State of Hawaii
Department of Health
4348 Waialea Avenue #648
Honolulu, HI 96816

Medical Marijuana Registry

PETITION TO ADD A DEBILITATING MEDICAL CONDITION IN 2017

Instructions

1. ALL items on the form MUST be completed.

2. Petitions and any supporting documents may be submitted as follows:
   a. Email to: medicalmarijuana@doh.hawaii.gov before the close of business (4:30 PM) on **Friday, June 16, 2017**. Please use the subject line: Petition to Add New Condition. Note that the DOH will not make public any information that is protected pursuant to Chapter 92F, HRS, the Uniform Information Practices Act.

   b. Postal mail to: 4348 Waialae Avenue, #648, Honolulu, Hawaii 96816. Mailed petitions must be received by **June 16, 2017.**

   c. Hand delivered to: Kinau Hale at 1250 Punchbowl Street, Honolulu, Hawaii 96813 before the close of business (4:30 PM) on Friday, June 16, 2017. Hand delivered petitions must be left with the security guard and addressed to the Medical Marijuana Registry Program **ATTN: Petition to Add New Condition**.

3. For best results, complete and thorough petitions that include substantiated and reputable research have the best chance of succeeding. DOH recommends that you do the following for items #2 - #8 on the petition form:
   a. Please cite research, published evidence, or findings using the standard American Medical Association (AMA) format for each piece of research, published evidence, or findings that you reference in your submittal or at a minimum the following:

      Author's Name; Title of Article; Name of Publication; Date of Publication; Volume/Section/Chapter/Page/Line as applicable; and URL (if applicable).

   b. Please attach a PDF copy of the cited material to your submittal. These documents will NOT be returned.

   c. Please be sure to indicate the specific section, page(s), lines, etc., of the attachment that you want reviewed/considered as evidence.

4. To view a list of current conditions click here: Current Debilitating Medical Conditions

State of Hawaii
Department of Health
4348 Waialea Avenue #648
Honolulu, HI 96816

Petitioner Content

(1) State the specific medical condition or its treatment for which the petition is being made.

Generalized Anxiety Disorder

(2) State the reason(s) why the medical condition or its treatment should be added to the list of qualifying debilitating medical conditions for which medical marijuana may be used. For best results, please cite research, published evidence, or scientific findings AND attach a PDF copy to your submittal. Indicate specific lines, page, section. Please use standard AMA format as outlined in the instructions.

Generalized Anxiety Disorder is a well-accepted chronic condition, which is frequently unresponsive to current standard pharmacotherapies.

As is stated in a recent review, "Generalized anxiety disorder (GAD) is a chronic, highly prevalent and debilitating disorder that commonly co-occurrs with mood disorders. Current available agents for GAD are limited either by their slow onsets of actions, unsatisfactory anxiolytic effects or potential for abuse/dependence." (Gao K; Sheehan DV; Calabrese JR. "Atypical antipsychotics in primary generalized anxiety disorder or comorbid with mood disorders." Expert Review of Neurotherapeutics. (2009) vol. 9(8): pages1147-58. (I was unable to obtain this reference as a PDF).

Importantly, many patients have found cannabis to be very useful in treating anxiety, with a high frequency of favorable response. This data is reviewed below.

(3) Describe the **extent to which** the medical condition is generally accepted by the medical community as a valid, existing medical condition. For best results, please cite research, published evidence, or scientific findings AND attach a PDF copy to your submittal. Indicate specific lines, page, section. Please use standard AMA format as outlined in the instructions.

Generalized Anxiety Disorder (GAD) is a chronic, debilitating medical condition which is well-accepted by the medical community. Approximately 3-6 % of people experience GAD. It has a diagnostic code in the standard Diagnostic and Statistical Manual (DSM) of Mental Disorders, DSM-5, of 300.02 (F41.1), indicating its general recognition as a valid and existing condition.
It is reviewed recently in the New England Journal of Medicine (Murray B. Stein, M.D., M.P.H., and Jitender Sareen, M.D., "Generalized Anxiety Disorder", New Engl J Med 2015; Volume 373: pages 2059-68.)

This PDF is attached. The key items to review are highlighted.

(4) Describe the symptoms and other physiological or psychological effects experienced by an individual suffering from the medical condition or its treatment and **the extent to which** these symptoms and physiological or psychological effects are debilitating. Note: "Debilitating" generally means impairing the ability of a person to accomplish activities of daily living. For best results, please cite research, published evidence, or scientific findings AND attach a PDF copy to your submittal. Indicate specific lines, page, section. Please use standard AMA format as outlined in the instructions.

Generalized anxiety disorder (GAD) is characterized by excessive and uncontrollable worry in addition to three or more associated symptoms including fatigue, difficulty concentrating, sleep trouble, irritability, restlessness and muscle tension (Diagnostic and Statistical Manual of Mental Disorders [DSM]. Generalized anxiety disorder is associated with significant impairment and also economic burden on the healthcare system. For example, GAD symptom scores have been linked with disability across multiple domains of daily life including self-care, interpersonal functioning and healthcare resource utilization. These features are described in numerous articles.

One such reference is: Sarah Kertz,* Joe Bigda-Peyton and Throstur Bjorgvinsson, "Validity of the Generalized Anxiety Disorder-7 Scale in an Acute Psychiatric Sample", Clin. Psychol. Psychother. (2013) Vol 20, pages 456–464. This PDF is attached. The key items to review are highlighted.

Two additional articles which each attest to the debilitating nature of untreated GAD are also attached:

Morgana M.M. Vieira , Thais B. Ferreira, Paulo A.F. Pacheco, Priscila O. Barros , Carla R.M. Almeida , Carlos Fernando Araújo-Lima , Renato G. Silva-Filho , Joana Hygino , Regis M. Andrade ,Ulisses C. Linhares , Arnaldo F.B. Andrade , Cleonice A.M. Bento; Enhanced Th17 phenotype in individuals with generalized anxiety disorder", Journal of Neuroimmunology (2010) Vol. 229, pages 212–218; and

Michelle G. Craske ,*, Peter P. Roy-Byrne , Murray B. Stein, Greer Sullivan , Cathy Sherbourne , Alexander Bystritsky; "Treatment for anxiety disorders: Efficacy to effectiveness to implementation", Behaviour Research and Therapy (2009), Vol. 47, pages 931–937

These PDFs are attached. The key items to review are highlighted.

(5) If one or more treatments for the medical condition, rather than the condition itself, are alleged to be the cause of a person's suffering, describe **the extent to which** the treatments causing suffering are generally accepted by the medical community as valid treatments for the medical condition. For best results, please cite research, published evidence, or scientific findings AND attach a PDF copy to your submittal. Indicate specific lines, page, section. Please use standard AMA format as outlined in the instructions.

N/A

(6) Describe the availability of conventional medical therapies other than those that cause suffering to alleviate symptoms caused by the medical condition or its treatment. For best results, please cite research, published evidence, or scientific findings AND attach a PDF copy to your submittal. Indicate specific lines, page, section. Please use standard AMA format as outlined in the instructions.

Among psychotherapeutic techniques, various kinds of cognitive behavioral therapy (CBT) have been found useful in controlled trials. The drugs of first choice include selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephine reuptake inhibitors (SNRIs), and the calcium-channel modulator pregabalin. Tricyclic antidepressants are also effective but have more adverse effects than SSRIs. Although benzodiazepines are effective anxiolytic agents for short-term use, they should not be given over the long term because of the danger of addiction. Buspirone, an azapirone, was found to be effective in a small number of trials, but the findings across trials are inconsistent. The response rate of GAD to CBT in published studies lies between 47% and 75%, while its response rate to drug treatment lies between 44% and 81%. Accordingly, around 20-50% of patients with GAD do not respond to these conventional therapies.

This is excerpted from the review by (Borwin Bandelow, Reinhard J. Boerner, Siegfried Kasper, Michael Linden, Hans-Ulrich Wittchen, Hans-Jürgen Möller,"The Diagnosis and Treatment of Generalized Anxiety Disorder", Dtsch Arztebl Int 2013; Vol 110(17): pages 300–10.)

This PDF is attached. The key items to review are highlighted.

I have found multiple conventional therapies which I have attempted over a several year period to be of limited efficacy; thus I have been very grateful to find that medical cannabis is useful for managing my GAD effectively.

(7) Describe **the extent to which** evidence supports a finding that the use of marijuana alleviates symptoms caused by the medical condition or its treatment. For best results, please cite research, published evidence, or scientific findings AND attach a PDF copy to your submittal. Indicate specific lines, page, section. Please use standard AMA format as outlined in the instructions.

There are numerous studies that have demonstrated that cannabis, and various of its components (both the close THC analog, nabilone, and CBD) are very frequently used to combat anxiety, and are very effective in alleviating these symptoms. Many of these studies are reviewed in this comprehensive and recent review by Zach Walsh:
Zach Walsh, Raul Gonzalez, Kim Crosby, Michelle S. Thiessen, Chris Carroll, Marcel O. Bonn-Miller; "Medical cannabis and mental

health: A guided systematic review"; Clinical Psychology Review (2017) vol. 51; pages 15–29.)
This PDF is attached. The key items to review are highlighted.

This review states (highlighted in the PDF):
3.1.2. Anxiety
Relaxation and relief of anxiety are among the most widely reported motives for both cannabis for therapeutic purposes (CTP) and non-medical cannabis (NMC). Evidence from cross-sectional studies is consistent regarding the anxiolytic effects of cannabis for therapeutic purposes. Our search identified 8 cross-sectional studies reporting relief of anxiety as a primary or secondary benefit of CTP.

Here are several of the articles that describe the common use of cannabis to effectively combat anxiety, as well as the ability of selected cannabinoids to reduce anxiety:

Webb, C. W., & Webb, S. M. "Therapeutic benefits of cannabis: A patient survey". Hawaii Journal of Medicine & Public Health, (2014) vol. 73, pages 109–111. (Note this study is from Hawaii).

