and Clinical Autoimmune Associations

None of the 62 patients were seropositive for any paraneoplastic neuronal nuclear or cytoplasmic autoantibody tested (Table 1). Five had N-type voltage-gated calcium channel antibody, 1 had P/Q-type voltage-gated calcium channel antibody, and 8 had voltage-gated potassium channel antibody (3 of whom had complex partial seizures). Four patients had muscle acetylcholine receptor-binding antibody, but none had clinical or electrophysiological evidence of myasthenia gravis. One third of the patients had type 1 diabetes mellitus (onset at or after neurological presentation in 50%). Those with coexisting IA-2 antibody were more likely to develop diabetes after neurological presentation than those without (diabetes mellitus developed after onset of neurological disorder in 4 [40%] of 10 patients with IA-2 antibody and in 3 [8%] of 38 patients without detectable IA-2 antibody; $P=.02$). Thyroid antibodies were detected in 33 patients (53%), most of whom had autoimmune thyroiditis; 25 patients (40%) had gastric parietal cell antibody; and 10 patients (16%) had vitiligo.

Autoantibody	No. (%) seropositive
Neuronal nuclear or cytoplasmic†	0 (0)
Cation channel‡	15 (24)
Thyroid	33 (53)
Striational	4 (6)
Gastric parietal cell	25 (40)
Islet cell tyrosine phosphatase-like protein	14 (23)
≥ 1 of the above antibodies	51 (82)

Table 3. Autoantibodies Coexisting With GAD65 Antibody in 62 Patients*.

*GAD65 = glutamic acid decarboxylase.

†Antineuronal nuclear autoantibody types 1 through 3; anti-Purkinje cell cytoplasmic autoantibody types 1, 2, and Tr; amphiphysin; and collapsin response-mediator protein-5 autoantibodies.

‡Neuronal Ca^{2+} channel (P/Q-type; N-type), muscle acetylcholine receptor, and voltage-gated potassium channel.

Therapy

Twenty-seven patients (44%) received a trial of immunosuppressant therapy. Improvement, when observed, was temporary. No patient was treated with long-term (maintenance) immunosuppression. Eight of 10 patients who received intravenous methylprednisolone (1 g/d for 3–7 days), after a median delay of 13.5 months (IQR, 6–51 months), were considered to have experienced improvement by the treating physician (5, mild, 2, moderate, and 1, marked). The delay from symptom onset to initiation of treatment was significantly shorter for patients who were reported as improved (median delay, 9.5 months; IQR, 6–31 months) than for those reported not improved (median delay, 90 months; IQR, 60–120 months; $P=.03$). A tapering course of oral prednisone, after a median postonset interval of 12 months (IQR, 3–36 months), appeared less beneficial.

Seven of 11 patients treated had no improvement reported; 1 had mild improvement, 2 had moderate improvement, and 1 had marked improvement reported. Again, improvement with oral prednisone was associated with a shorter delay from onset to treatment (median, 5 months; IQR, 1.13–10.8 months) compared with nonresponders (median, 24 months; IQR, 12–108 months; $P<.05$). A course of intravenous immune globulin was administered to 6 patients after a median of 24 months (IQR, 8–108 months). Improvement (marked) was reported in a single patient (treated 23 months after onset). Plasmapheresis, started after a median of 20 months (IQR, 2.5–34.5 months), was reported beneficial in 3 of 5 patients treated by this modality (mild in 1 and moderate in 2).

Discussion

Our report extends the spectrum of subacute neurological symptoms and signs associated with high serum levels of GAD65 antibody to include disorders of the brainstem, extrapyramidal system, and spinal cord. The heterogeneity of the neurological presentation in patients with GAD65 autoimmunity is consistent with the widespread distribution of GABAergic neurons in the CNS. Our cases also included variable components of the previously recognized GAD65 autoimmune neurological syndromes, including stiff-man phenomena, idiopathic cerebellar ataxia, and seizure disorders, but in the context of more complex clinical syndromes. The

extremely high levels of GAD65 autoantibody in our patients strongly support an underlying autoimmune origin. The frequency and incidence of these unappreciated phenotypes of GAD65 autoimmunity remain to be determined.

After initial evaluation and testing (but before recognition of GAD65 antibody positivity), most patients in this study were considered to have a neurodegenerative disorder. It merits emphasis that in no case was GAD65 autoimmunity suspected in the initial differential diagnosis. In all cases, GAD65 antibody was detected incidentally during immunofluorescence screening of thousands of patients (currently 40,000 annually) for paraneoplastic autoantibodies. Subsequent RIA testing revealed that in all cases the GAD65 antibody value exceeded 20 nmol/L (range, 69–13,900 nmol/L). Values less than 20 nmol/L are generally not detected by immunofluorescence assay.