Woolridge, E., Barton, S., Samuel, J., Osorio, J., Dougherty, A., & Holdcroft, A. "Cannabis use in HIV for pain and other medical symptoms". Journal of Pain and Symptom Management (2005), vol. 29, pages 358–367.

Walsh, Z., Callaway, R., Belle-Isle, L., Capler, R., Kay, R., Lucas, P., & Holtzman, S. "Cannabis for therapeutic purposes: Patient characteristics, access, and reasons for use." The International Journal on Drug Policy, (2013) 24(2013), 511–516.

Crippa, J. A. S.,Derenusson,G. N.,Ferrari,T.B.,Wichert-Ana,L.,Duran,F. L.,Martin-Santos,R., Hallak, J. E. C. "Neural basis of anxiolytic effects of cannabidiol (CBD) in generalized social anxiety disorder: A preliminary report" Journal of Psychopharmacology (2011), vol. 25(1), pages 121–130.

Bergamaschi, M.M., Queiroz, R. H. C., Chagas, M. H. N., de Oliveira, D. C. G., De Martinis, B.S., Kapczinski, F., Crippa, J. A. S. Cannabidiol reduces the anxiety induced by simulated public speaking in treatment-naive social phobia patients. Neuropsychopharmacology, (2011), vol. 36(6), pages 1219–1226.

Fabre LF, McLendon D. "The efficacy and safety of nabilone (a synthetic cannabinoid) in the treatment of anxiety." J Clin Pharmacol. (1981) Vol. 21, pages 377S-382S. (the complete PDF of this was not available).

These PDFs are attached. The key items to review are highlighted.

(8) Provide any information, studies, or research reports regarding any beneficial or adverse effects from the use of marijuana in patients with the medical condition. For best results, please cite research, published evidence, or scientific findings AND attach a PDF copy to your submittal. Indicate specific lines, page, section. Please use standard AMA format as outlined in the instructions.

See Number 7 above also.

(9) Attach letters of support from physicians or other licensed health care professionals knowledgeable about the medical condition.

This letter is pending. It will be emailed directly to HI DOH under separate cover if the professional is unable to send to me in time to attach it here.

You **MUST** provide a Number and Name for each Attachment referenced above and provide a list of these attachments here. This way we can ensure that your petition was submitted with all of the applicable attachments:

State of Hawaii
Department of Health
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REF1.) Murray B. Stein, M.D., M.P.H., and Jitender Sareen, M.D., "Generalized Anxiety Disorder", New Engl J Med 2015; Volume 373: pages 2059-68.

REF2.) Sarah Kertz,* Joe Bigda-Peyton and Throstur Bjorgvinsson, "Validity of the Generalized Anxiety Disorder-7 Scale in an Acute Psychiatric Sample", Clin. Psychol. Psychother. (2013) Vol 20, pages 456–464

REF3.) Morgana M.M. Vieira, Thais B. Ferreira, Paulo A.F. Pacheco, Priscila O. Barros, Carla R.M. Almeida, Carlos Fernando Araújo-Lima, Renato G. Silva-Filho, Joana Hygino, Regis M. Andrade, Ulisses C. Linhares, Arnaldo F.B. Andrade, Cleonice A.M. Bento; Enhanced Th17 phenotype in individuals with generalized anxiety disorder", Journal of Neuroimmunology (2010) Vol. 229, pages 212–218

REF4.) Michelle G. Craske a,*, Peter P. Roy-Byrne b, Murray B. Stein c, Greer Sullivan d, Cathy Sherbourne e, Alexander Bystritsky; "Treatment for anxiety disorders: Efficacy to effectiveness to implementation", Behaviour Research and Therapy (2009), Vol. 47, pages 931–937

REF5.) Borwin Bandelow, Reinhard J. Boerner, Siegfried Kasper, Michael Linden, Hans-Ulrich Wittchen, Hans-Jürgen Möller,"The Diagnosis and Treatment of Generalized Anxiety Disorder", Dtsch Arztebl Int 2013; Vol 110(17): pages 300–10.

REF6.) Zach Walsh, Raul Gonzalez, Kim Crosby, Michelle S. Thiessen, Chris Carroll, Marcel O. Bonn-Miller; "Medical cannabis and mental health: A guided systematic review"; Clinical Psychology Review (2017) vol. 51; pages 15–29

REF7.) Webb, C. W., & Webb, S. M. "Therapeutic benefits of cannabis: A patient survey". Hawaii Journal of Medicine & Public Health, (2014) vol. 73, pages 109–111. (Note this study is from Hawaii).

REF8.) Woolridge, E., Barton, S., Samuel, J., Osorio, J., Dougherty, A., & Holdcroft, A. "Cannabis use in HIV for pain and other medical symptoms". Journal of Pain and Symptom Management (2005), vol. 29, pages 358–367.

REF9.) Walsh, Z., Callaway, R., Belle-Isle, L., Capler, R., Kay, R., Lucas, P., & Holtzman, S. "Cannabis for therapeutic purposes: Patient characteristics, access, and reasons for use." The International Journal on Drug Policy, (2013) 24(2013), 511–516.

REF10.) Crippa, J. A. S.,Derenusson,G. N.,Ferrari,T.B.,Wichert-Ana,L.,Duran,F. L.,Martin-Santos,R., Hallak, J. E. C. "Neural basis of anxiolytic effects of cannabidiol (CBD) in generalized social anxiety disorder: A preliminary report" Journal of Psychopharmacology (2011), vol. 25(1), pages 121–130.

REF11.) Bergamaschi, M.M., Queiroz, R. H. C., Chagas, M. H. N., de Oliveira, D. C. G., De Martinis, B.S., Kapczinski, F., Crippa, J. A. S. Cannabidiol reduces the anxiety induced by simulated public speaking in treatment-naive social phobia patients. Neuropsychopharmacology, (2011), vol. 36(6), pages 1219–1226.

REF12.) Fabre LF, McLendon D. "The efficacy and safety of nabilone (a synthetic cannabinoid) in the treatment of anxiety." J Clin Pharmacol. (1981) Vol. 21, pages 377S-382S. (the complete PDF of this was not available).

All these PDFs are attached, with the first characters of the file name "REFx". The key items to review in each PDF are highlighted.

CLINICAL PRACTICE

Caren G. Solomon, M.D., M.P.H., *Editor*

Generalized Anxiety Disorder

Murray B. Stein, M.D., M.P.H., and Jitender Sareen, M.D.

This *Journal* feature begins with a case vignette highlighting a common clinical problem. Evidence supporting various strategies is then presented, followed by a review of formal guidelines, when they exist. The article ends with the authors' clinical recommendations.

A 46-year-old married woman presents with insomnia, headaches, muscle tension, and back pain. She describes a long-term pattern of worrying about several life situations, including health, finances, and her job, and she notes increased anxiety associated with her teenager's leaving home to attend college. She drinks alcohol daily to reduce the tension and help her sleep. In reviewing her history, you note that she has visited your office many times over the past year because of physical symptoms. What do you advise?

From the Department of Psychiatry and the Department of Family Medicine and Public Health, University of California, San Diego, La Jolla, and the Veterans Affairs San Diego Healthcare System, San Diego — both in California (M.B.S.); and the Departments of Psychiatry, Psychology, and Community Health Sciences, University of Manitoba, Winnipeg, Canada (J.S.). Address reprint requests to Dr. Stein at the University of California, San Diego, 9500 Gilman Dr., Mail Code 0855, La Jolla, CA 92093-0855, or at mstein@ucsd.edu.

N Engl J Med 2015;373:2059-68.
DOI: 10.1056/NEJMcp1502514

An audio version of this article is available at NEJM.org

THE CLINICAL PROBLEM

GENERALIZED ANXIETY DISORDER IS CHARACTERIZED BY CHRONIC AND persistent worry. This worry, which is multifocal (e.g., about finances, family, health, and the future), excessive, and difficult to control, is typically accompanied by other nonspecific psychological and physical symptoms (Table 1). The term "generalized anxiety disorder" may incorrectly suggest that symptoms are entirely nonspecific, and this misconception may sometimes lead to the inappropriate use of this diagnosis for virtually any anxious patient. A new term — generalized worry disorder — was considered, though not adopted, for the fifth edition of the *Diagnostic and Statistical Manual of Medical Disorders* (DSM-5).[1] However, excessive worry is, indeed, the core and defining feature of generalized anxiety disorder.

According to representative epidemiologic surveys, the estimated prevalence of generalized anxiety disorder in the general population of the United States is 3.1% in the previous year and 5.7% over a patient's lifetime; the prevalence is approximately twice as high among women as among men.[2] The age at onset is highly variable; some cases of generalized anxiety disorder begin in childhood, most begin in early adulthood, and another peak of new-onset cases occurs in older adulthood, often in the context of chronic physical health conditions.[3] Generalized anxiety disorder is, by definition, a chronic disorder; 6 months is the minimum duration of anxiety for diagnosis, and most patients have had the disorder for years before seeking treatment.

Generalized anxiety disorder is particularly prevalent in primary care settings, where it occurs among 7 to 8% of patients.[4] Patients rarely, however, report the symptom of worry. The predominant presentation in primary care (rather than mental health) settings is physical symptoms such as headaches or gastrointestinal distress.[5] In children, generalized anxiety disorder often manifests as recurrent

Table 1. Criteria for the Diagnosis of Generalized Anxiety Disorder.*

Excessive anxiety and worry about various events have occurred more days than not for at least 6 months.
The person finds it difficult to control the worry.
The anxiety and worry are associated with at least three of the following six symptoms (only one symptom is required in children): restlessness or a feeling of being keyed up or "on edge," being easily fatigued, having difficulty concentrating, irritability, muscle tension, and sleep disturbance.
The anxiety, worry, or associated physical symptoms cause clinically significant distress or impairment in important areas of functioning.
The disturbance is not due to the physiological effects of a substance or medical condition.
The disturbance is not better accounted for by another mental disorder.

* All the features listed must be present in order to make a diagnosis of generalized anxiety disorder. Adapted from the American Psychiatric Association, *Diagnostic and Statistical Manual of Mental Disorders*, fifth edition.[1]

abdominal pain and other somatic symptoms[6] that may cause them to stay out of school.

Major depression is a common coexisting condition, although major depression may be difficult to distinguish from generalized anxiety disorder because many symptoms of generalized anxiety disorder (e.g., fatigue and insomnia) overlap with those of major depression. Persistent anhedonia (the inability to experience pleasure), which is characteristic of major depression, is not a symptom of generalized anxiety disorder. Patients with generalized anxiety disorder often describe a sense of helplessness, whereas patients with major depression may feel hopeless. Nevertheless, persons with generalized anxiety disorder are at increased risk for deliberate self-harm, including suicide attempts.[7] In many patients, generalized anxiety disorder is an underlying waxing-and-waning condition, with episodic bouts of major depression emerging during particularly stressful life circumstances. This dual occurrence of generalized anxiety disorder and major depression constitutes what is sometimes referred to as "anxious depression," a particularly common clinical presentation in primary care settings.[8]

The differential diagnosis of generalized anxiety disorder is broad. Health anxiety disorder (formerly known as hypochondriasis) is diagnosed when the worries are restricted to a preoccupation with illness. Obsessive–compulsive disorder, which is diagnosed when the ruminations are tied to irrational beliefs (e.g., beliefs about contamination), is often associated with compulsions (such as hand washing). Social anxiety disorder is diagnosed when the fear and worry are constrained to scrutiny by others and embarrassment when the person has to interact with or perform in front of others. In panic disorder, the anxiety is marked by abrupt, unexpected, transient episodes of fear and physical symptoms, and in post-traumatic stress disorder, a history of life-threatening trauma precedes the onset of anxiety, which coalesces around reminders of the traumatic event or events.

Patients with generalized anxiety disorder have increased risks of other mental and physical health conditions (e.g., chronic pain syndromes, asthma or chronic obstructive pulmonary disease, and inflammatory bowel disease).[9] Approximately 35% of people with generalized anxiety disorder self-medicate with alcohol and drugs to reduce the symptoms of anxiety, and this pattern of use is thought to contribute to the increased risk of alcohol- and drug-use problems among these persons.[10] Given the high rates of coexisting conditions, management of generalized anxiety disorder requires attention to a potentially complex array of psychological and physical symptoms, which may be mutually reinforcing.

Well-established risk factors for generalized anxiety disorder include female sex, low socioeconomic status, and exposure to childhood adversity (e.g., physical or sexual abuse, neglect, and parental problems with intimate-partner violence, alcoholism, and drug use).[11] Recent evidence suggests that exposure to physical punishment in childhood is associated with an increased risk of generalized anxiety disorder in adulthood.[12] However, these risk factors are nonspecific and can also be associated with risks of other anxiety and mood disorders.

Studies involving twins have shown evidence of a moderate genetic risk of generalized anxiety disorder, with heritability estimated at between 15 and 20%.[13] Candidate and genome-wide association studies involving persons with generalized anxiety disorder and other anxiety disorders have suggested some genetic associations,[13,14] but these findings have yet to be widely replicated.

A psychological construct known as intolerance of uncertainty — the tendency to react

KEY CLINICAL POINTS

GENERALIZED ANXIETY DISORDER

- Generalized anxiety disorder is characterized by persistent anxiety and uncontrollable worry that occurs consistently for at least 6 months.
- This disorder is commonly associated with depression, alcohol and substance abuse, physical health problems, or all these factors.
- In primary care, patients with this disorder often present with physical symptoms such as headaches, muscle tension, gastrointestinal symptoms, back pain, and insomnia.
- Brief validated screening tools such as the Generalized Anxiety Disorder 7 (GAD-7) scale should be used to assess the severity of symptoms and response to treatment.
- First-line treatments for generalized anxiety disorder are cognitive behavioral therapy, pharmacotherapy with a selective serotonin-reuptake inhibitor (SSRI) or a serotonin–norepinephrine reuptake inhibitor (SNRI), or cognitive behavioral therapy in conjunction with either an SSRI or an SNRI. Pregabalin and buspirone are suitable second-line or adjunctive medications.
- Although there is controversy regarding the long-term use of benzodiazepines owing to the potential for misuse and concerns about long-term adverse cognitive effects, these agents can, with careful monitoring, be used on a long-term basis in selected patients with treatment-resistant generalized anxiety disorder.

negatively to situations that are uncertain — has been shown to be a relatively specific characteristic of persons with generalized anxiety disorder.[15] Although it is unclear whether the origin of this construct is experiential or genetic, the observation that a reduction in intolerance of uncertainty is an important mediator of outcomes of cognitive behavioral therapy provides support for its central role in this disorder.[16]

Functional neuroimaging studies involving patients with generalized anxiety disorder have suggested increased activation within parts of the limbic system (e.g., the amygdala) and reduced activation in the prefrontal cortex, with additional evidence of diminished functional connectivity between these regions.[17-19] In addition, preliminary data suggest that effective treatments for this disorder may remediate these functional abnormalities in the brain. For example, functional magnetic resonance imaging in patients with generalized anxiety disorder[20] has shown increased activation of the amygdala while the patients are viewing faces that express emotion, and this activation is attenuated with cognitive behavioral therapy.[21]

STRATEGIES AND EVIDENCE

ASSESSMENT

Patients with generalized anxiety disorder generally have an affirmative response to the question "Do you worry excessively about minor matters?" That question is worth asking of patients with insomnia, a depressed mood, chronic gastrointestinal and other pain symptoms, or other unexplained recurrent health concerns.

Brief questionnaires such as the Generalized Anxiety Disorder 7-Item (GAD-7) Questionnaire[22] (Fig. 1), which take only minutes for the patient to complete, can be used to screen for the disorder as well as to longitudinally monitor outcomes. However, the advisability of routine screening for generalized anxiety disorder remains controversial.

Table 1 lists the DSM-5 diagnostic criteria for generalized anxiety disorder. Patients with suspected generalized anxiety disorder should routinely be asked whether they use alcohol or drugs to reduce anxiety or tension, and they should be screened for depression and the risk of suicide.

MANAGEMENT

Randomized, controlled trials provide strong evidence of the benefits of certain types of pharmacotherapy, psychotherapy, or both for generalized anxiety disorder.[23-25] A stepped-care approach is recommended (Table 2). The initial choice of treatment should depend largely on patient preference (with the majority of patients choosing psychotherapy).[26] Physicians who are not psychiatrists often prescribe medications for and monitor outcomes in these patients; in patients for whom psychotherapy is preferred or pharmacologic management is more complicated, refer-

Over the past 2 weeks, how often have you been bothered by the following problems? (*Use "✓" to indicate your answer*)	Not at All	Several Days	More Than Half the Days	Nearly Every Day
1. Feeling nervous, anxious, or on edge	0	1	2	3
2. Not being able to stop or control worrying	0	1	2	3
3. Worrying too much about different things	0	1	2	3
4. Having trouble relaxing	0	1	2	3
5. Being so restless that it is hard to sit still	0	1	2	3
6. Becoming easily annoyed or irritable	0	1	2	3
7. Feeling afraid, as if something awful might happen	0	1	2	3

Figure 1. Generalized Anxiety Disorder 7-Item Questionnaire.
The total score (0 to 21) is the sum of the individual items. Total scores of 5 to 9 indicate mild, probably subclinical anxiety, and monitoring is recommended. Total scores of 10 to 14 indicate moderate, possibly clinically significant anxiety, and further evaluation and treatment (if needed) are recommended. Total scores of 15 to 21 indicate severe, probably clinically significant anxiety, and treatment is probably warranted. Data are from Spitzer et al.[22]

ral is warranted, but the primary care physician should play a role in encouraging and supporting the patient's therapeutic work with the psychotherapist.

Primary care physicians who are treating patients with generalized anxiety disorder can be supported by a collaborative-care approach that includes the involvement of case managers (e.g., nurses or social workers) who deliver evidence-based psychotherapies and facilitate access to psychiatric consultation when needed. This approach has been shown to be more effective than treatment as usual.[27,28]

Lifestyle Modifications

Before patients embark on a course of pharmacotherapy or psychotherapy, they should be directed to unbiased sources of information about anxiety disorders (e.g., the Anxiety and Depression Association of America; www.adaa.org). Clinical experience and randomized, controlled trials provide support for the prescription of exercise for anxiety, though effect sizes are modest.[29]

Since insomnia is a prominent symptom of generalized anxiety disorder, the patient should be encouraged to practice positive sleep-hygiene behaviors (i.e., to maintain a regular sleep schedule, avoid smoking or the use of nicotine during the evening, and avoid alcohol and the prolonged use of devices with light-emitting screens, such as smartphones, laptops, and television, before bedtime). However, randomized trials are lacking to support specific benefits of sleep hygiene for patients with generalized anxiety disorder.