Retrospective clues that the patient's underlying disease process may be autoimmune included multifocal neurological manifestations that did not conform to any currently recognized neurodegenerative syndrome, inflammatory cerebrospinal fluid in some cases, and components of more classic GAD65 autoimmune neurological syndromes (SMS, ataxia, and seizures). Coexistence of other organ-specific autoimmune disorders was also a clue, particularly diabetes mellitus, hypothyroidism or hyperthyroidism, pernicious anemia, or vitiligo, as well as antibody markers of organ-specific autoimmunity (particularly pancreatic islet cell [IA-2], thyroid [peroxidase or thyroglobulin], gastric parietal cell, cation channel, or striational antibodies). The onset of type 1 diabetes mellitus (diagnosed by serum autoantibody profile rather than by age of onset, habitus, or insulin requirement) within months of the onset of a neurological disorder should prompt consideration of an autoimmune basis for the neurological disorder, especially if the GAD65 antibody level exceeds 20 nmol/L.

Because African Americans represent less than 10% of current neurology patients at the Mayo Clinic, the relatively high proportion of African Americans in the Mayo Clinic patients in this series (23%) suggests that this ethnic group has a heightened susceptibility to GAD65 autoimmune disorders. Although we do not have a denominator for ethnicity of non-Mayo patients, the 56% frequency of African Americans in

the non-Mayo Clinic patients is remarkable. Other autoimmune neurological disorders that occur more commonly or with greater severity among African American patients include neurological complications of systemic lupus erythematosus (higher frequency than in whites or Hispanics), the disproportionate frequency and severity of neuromyelitis optica, and juvenile-onset myasthenia gravis (fewer spontaneous or treatment-induced remissions than in white children). A heightened susceptibility to GAD65 autoimmunity has not previously been recognized in African Americans. However, we suspect that the prevalence of type 1 diabetes mellitus is underestimated in this ethnic group because African Americans are considered more at risk of type 2 diabetes mellitus than are whites. Among patients with classic SMS whose serological status was determined in Mayo Clinic's Neuroimmunology Laboratory, SMS appears to be disproportionately frequent and severe in African Americans compared with patients in whom paraneoplastic neuronal nuclear or cytoplasmic autoantibodies are identified (V. A. L., unpublished data, 1990–2003). The typical African American presentation of GAD65 autoimmunity in this study was a mixed syndrome of brainstem manifestations (ophthalmoplegia, dysarthria, and dysphagia), cerebellar ataxia, stiff-man phenomena (including axial and neck rigidity), and sometimes seizures.

The specific immunologic mechanism has not been fully elucidated in GAD65 autoimmune neurological disorders; a targeted immune attack directed against GAD65 antigen is unproved. Moreover, 51 (82%) of our 62 patients had 1 or more organ-specific autoantibodies accompanying the neuronal cytoplasmic GAD65-specific antibody. In 15 (24%) of patients, recognized companion autoantibodies were directed at plasma membrane cation channels. Perhaps the immunologic picture will parallel the myasthenia gravis story, in which muscle cytoplasmic (striational) autoantibodies were discovered as a marker almost 2 decades before discovery of the pathogenic muscle antibodies directed at acetylcholine receptors in the postsynaptic membrane. Thus, we consider it plausible that an as yet unidentified plasma membrane autoantigen may be pertinent to the pathogenesis of neurological disorders currently recognized by their association with GAD65 antibody.

Patients with a clinical syndrome related to GAD65 autoimmunity may benefit from early and maintained

immunosuppression therapy. Beneficial responses have been reported for SMS and cerebellar ataxia in the context of high-titer GAD65 antibody. However, many patients did not benefit, and as in other autoimmune neurological disorders, this may occur when treatment is delayed. The frequency of reported improvement related to immunotherapy in our study, particularly with treatment initiated within 6 to 12 months after symptom onset, warrants emphasis. However, we acknowledge the bias inherent in physician-reported treatment response. Unfortunately, guidelines for management of patients with immune-mediated CNS disorders are lacking (except for multiple sclerosis). In some of our patients, beneficial responses were reported after treatment with intravenous methylprednisolone, intravenous immune globulin, or plasmapheresis. However, no patient received long-term immunosuppression. In cases that showed improvement after a short trial of immunosuppression, an argument could be made for maintenance immunosuppression with mycophenolate, azathioprine, or cyclophosphamide. Prospective studies that are both randomized and controlled are needed to substantiate anecdotal treatment data of this type in the face of otherwise devastating neurological conditions.

Conclusions

The neurological spectrum associated with GAD65 autoimmunity is broader than previously recognized and includes brainstem, extrapyramidal, and spinal cord syndromes. It appears from our observations that African Americans are disproportionately affected. Most patients were initially considered to have a neurodegenerative disorder. In patients with recent-onset neurological disorders and high serum levels of GAD65 antibody, immunotherapy should be considered.

Adapted from Mayo Clinic Proceedings September 2006;81(9):1207-1214. References and 2 tables omitted. The complete article is available online at www.mayoclinicproceedings.com.