Pharmacotherapy

Pharmacologic treatment of generalized anxiety disorder results in a reduction in symptoms and disability and improved health-related quality of life.[30] Studies provide support for the efficacy of most (but not all) antidepressants, several benzodiazepines, buspirone, and pregabalin in the treatment of generalized anxiety disorder (Table 3).[31]

Selective serotonin-reuptake inhibitors (SSRIs) and serotonin–norepinephrine reuptake inhibitors (SNRIs) are generally considered to be first-line pharmacotherapies for generalized anxiety disorder, with response rates in the range of 30 to 50%.[23,32] A recent meta-analysis suggested the possibility of publication and reporting biases in clinical trials of these agents for the treatment of anxiety, but the authors concluded that these biases probably did not lead to a systematic inflation of effect sizes.[33] No SSRI or SNRI has been shown to be superior to any other in the

Table 2. Stepped-Care Approach for Management of Generalized Anxiety Disorder.*

Assessment Phase
Gather a detailed history of symptoms of generalized anxiety disorder and effect on functioning.
Ensure that generalized anxiety disorder is the principal or one of the principal diagnoses.
Evaluate patient for common co-occurring mental health conditions (e.g., depression, other anxiety problems, and substance-use disorders).
Evaluate patient for suicidal ideation, plans, or attempts.
Rule out treatable physical conditions such as thyroid and cardiac problems.
Use the Generalized Anxiety Disorder 7-Item Questionnaire or another suitable measure to gauge severity and track progress.
Step 1. All known or suspected cases of generalized anxiety disorder
Educate patient and family members about generalized anxiety disorder with use of self-help sites (e.g., that of the Anxiety and Depression Association of America [www.adaa.org]).
Educate patient about lifestyle changes that can reduce symptoms of generalized anxiety disorder. Discuss strategies for improving quality and quantity of sleep and encourage regular exercise (such as aerobic exercise and yoga). Encourage patient to minimize caffeine and alcohol use and to avoid nicotine and illicit drugs.
Monitor patient's progress with lifestyle changes.
Step 2. Diagnosed generalized anxiety disorder that has not improved after education and active monitoring in primary care
Suggest low-intensity psychological interventions such as individual nonfacilitated self-help (e.g., books and high-quality websites), individual guided self-help, educational groups, computer-assisted cognitive behavioral therapy.
Step 3. Generalized anxiety disorder with an inadequate response to step 2 interventions
Provide choice of a high-intensity psychological intervention or a drug treatment according to patient's preference and then refer patient for individual or group-based cognitive behavioral therapy (8–16 sessions) or for prescription of first-line pharmacologic treatments (SSRIs or SNRIs).
Step 4. Complex or treatment-refractory generalized anxiety disorder
Refer patient for specialized care by a mental health professional who will prescribe other first-line pharmacologic treatments or adjunctive treatment with a long-acting benzodiazepine (to be avoided among patients who are receiving opioids and among the elderly), buspirone, pregabalin, or quetiapine, and who will consider more intensive cognitive behavioral therapy, other forms of psychotherapy (such as psychodynamic therapy and acceptance and commitment therapy), or both.

* Adapted from United Kingdom's National Institute for Health and Care Excellence guidelines: (www.nice.org.uk/guidance/cg113/chapter/1-recommendations). SNRI denotes serotonin–norepinephrine reuptake inhibitor, and SSRI selective serotonin-reuptake inhibitor.

treatment of generalized anxiety disorder, so the choice of drug should be based on cost and on the patient's prior response to or the physician's familiarity with a particular agent. When SSRIs and SNRIs are used for generalized anxiety disorder, they are administered at the same doses as those used for the treatment of major depression, with the same expectation of time to response (4 to 6 weeks) and with the same precautions and anticipated adverse effects.[34]

The evidence base is growing for the use of SSRIs and SNRIs for the treatment of anxiety disorders, including generalized anxiety disorder, in children and adolescents.[35] However, these medications should be prescribed to children and adolescents only when psychological approaches have failed, and only then by experienced behavioral pediatricians or psychiatrists.

Several randomized, controlled trials have shown a benefit of a newly marketed antidepressant, vilazodone, in patients with generalized anxiety disorder,[36] but this agent has no known advantages over generically available SSRIs or SNRIs. Trials involving patients with generalized anxiety disorder have not consistently shown efficacy of certain other antidepressants, including bupropion and the recently marketed vortioxetine,[37] and these agents are not recommended.

The efficacy of tricyclic antidepressants such as imipramine is similar to that of SSRIs,[32] but tricyclic antidepressants have a less favorable safety profile. Their role in treating generalized

Table 3. Medications Commonly Prescribed for the Treatment of Generalized Anxiety Disorder.*

Medication	Starting Dose	Target Dose†	Common Side Effects	Comments
	mg/day			
SSRI			Nausea, somnolence, insomnia, jitteriness, diarrhea, sexual dysfunction	
Sertraline	25	100–200		
Paroxetine‡	10	20–60		
Paroxetine CR	12.5	25–75		
Citalopram	10	20–40		Dose should not exceed 40 mg/day because of concerns about prolongation of QT interval
Escitalopram‡	5	10–20		
SNRI			Nausea, somnolence, insomnia, dizziness, sexual dysfunction, hypertension	
Venlafaxine XR‡	37.5	75–225		
Duloxetine‡	20	20–60		
Benzodiazepine			Somnolence, dizziness	Use with caution in the elderly and in patients with past or present substance-use problems; may be used as monotherapy or as an adjunct to SSRI or SNRI
Diazepam	2.5–5.0	10–40		Usually administered in two divided doses
Clonazepam	0.25–0.50	1.0–2.0		May be administered once daily or in two divided doses
Lorazepam	0.5–1.0	1.0–4.0		Usually administered in two divided doses
Alprazolam	1.0–2.0	2.0–6.0		Usually administered in three divided doses
Tricyclic antidepressant			Orthostasis, cardiac arrhythmias, weight gain, potentially lethal in overdose	
Imipramine	10	50–200		
Other medication				May be used as monotherapy or as an adjunct to SSRI or SNRI
Buspirone‡	10–20	20–60	Dizziness, sweating, nausea, insomnia	
Pregabalin	150	150–600	Somnolence, dizziness	Usually administered in two or three divided doses
Gabapentin	100–200	100–1800	Somnolence, dizziness	Usually administered in two or three divided doses
Quetiapine	25	50–200	Somnolence, dizziness, weight gain, and other metabolic side effects	

* This list is not comprehensive. CR denotes controlled release, and XR extended release.
† In older adults, target doses should be at the lower end of the range.
‡ This drug has been approved by the Food and Drug Administration (FDA) for the treatment of generalized anxiety disorder.

anxiety disorder is currently uncertain, though they may be useful in persons who have had a response to them in the past and may be considered in patients who do not have a response to SSRIs or SNRIs.

Referral to a psychiatrist is indicated for patients who do not have a response to SSRIs or SNRIs or who have had adverse effects from these drugs that could not be managed, or when the clinical picture is complicated by a coexisting

condition (such as a substance-use disorder or suicidality). In such instances, alternative or adjunctive therapies may be prescribed; these include buspirone (a nonbenzodiazepine, nonantidepressant azapirone class of drug that appears to be effective only for generalized anxiety disorder and not for other anxiety disorders),[38] pregabalin (which, although not approved by the Food and Drug Administration [FDA] for generalized anxiety disorder, has been shown to be efficacious in several randomized clinical trials),[39] and quetiapine (also not FDA-approved for generalized anxiety disorder, but its use is similarly supported by data from randomized trials).[40] Treatment with quetiapine or other atypical antipsychotic agents should be undertaken with due regard to the adverse metabolic effects of this drug class and with close monitoring of the patient's weight, lipid levels, and glycated hemoglobin level. Although limited data have suggested efficacy of antihistamines such as hydroxyzine for generalized anxiety disorder, these agents are not recommended because of their tendency to sedate and the absence of longer-term data to support their use.[41]

Benzodiazepines such as diazepam and clonazepam (both of which are long-acting agents) are also efficacious in the treatment of generalized anxiety disorder,[42] but because of concerns about misuse and dependence, some physicians do not administer them for generalized anxiety disorder and other anxiety disorders. Most prescribing guidelines suggest that benzodiazepines should be used only on a short-term basis (3 to 6 months), a time frame that is inconsistent with the typically chronic nature of generalized anxiety disorder. However, many specialists believe that, with close monitoring, benzodiazepines are a reasonable option in selected patients (i.e., those without current or past alcohol-use or other substance-use problems) for whom preferred agents are ineffective or associated with a poor side-effect profile.[23,43] Observational data have raised concern regarding an increased risk of dementia associated with long-term benzodiazepine use,[44] but it is unclear whether this relationship is causal. Benzodiazepines should not be used with opioid medications because of the risk of drug interactions, and the use of these agents should be minimized in the elderly, in whom risks such as falls are likely to outweigh benefits.

Psychotherapy

Randomized, controlled trials have evaluated a number of psychotherapeutic techniques for generalized anxiety disorder, including cognitive behavioral therapy, psychodynamic therapies (which address underlying conflicts that are thought to be the source of anxiety), mindfulness-based therapies (including acceptance and commitment therapy, which encourages a focus on the present and on core values that transcend symptoms and illness),[45] and applied relaxation therapy (which teaches approaches to inducing a relaxed state). Among these forms of therapy, the evidence is strongest for the use of cognitive behavioral therapy in the treatment of generalized anxiety disorder, for which it can be considered a first-line treatment.[25]

The framework of cognitive behavioral therapy posits that patients with generalized anxiety disorder overestimate the level of danger in their environment, have difficulty with uncertainty, and underestimate their capacity to cope. Cognitive behavioral therapy for generalized anxiety disorder involves cognitive restructuring to help patients understand that their worry is counterproductive, exposure therapy to enable patients to learn that their worry and avoidance behaviors are malleable, and relaxation training.

Methods of delivery of cognitive behavioral therapy include weekly individual sessions (60 minutes each for 12 to 16 sessions), 8 to 12 weekly group-based sessions, computer-assisted therapy with minimal assistance from a therapist in primary care, and therapy delivered by means of the telephone in rural areas.[46] These methods have been tested and have been shown to be efficacious, with moderate-to-large effect sizes as compared with the control method (the use of a waiting list).[25]

Whereas cognitive behavioral therapy, which teaches skills to manage anxiety, would be expected to have more durable effects than medications (which stop working when the patient stops taking them), data are lacking from head-to-head trials comparing cognitive behavioral therapy with pharmacotherapy and including long-term follow-up. Patient preference regarding the method of delivery of cognitive behavioral therapy should be assessed. Cognitive behavioral therapy that is fully delivered by means of the Internet may be an ideal starting point for

some patients,[47] particularly those who do not have ready access to a therapist.

Combined Medications and Psychotherapy

Evidence from randomized trials on the most effective strategy for patients who do not have a response or who have only a partial response to psychotherapy or medication alone is lacking, but practice guidelines recommend the use of combination therapy. In children and adolescents[48] and in older adults,[49] there is some evidence that cognitive behavioral therapy combined with pharmacotherapy yields the best results, though most experts would still recommend starting with cognitive behavioral therapy and sequentially adding pharmacotherapy if needed.

AREAS OF UNCERTAINTY

Although cognitive behavioral therapy and SSRI or SNRI agents are effective in reducing symptoms in up to 50% of patients with generalized anxiety disorder, it remains unclear how best to treat patients who have no response or only a partial response to those therapies. Furthermore, although most experts suggest that patients with generalized anxiety disorder who are treated with medication should continue to receive medication for at least 1 year, the most appropriate duration of maintenance treatment is not known.

Data from randomized trials are lacking to assess the effects of combinations of currently used therapies and also to assess complementary therapies (such as yoga and massage). Data are also lacking on the extent of use, usefulness, and safety of medicinal marijuana for generalized anxiety disorder.

GUIDELINES

Several organizations have published guidelines for the treatment of anxiety disorders, including generalized anxiety disorder; these include the World Federation of Societies of Biological Psychiatry[50] and the Canadian Anxiety Guidelines Initiative Group.[51] The recommendations in this article are generally consistent with these guidelines.

CONCLUSIONS AND RECOMMENDATIONS

The woman described in the case vignette has generalized anxiety disorder and is self-medicating with alcohol to reduce tension. Using a stepped-care approach (Table 2), the physician should perform a careful assessment of her symptoms (with a standardized scale such as GAD-7) and of coexisting conditions, level of disability, and risk of suicide. She should be given information about lifestyle modifications including exercise, sleep hygiene, and reduced caffeine intake and should be strongly advised not to use alcohol to reduce symptoms of anxiety.

Reasonable initial strategies, supported by data from randomized trials, would be to administer an SSRI or an SNRI, refer the patient for cognitive behavioral therapy, or both, with the choice guided by the patient's preference. Benzodiazepines should be avoided, given her pattern of alcohol use to reduce anxiety. Her outcome during treatment should be monitored. If improvement (e.g., a 50% or more decrease in the GAD-7 score, as compared with the pretreatment score) is not seen after 3 months of treatment, a different — or adjunctive — treatment should be offered, and referral to a mental health specialist should be strongly considered if it has not already been recommended.

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Assessment

Validity of the Generalized Anxiety Disorder-7 Scale in an Acute Psychiatric Sample

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Generalized anxiety disorder (GAD) is one of the most prevalent psychiatric presentations; however, GAD has the lowest diagnostic reliability of the anxiety disorders and is poorly recognized in clinical practice. A more reliable assessment of GAD could lead to earlier detection and treatment of the disorder, which has an otherwise debilitating course and significant associated impairment. The 7-item GAD Scale (GAD-7) has shown promise as a measure with good clinical utility and strong psychometric properties in primary care and community settings but has yet to be assessed in acute psychiatric populations. This study examined the validity of the GAD-7 in a sample of 232 patients enrolled in a partial hospital programme. Patients completed a diagnostic interview and a battery of self-report measures before and after treatment. Findings suggest that the GAD-7 has good internal consistency and good convergent validity with worry, anxiety, depression and stress, and the measure was sensitive to change over the course of a short intensive cognitive–behavioural therapy partial hospital programme. However, the confirmatory analysis failed to support the hypothesized unidimensional factor structure; and although the GAD-7 demonstrated good sensitivity (.83), specificity was poor (.46) in identifying patients with GAD. Overall, the GAD-7 appears to be a valid measure of generalized anxiety symptoms in this sample, on the basis of good internal consistency, convergent validity and sensitivity to change, but does not perform well as a screener for GAD. Copyright © 2012 John Wiley & Sons, Ltd.

Key Practitioner Message:

- The GAD-7 Scale is an easy-to-score, self-report measure of core generalized anxiety disorder symptoms.
- The GAD-7 Scale has good internal consistency and convergent validity with depression, anxiety, stress and worry, and is sensitive to change.
- The GAD-7 Scale appears to be a good measure of generalized anxiety symptoms in an acute psychiatric sample.
- The GAD-7 Scale does not perform well as a screener for GAD and should not be used to identify cases of GAD in acute psychiatric samples.

Keywords: GAD-7, Generalized Anxiety Disorder, Worry, Psychometric Properties

Generalized anxiety disorder (GAD) is characterized by excessive and uncontrollable worry in addition to three or more associated symptoms including fatigue, difficulty concentrating, sleep trouble, irritability, restlessness and muscle tension (Diagnostic and Statistical Manual of Mental Disorders [DSM], Fourth Edition, Text Revision; American Psychiatric Association, 2004). Lifetime prevalence rates for GAD have been estimated at 5.7% for adults in the USA, with a 12-month prevalence of 3.1% (National Comorbidity Survey-Replication, 2011). GAD presentation is exceedingly common across healthcare settings; in primary care, for instance, prevalence estimates for GAD range from 3.7% to 14.8% (Olfson, 2000; Olfson *et al.*, 1997).

Generalized anxiety disorder is associated with significant impairment and economic burden on the healthcare system. For example, GAD symptom scores have been linked with disability across multiple domains of daily life including self-care, interpersonal functioning and healthcare resource utilization (Ruiz *et al.*, 2011). Furthermore, GAD is associated with an increased healthcare cost of $2138 per individual over the course of a person's lifetime (Marciniak *et al.*, 2005), and estimated health costs for individuals with GAD are 64% higher than those without GAD (Olfson & Gameroff, 2007).

High rates of impairment are likely a function of the debilitating course of GAD. Studies of the trajectory of GAD have yielded inconsistent findings, with results

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suggesting that the disorder is either chronic or recurrent (Angst, Gamma, Baldwin, Ajdacic-Gross, & Rossler, 2009; Bruce *et al.*, 2005; Wittchen, 2002). Evidence for the chronicity of the disorder was found in a longitudinal study in which 42% of individuals with GAD (based on DSM-III-R criteria) still had the disorder 12 years later; furthermore, individuals with GAD were symptomatic for 74% of the study's duration (Bruce *et al.*, 2005). Other work suggests that the disorder is recurrent, with a chronicity rate of 20% (Angst *et al.*, 2009). Regardless of the exact nature of the course, without treatment, the prognosis is poor (Yonkers, Dyck, Warshaw, & Keller, 2000; Yonkers, Warshaw, Massion, & Keller, 1996). Methods for increasing early recognition and appropriate treatment referrals could have wide-ranging benefits.

Unfortunately, identifying GAD appears to be challenging. GAD has the lowest diagnostic reliability of any anxiety disorder (Brown, DiNardo, Lehman, & Campbell, 2001) and is poorly recognized in clinical practice (Beesdo *et al.*, 2009). Estimates suggest that when individuals with GAD seek help for symptoms, only 50–65% are identified as suffering from a psychiatric problem; and of those, only 34% are diagnosed with GAD (Hoyer *et al.*, 1998; Weiller, Bisserbe, Maier, & Lecrubier, 1998).

The poor recognition of GAD may be due in part to high rates of comorbidity with other psychiatric disorders and general medical conditions. Comorbidity rates suggest that 90% of individuals from the general population with lifetime GAD have also met criteria for another psychiatric disorder (Carter, Wittchen, Pfister, & Kessler, 2001). Two-thirds of individuals with current GAD also meet criteria for at least one additional psychiatric disorder (Wittchen, Zhao, Kessler, & Eaton, 1994). More specifically, GAD is frequently comorbid with major depressive disorder and other anxiety disorders and has been linked closely with chronic pain conditions, medically unexplained somatic symptoms and sleep disorders (Nutt, Argyropoulos, Hood, & Potokar, 2006).

A practical assessment of GAD could lead to earlier detection and treatment of a disorder with an otherwise debilitating course. Individual distress could be reduced by limiting impairment, disability status and overall healthcare usage; and associated costs of GAD to society could also be lessened. The 7-item GAD Scale (GAD-7; Spitzer, Kroenke, Williams, & Lowe, 2006) has shown promise as a scale with good clinical utility and strong psychometric properties. The measure is a short, self-report questionnaire that is easily scored and was developed specifically to increase recognition of GAD in a primary care patient sample (Spitzer *et al.*, 2006). It has demonstrated good reliability and construct validity, as evidenced by its associations with depression, self-esteem, quality of life, life satisfaction and resilience (Kroenke, Spitzer, Williams, Monahan, & Löwe, 2007; Löwe *et al.*, 2008; Spitzer *et al.*, 2006). Importantly, the measure performs well diagnostically and accurately identified 89% of individuals with GAD in a primary care sample (Spitzer *et al.*, 2006).

Although the GAD-7 Scale has promising psychometrics, it has not yet been evaluated in a psychiatric population. The measure's reliability and validity in psychiatric samples is especially important to understand, as the measure has recently attracted the attention of the National Network of Depression Centers, an organization including 21 leading depression centres and academic medical centres across the nation. The GAD-7 Scale is included in their Common Assessment Package project, an initiative developed to standardize assessment of depression and bipolar diagnosis in both research and clinical settings and is being administered nationwide. The current study evaluates preliminary psychometric properties of the measure, including convergent validity, factor structure, sensitivity, specificity and responsiveness to change, in a naturalistic, partial hospital setting. This setting presents a substantial assessment challenge, as patients are often admitted with complex diagnostic presentations and acute symptom severity.

On the basis of previous work, we hypothesized that the GAD-7 items would demonstrate moderate-to-strong correlations with one another and with the total score and evidence good internal consistency. We predicted that the GAD-7 total score would correlate positively with worry, anxiety and stress, and to a lesser degree with depression, and that patients who meet criteria for GAD would report higher scores than those who do not. We also examined the measure's factor structure, hypothesizing that all items load onto a unidimensional factor. Sensitivity and specificity were calculated, and it was expected that the measure would show good sensitivity and adequate specificity in identifying patients with GAD. Finally, we predicted that the measure would be sensitive to change over the course of a brief, partial hospitalization.