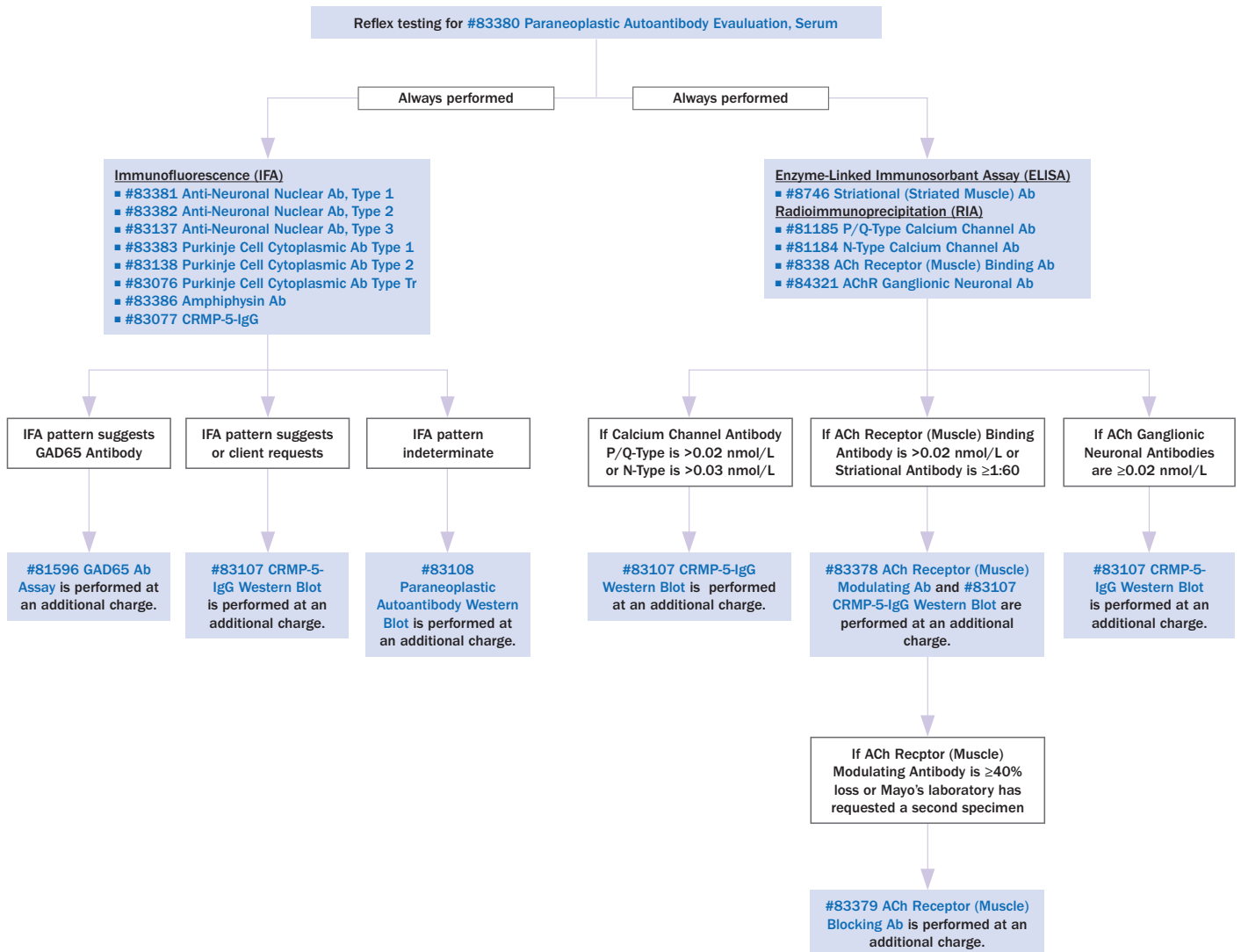
Question:

Where do I obtain GAD65 testing?

Answer:

Mayo Medical Laboratories offers 2 ways to obtain GAD65 antibody testing. Mayo's preferred approach is to order [#83380 Paraneoplastic Autoantibody Evaluation, Serum](#). This evaluation is designed to efficiently screen and, when appropriate, automatically confirm the presence of various serological markers of paraneoplastic autoimmunity (see algorithm).

As an alternative approach, testing for GAD65 alone is available as [#81596 Glutamic Acid Decarboxylase \(GAD65\) Antibody Assay, Serum](#) or [#84221 Glutamic Acid Decarboxylase \(GAD65\) Antibody Assay, Spinal Fluid](#).



Paraneoplastic Evaluation Algorithm

2007 Education Calendar

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Practical Surgical Pathology

September 13–15, 2007

Mayo Clinic • Rochester, Minnesota

Quality Phlebotomy: Beyond the Basics

September 24, 2007

Marriott Washington Dulles Airport • Dulles, Virginia

State-of-the-Art Thrombophilia: A Practical Clinical Conference

October 4–6, 2007

The Kahler Grand Hotel • Rochester, Minnesota

Disease Management Strategies . . .

Breast Cancer

September 20, 2007

Presenter: Matthew P. Goetz, MD

Oral Anticoagulation Monitoring

October 11, 2007

Presenter: Robert D. McBane, MD

Overview of Inflammatory Bowel Disease

November 13, 2007

Presenter: Edward V. Loftus Jr., MD

Celiac Disease

December 11, 2007

Presenter: Joseph A. Murray, MD



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