METHOD

Participants

Participants were 232 patients presenting for treatment at a partial hospital programme in a private psychiatric hospital in New England. About half of patients (47.5%) were referred by their outpatient treatment providers for an increased level of care, whereas the other half (52.5%) were transitioning from an inpatient hospitalization. Specific patient referral information was available for 82% of patients in this sample and indicated that patients referred from inpatient sources were comparable with those from outpatient sources on GAD-7 scores, $t(186) = 1.59$, $p = 0.11$, M = 11.38, standard deviation (SD) = 6.57 and M = 12.94, SD = 6.88, respectively. Age of participants ranged from 18 to 68 years, with a mean age of 34.64 years (SD = 13.42), and 60% were women ($n = 139$). Ethnic composition of the sample was primarily European American (82%; $n = 190$), followed by Asian (5.6%; $n = 13$). The majority of the sample

had never married (59.1%; $n = 137$). The partial hospital programme serves patients with a wide range of Axis I symptoms. Diagnostic comorbidity in this population is common; and in this sample, 43.1% ($n = 100$) met criteria for more than one DSM-IV disorder, with an average of 1.56 diagnoses (SD = 0.86). Major depressive disorder is the most common diagnosis (66%; $n = 135$), followed by GAD (30%; $n = 30\%$), bipolar disorder (19%; $n = 40$), social anxiety disorder (18%; $n = 42$), panic disorder (12%; $n = 27$), obsessive–compulsive disorder (11%; $n = 25$), mood disorder with psychotic features (11%; $n = 25$), psychotic disorder (9%; $n = 19$) and post-traumatic stress disorder (8%; $n = 19$).

Measures

Miniature International Neuropsychiatric Interview (MINI; Sheehan et al., *1998)*

The MINI is a structured diagnostic interview that assesses for Axis I symptoms (e.g., mood, anxiety, alcohol abuse and psychosis). Each MINI diagnostic module consists of a series of screening items followed by questions about specific symptoms. The MINI demonstrates strong reliability and convergent validity with the Structured Clinical Interview for DSM-IV, with inter-rater reliabilities ranging from kappas of 0.89 to 1.0 (Sheehan *et al.*, 1998).

Generalized Anxiety Disorder-7 Scale (Spitzer et al., *2006)*

The GAD-7 Scale is a 7-item self-report measure designed to screen for the presence of GAD. Items consist of statements about worry (e.g., 'Not being able to stop or control worrying') and general somatic tension (e.g. 'Trouble relaxing'), which are rated on a 4-point Likert-type scale indicating symptom frequency, ranging from 0 (*not at all sure*) to 3 (*nearly every day*). Higher scores indicate higher levels of GAD symptoms. The GAD-7 has demonstrated adequate validity, good clinical utility and generally strong psychometric properties in primary care settings and the general population (Kroenke *et al.*, 2007; Löwe *et al.*, 2008; Spitzer *et al.*, 2006).

Center for the Epidemiological Studies of Depression-10 (CES-D-10; Andresen, Malmgren, Carter, & Patrick, 1994)

The CES-D-10 is a widely used, brief, self-report instrument. Items assess for symptoms of depression (e.g., 'I felt depressed'), and response anchors range from 0 (*rarely or none of the time*) (less than 1 day) to 3 (*most or all of the time*) (5–7 days). Participants indicate how true each statement is of them over the past week. Previous work suggests that the CES-D-10 is a reliable and valid measure (Andresen *et al.*, 1994) and had high internal consistency in this study ($\alpha = 0.89$).

Depression, Anxiety and Stress Scale-21 (DASS-21: Gloster et al., *2008)*

The DASS is a well-validated, three-factor, 21-item measure designed to assess the core symptoms of anxiety, depression and overall stress. Items on the DASS consist of statements about depression (e.g., 'I felt that I had nothing to look forward to'), anxiety (e.g., 'I was worried about situations in which I might panic and make a fool of myself') and stress (e.g., 'I tended to over-react to situations'), and are rated at a 4-point Likert-type scale from 0 (*did not apply to me*) to 4 (*applied to me very much or most of the time*), over the past week. Internal consistency in the present study was very high for depression ($\alpha = 0.94$) and high for anxiety ($\alpha = 0.84$) and stress ($\alpha = 0.88$).

Penn State Worry Questionnaire—Abbreviated (PSWQ-A; Hopko et al., *2003)*

The PSWQ-A is a single-factor, 8-item measure designed to assess worry severity. Derived from Meyer, Miller, Metzger, and Borkovec's (1990) original 16-item instrument, items on the PSWQ-A consist of statements about worry (e.g., 'Many situations make me worry') that participants rate on a 5-point Likert-type scale ranging from 1 (*not at all typical of me*) to 5 (*very typical of me*). Reliability in the present study was very high ($\alpha = 0.95$).

Schwartz Outcome Scale (SOS; Blais et al., *2008)*

The SOS is a well-validated and reliable, single-factor, 10-item measure designed to examine a broad domain of psychological health in a variety of settings (Young, Waehler, Laux, McDaniel, & Hilsenroth, 2003). Each item assesses for psychological well-being (e.g., 'My life is according to my expectations'). Participants rate items on a 7-point Likert scale from 0 (*never*) to 6 (*all or nearly all of the time*) over the past week. Internal consistency of the SOS was very high in the present study ($\alpha = 0.94$).

Treatment Setting: Behavioral Health Partial Program (BHPP)

The BHPP is a partial hospitalization programme utilizing individual and group cognitive–behavioural therapy to treat a patient population suffering from a variety of Axes I and II personality disorders. Group and individual treatment focuses on the acquisition of cognitive–behavioural therapy skills, with an emphasis on skills related to self-monitoring, cognitive restructuring, behavioural activation and effective communication strategies (for a review of the BHPP treatment approach, see Neuhaus, 2006). The average length of stay in the programme is 8.2 (SD = 3.2) days. Patients are assigned a case manager who coordinates all aspects of treatment in conjunction with a programme

psychiatrist who also provides medication management. Treatment includes group and individual psychotherapies.

Procedure

Approval for the study was granted by the hospital's Internal Review Board, and all participants gave informed consent. Before receiving any form of treatment at the BHPP, patients completed a demographics survey and a battery of self-report measures. Patients completed the same battery post-treatment. All self-report measures were completed on a computer. Patients were also administered the MINI to assess for current Axis I diagnoses. The MINI was administered by trained psychology interns, doctoral students in psychology and one post-baccalaureate mental health counsellor, who met weekly for supervision with a post-doctoral fellow in clinical psychology.

Data Analysis

All GAD-7 data for psychometric analyses were collected pre-treatment, and scores from post-treatment were used only to assess for sensitivity to change. SPSS version 17.0 (IBM Corporation, Armonk, NY) was used for all analyses, with the exception of the factor analyses that used AMOS 17.0, with maximum likelihood estimation.

We examined item characteristics and internal consistency by estimating item means, inter-item correlations and corrected-item total correlations. Internal consistency was estimated using Cronbach's alpha. Construct validity was examined by first assessing convergent validity with correlations between GAD-7 scores and measures of CES-D-10 and DASS—depression, DASS—anxiety, DASS—stress and SOS—overall well-being. Construct validity was also assessed with an examination of GAD-7 means in both the overall sample and a sample of individuals who met criteria for GAD based on the MINI. A factor analysis was conducted to determine if a unidimensional structure fit the data from this sample and tested for invariance across GAD and non-GAD groups. Four criteria were used to examine model fit. First, root mean square error of approximation (RMSEA) was used to assess absolute model fit, with values <.05 considered a 'close fit', values between 0.05 and 0.08 considered 'reasonably close fit' and >.08 an 'unacceptable' model (Steiger, 1990). The Tucker–Lewis Index (TLI), Comparative fit index (CFI) and incremental fit index (IFI) were used to examine relative model fit (compared with the null model), with values >.95 required for a well-fitting model (Bentler, 1990; Hu & Bentler, 1999). The suggested cutoff score of 10 (Spitzer *et al.*, 2006) was used to compare those identified by the GAD-7 as likely having GAD with those who met criteria based on the MINI. Sensitivity, specificity, positive and negative predictive values were also calculated for a range of alternative cutoff scores. Finally, changes in GAD-7 scores over the course of treatment were examined using a repeated measures, mixed analysis of variance design, with GAD diagnosis as the independent factor and time of assessment (before and after treatment) as the between subjects factor.

RESULTS

Sample Characteristics

The overall mean GAD-7 score in this sample was 10.6 (SD = 5.8), which is in the 97th percentile reported previously in the general population (Löwe *et al.*, 2008) and falls in the moderate anxiety category based on data from primary care samples (Spitzer *et al.*, 2006). Age did not correlate significantly with GAD-7 scores, $r = 0.09$, $p = 0.19$. Female participants reported higher scores compared with men, $t(225) = -2.75$, $p = 0.006$, M = 13.15 (SD = 6.37) and M = 10.82 (SD = 6.03), respectively.

Item Characteristics and Internal Consistency

Means of each item of the GAD-7 and the total score are presented in Table 1. Mean item scores range from 1.0 to 1.9.

Table 1. Item characteristics and factor loadings for Generalized Anxiety Disorder-7 items

No.	Item	M	SD	Corrected item-total correlation*	Factor loading	Cronbach's α
1	Feeling nervous, anxious or on edge	1.9	1.1	0.80	0.88	
2	Not being able to stop or control worrying	1.7	1.1	0.81	0.94	
3	Worrying too much about different things	1.8	1.1	0.79	0.92	
4	Trouble relaxing	1.7	1.1	0.74	0.73	
5	Being so restless that it is hard to sit still	1.0	1.0	0.52	0.56	
6	Becoming easily annoyed or irritable	1.3	1.1	0.53	0.59	
7	Feeling afraid as if something awful might happen	1.2	1.1	0.64	0.70	
	GAD-7 sum score	10.6	5.8	—	—	0.91

GAD-7 = Generalized Anxiety Disorder-7. SD = standard deviation.
*Correlation between the respective item and the total sum score (without the respective item).

Item-total correlations ranged from 0.52 to 0.81. Internal consistency was excellent, Cronbach's $\alpha = 0.91$ (compared with 0.89, as reported by Löwe *et al.*, 2008). Inter-item correlations ranged from 0.44 to a high of 0.88. Thus, the GAD-7 showed strong internal consistency in this sample.

Construct Validity

Convergent validity of the GAD-7 was assessed by examining correlations with CES-D-10, DASS-21, PSWQ-A and SOS. As expected, GAD-7 showed significant positive correlations with PSWQ-A—worry ($r = 0.64$), DASS—anxiety ($r = 0.77$) and DASS—stress ($r = 0.79$) scores. The GAD-7 Scale correlated comparably with two measures of depression, $r = 0.64$ for DASS—depression and $r = 0.74$ for CES-D-10—depression. Higher GAD-7 scores were also negatively associated with SOS—overall well-being, $r = -0.53$. See Table 2 for correlations.

We also compared scores of individuals with and without a diagnosis of GAD ($n = 69$ and $n = 149$, respectively, and $n = 4$ patients did not complete the diagnostic interview). Those who met criteria for GAD reported significantly higher scores, $t(169.32) = -4.27$, $p = 0.001$ (Levene's test was significant, $F(221) = 12.16$, $p = 0.001$, so equal variances were not assumed). Individuals with GAD reported a mean score approximately 3 points greater than those without the diagnosis, M = 14.57 (SD = 4.90) and M = 11.17 (SD = 6.60), respectively. Overall, the measure appears to have strong construct validity, as evidenced by good convergent validity and higher scores in patients with a diagnosis of GAD.

A confirmatory factor analysis was conducted to further examine validity. On the basis of previous work (Löwe *et al.*, 2008), the measure was expected to reflect a unidimensional construct. All seven items were set to load on one higher-order GAD factor. Results showed that, overall, the model did not fit the data well, $\chi^2 = 81.921$, degrees of freedom (df) = 14, $p < 0.001$, CFI = 0.94, TLI = 0.91, IFI = 0.94 and RMSEA = 0.14, with all values outside of the range indicative of a good fit. An examination of the factor loadings suggested that items 5 ('Being so restless that it is hard to sit still') and 6 ('Becoming easily annoyed or irritable') loaded only moderately onto the latent factor compared with the other items (Table 1).

To determine the areas of misfit in the hypothesized model, we conducted exploratory analyses. Examination of the modification indices suggested that allowing the error terms of items 4 and 5, 5 and 6, and 4 and 6 to covary would improve the model. Associations between these items are consistent with the conceptualization of GAD, as they reflect the associated symptoms criterion of the GAD diagnosis. We respecified the model, allowing the pairs of error variances to covary, and re-estimated the model. The respecified model appeared to fit the data better than the first model, $\chi^2 = 33.63$, df = 11, $p < 0.001$, CFI = 0.98, TLI = 0.96, IFI = 0.98, RMSEA = 0.09, although the RMSEA value suggested an inadequate model. Given that the remaining suggested modifications could not be conceptually justified, the model respecification process was concluded.

We next tested for model invariance across GAD and non-GAD groups. A baseline model with both groups was estimated simultaneously, $\chi^2 = 58.85$ df = 22, CFI = 0.97, TLI = 0.94, IFI = 0.97, RMSEA = 0.09, suggesting an overall adequate fit to the data based on CFI and IFI values >0.95. Next, factor loadings, covariances and the variance of the latent GAD factor were constrained to equality, resulting in a $\chi^2 = 75.6$, df = 32, $p < 0.001$. Because the change in chi-square was non-significant, χ^2 change = 16.75, df change = 10, $p = 0.08$, we conclude that the model is invariant across GAD diagnosis. Overall, a unidimensional factor structure provides a

Table 2. Generalized Anxiety Disorder-7 correlations with depression, anxiety, stress,and overall well-being

	1.	2.	3.	4.	5.	6.	7.
1. GAD-7							
2. CES-D-10—depression	0.74						
3. DASS—depression	0.64	0.85					
4. DASS—anxiety	0.77	0.68	0.64				
5. DASS—stress	0.79	0.73	0.68	0.78			
6. PSWQ-A—worry	0.64	0.55	0.46	0.44	0.45		
7. SOS—overall well-being	−0.55	−0.73	−0.72	−0.39	−0.49	−0.56	
n	232	225	229	226	224	223	224
Mean	10.6	16.6	5.9	3.8	5.9	28.2	34.7
SD	5.8	7.4	5.1	3.5	4.6	9.3	13.4
Range	0–21	0–30	0–21	0–21	0–21	8–40	10–70

All correlations are significant, $p < 0.001$. GAD-7 = Generalized Anxiety Disorder-7. CES-D-10 = Center for the Epidemiological Studies of Depression-10. DASS = Depression, Anxiety and Stress Scale-21. PSWQ-A = Penn State Worry Questionnaire—Abbreviated. SOS = Schwartz Outcome Scale. SD = standard deviation.

good fit to the data only when items 4, 5 and 6 are allowed to covary, and the model is comparable across patients with and without a diagnosis of GAD.

Cutoff Scores, Sensitivity and Specificity

Sensitivity and specificity values for the GAD-7 Scale were calculated next. Previous work suggests that a cutoff score of 10 maximizes sensitivity and specificity in identifying cases of GAD (Spitzer *et al.*, 2006). In this sample, a cutoff score of 10 resulted in good sensitivity (83%) but poor specificity (46%) and a high false positive rate (0.54). Values were also calculated for a range of other cutoff scores (ranging up to 17), but none adequately balanced sensitivity and specificity. That is, cutoff scores with good sensitivity resulted in poor specificity, whereas those with good specificity resulted in poor sensitivity (see Table 3 for sensitivity, specificity and positive and negative predictive power). A receiver operating characteristic curve analysis estimated the area under the curve at 0.65 (95% confidence interval = 0.59–0.73). See Figure 1.

Improvements during Treatment

We also examined changes in GAD-7 scores across the patient's treatment in the programme. A mixed-design, repeated-measures analysis of variance, with GAD diagnosis as the independent factor and time of GAD-7 assessment (admission and discharge) as the between-subjects factor, was conducted. Results showed a significant main effect for time, $F(1, 166) = 84.73$, $p < 0.001$, partial $\eta^2 = 0.34$, and GAD diagnosis, $F(1, 166) = 8.9$, $p = 0.003$, partial $\eta^2 = 0.05$, but no interaction between time and GAD diagnosis, $F(1, 166)$—0.29, $p = 0.59$. Because the previous *t*-test indicated that those with GAD report higher GAD-7 scores, the main effect finding here will not be discussed. The effect for time indicated that scores decreased from admission (M = 12.52, standard error = 0.54) to discharge (M = 8.9, standard error = 0.50), regardless of GAD diagnosis.

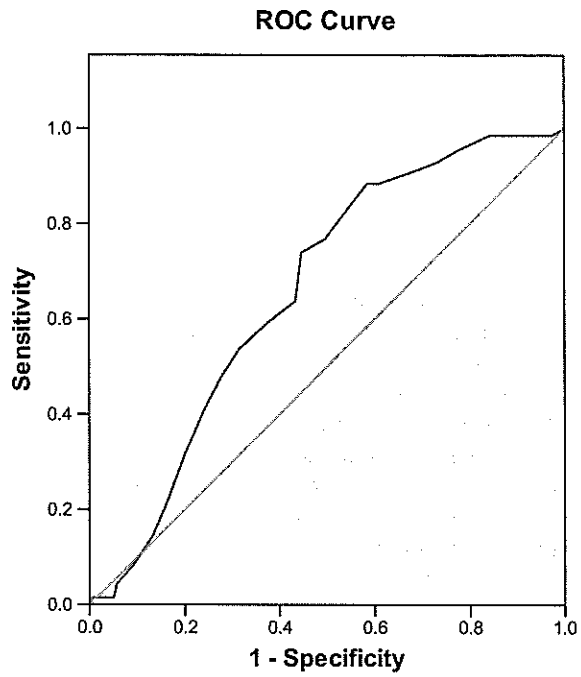

Figure 1. Receiver operating characteristic curve (ROC) for Generalized Anxiety Disorder-7 (GAD-7) against a Miniature International Neuropsychiatric Interview diagnosis of GAD

DISCUSSION

This study examined the validity of the GAD-7 Scale in a sample of patients in an acute partial hospital programme. Patients in this setting frequently have complex diagnostic presentations and comorbidity is the norm, rather than the exception. Although the GAD-7 Scale has demonstrated strong psychometric properties in primary care (Spitzer *et al.*, 2006) and the general population (Löwe *et al.*, 2008), it is yet to be examined in a psychiatric population.

In this sample, GAD-7 scores were uniformly high across the sample. The average GAD-7 score fell in the 97th percentile, compared with individuals from the general population (Löwe *et al.*, 2008). Those who met diagnostic criteria for GAD reported higher scores than those who do

Table 3. Sensitivity, specificity and false positive rates for possible Generalized Anxiety Disorder-7 cutoff scores

GAD-7 cutoff score	Sensitivity	Specificity	False positive rate	PPV	NPV
10	0.83	0.46	0.54	0.40	0.86
11	0.77	0.50	0.50	0.40	0.83
12	0.74	0.55	0.45	0.41	0.83
13	0.64	0.57	0.43	0.39	0.78
14	0.59	0.62	0.38	0.41	0.78
15	0.54	0.69	0.31	43	0.77
16	0.48	0.73	0.27	0.43	0.76
17	0.41	0.76	0.24	0.42	0.75

PPV = positive predictive value. NPV = negative predictive value.

not, and the GAD group mean is nearly identical to that found in previous work (Spitzer *et al.*, 2006); however, patients without GAD also reported high scores. The average score in patients who do not meet criteria for GAD falls in the 97th percentile on the basis of norms from the general population (Löwe *et al.*, 2008). Further, both group scores fall above the suggested cutoff score for identifying individuals with GAD. Given that all patients had been admitted to the programme at the time of assessment, it seems unlikely that non-GAD patients are over-reporting symptoms in order to access services.

Our hypothesis that the GAD-7 Scale would have good internal consistency and item-total correlations was supported. The GAD-7 Scale displayed excellent internal consistency in this sample. Cronbach's alpha was excellent, and inter-item correlations ranged from moderate to high. Item-total correlations also ranged from moderate to high, with the 'restless' and 'irritable' items correlating the lowest of the seven items. Similarly, these items showed the weakest item-total correlations in a study of the general population, (Löwe *et al.*, 2008).

The hypothesis that the measure would show good convergent validity was largely supported. The GAD-7 correlated highly with DASS—anxiety and DASS—stress scores, comparable with previous work (Löwe *et al.*, 2008; Spitzer *et al.*, 2006). The measures also showed a moderate negative association with SOS—overall well-being scores, in line with our prediction. Of note, the GAD-7 also correlated strongly with both DASS—depression and CES-D-10—depression scores and only moderately with PSWQ-A—worry scores. GAD and depression are often comorbid (Hettema, 2008), and other work suggests that worry levels are comparable in individuals with GAD and depression (Starcevic, 1995). Therefore, the correlation of the GAD-7 with depression measures is not surprising. Future work might strive to better understand if the GAD-7 is a measure of GAD symptoms specifically or negative affect more generally.

A confirmatory factor analysis, specified so that each of the seven items loaded onto one latent GAD factor, failed to support our hypothesis and the model detailed elsewhere (Löwe *et al.*, 2008). Follow-up exploratory analyses suggested that several of the items, specifically items 4, 5 and 6, shared unique variance beyond that explained by the GAD factor. An examination of the items suggests that perhaps these items also reflect a somatic tension/autonomic arousal factor. A recent review has suggested that it may be beneficial to examine the subtypes of GAD (Portman, Starcevic, & Beck, 2011), on the basis of findings that individuals who would otherwise meet diagnostic criteria for GAD (based on endorsement of the associated symptoms criterion) fail to receive the diagnosis because they do not meet the excessive worry criterion (Roth *et al.*, 2008). Portman *et al.* (2011) hypothesize that there may be subtypes of GAD, including an excessive worry type, a somatic tension/autonomic arousal subtype and a combined subtype. To date, no work has tested this hypothesis empirically, although our findings here suggest that this may be valuable to explore in future work.

The hypothesis that the GAD-7 Scale would show good sensitivity was supported, whereas the prediction of good specificity was not. High scores on the GAD-7 across participants, as discussed previously, impacted the measure's utility to discriminate those who met criteria for GAD from those who did not. Previous research suggested that a cutoff score of 10 resulted in good sensitivity and specificity in a primary care sample, with sensitivity of 89% and specificity of 82% (Spitzer *et al.*, 2006). Contrary to these findings, however, a cutoff score of 10 in this sample resulted in good sensitivity (0.83) but poor specificity (0.46). Further, the cutoff score resulted in an unacceptable rate of false positives. We examined the potential utility of a range of other cutoff scores, up to 17, to determine if a higher score might perform better in our more symptomatic sample; however, the alternative cutoff scores were similarly limited and unable to balance sensitivity and specificity so that both were acceptable.

Findings from this study indicate that the GAD-7 Scale is sensitive to changes in symptoms over time, in line with the hypothesis. There was no interaction between time of assessment and GAD group, which suggests that all patients saw symptom improvement over time, regardless of GAD diagnosis. Given the chronic nature of GAD (Bruce *et al.*, 2005), the ability of the GAD-7 Scale to detect symptom improvement over a short period is a significant strength. The main effect for time indicates that patient scores decreased approximately 3.5 points, from a mean score of 12.5 to 8.9. This change suggests that, on average, patients are moving across the previously identified clinical cutoff point of the GAD-7, from a group identified as likely having GAD to a non-GAD group; however, this particular finding should be interpreted with caution, given the poor specificity of the cutoff score in this sample.

This study has several strengths. It is the first to examine the psychometric properties of the GAD-7 in a psychiatric sample and the diagnostically heterogeneous nature of the sample has strong external validity, suggesting that findings are likely to be highly generalizable. The inclusion of a range of symptom measures, including depression, anxiety, worry, stress and overall well-being scores, also allowed for a thorough assessment of convergent validity. Despite these strengths, however, the study is not without limitations. First, the acute nature of the symptoms in the current sample could have influenced the results, which may differ in outpatient clinics. Future work would benefit from examining the psychometric properties of the GAD-7 in a traditional outpatient setting. In addition, the sample was relatively homogenous in terms of ethnic and racial demographics, and these findings would need to be replicated in more diverse samples.

Overall, the GAD-7 Scale is a short, easily administered and scored self-report questionnaire with strong construct validity in this acutely symptomatic psychiatric sample, as evidenced by good internal consistency, good convergent validity and higher scores in patients with GAD compared with those without GAD. It should be noted that the GAD-7 Scale also correlated highly with DASS and CES-D-10—depression scores and moderately with PSWQ-A—worry scores. The measure was responsive to symptom improvement over the course of treatment in a partial hospitalization programme and may be a valuable indicator of fluctuations in anxiety over time. However, the initial hypothesized factor structure failed to provide a good fit to the data; and the GAD and non-GAD groups reported scores higher than the suggested cutoff score, which resulted in good sensitivity but poor specificity. In summary, the GAD-7 is an internally consistent and valid measure of general anxiety and worry in a sample of acutely symptomatic patients, but the measure is unlikely to be useful as a screening tool for GAD in this sample.

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Enhanced Th17 phenotype in individuals with generalized anxiety disorder

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ABSTRACT

The generalized anxiety disorder (GAD) is often a debilitating chronic condition, characterized by long-lasting anxiety that is not focused on any object or situation. Besides being clearly linked to increased susceptibility to infectious diseases, anxiety is also known to contribute to the pathogenesis of many inflammatory/autoimmune disorders. The present work aimed to explore the T cell profile following *in vitro* activation in cultures obtained from a group of individuals with GAD, comparing them with healthy control individuals. Our results demonstrated that cell cultures from GAD group proliferated less following T cell activation as compared with the control group. The analysis of the cytokine profile revealed Th1 and Th2 cytokine deficiencies in the anxious group, as compared with the control subjects. On the other hand, this cellular and humoral immune damage was followed by enhanced production of Th17-derived cytokines. In particular, the levels of TNF-α and IL-17 were significantly higher in cell cultures containing activated T cells from GAD individuals. Therefore, besides a deficiency on Th1 phenotype, an elevated proinflammatory status of these individuals might be related to both glucocorticoid immune resistance and lower IL-10 levels produced by activated T cells. In conclusion, our results demonstrated a T cell functional dysregulation in individuals with GAD, and can help to explain the mechanisms of immune impairment in these subjects and their relationship with increased susceptibility to infections and autoimmune diseases.

1. Introduction

Nervous and immune systems are able to counterattack a variety of danger signals in an adaptive and coordinated manner. Although the first primarily reacts to real or perceived stressful life events and the least responds to viruses, bacteria and parasites, they are biologically connected to each other, acting together in order to maintain or re-establish homeostasis.

The cross-talk between these two systems is more studied in the context of stress responses that involve primarily the activation of hypothalamus–pituitary adrenal (HPA) axis resulting in release of cathecolamines (norepinephrine and epinephrine) and glucocorticoids from medullar and cortical regions of adrenal, respectively (Steinman, 2004).

Immune cells have different sets of receptors for different products secreted by activated HPA axis, and these neuroendocrine products have the potential to influence immune function (Butts and Sternberg, 2008; Padgett and Glaser, 2003). Although the activation of HPA axis is fundamental for the adaptive response to stress, if the stressor is excessive or prolonged, adaptations can increase the risk of immunological disorders, such as those observed in patients suffering of generalized anxiety disorder (GAD).

The GAD is often a debilitating chronic condition, characterized by long-lasting anxiety that is not focused on any object or situation. In times of high stress, GAD patients frequently present some physiological symptoms such as headache, sweating, muscle spasms, palpitations, and hypertension, which in some cases lead to fatigue or even exhaustion (Khouzam, 2009).

Many studies have demonstrated that chronic anxiety state has also a deleterious impact on immune function (Boscarino, 2004; Sareen et al., 2005; Schneiderman et al., 2005; Godbout and Glaser, 2006). Authors have demonstrated that anxiety can lead to damaged cellular and humoral immune responses (Arranz et al., 2007; Zhou et al., 2005; Koh and Lee, 2004), and, consequently, increase the incidence of viral and bacterial infections (Takkouche et al., 2001; Aviles et al., 2004; Cohen et al., 1999). Anxiety has also been related to impaired immune response to several antiviral/bacterial vaccines, such as hepatitis B virus (Glaser et al., 1992; Jabaaij et al., 1996), pneumococcal bacteria (Glaser et al. 2000), rubella virus (Morag et al. 1999), meningitis virus (Burns et al.

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2002), and influenza virus (Kiecolt-Glaser et al. 1996; Miller et al. 2004; Vedhara et al. 1999).

Efficient host defense against invading pathogenic microorganisms is coordinated by the action of highly heterogeneous poll of effector T cells. In this context, Th1-mediated response is characterized by the production of IL-2 and IFN-γ and is involved in cellular immunity against intracellular microorganisms (Gutcher and Becher, 2007). These cytokines activate the bactericidal activities of macrophages, induce opsonizing IgG1 and IgG3 secretion by B cells, mobilize and enhance the cytotoxic activities of $CD8^+$ T cells and NK cells (Crozat et al., 2009). On the other hand, human Th2 cells produce IL-4, IL-5, and IL-13 and are required to control helminthes infections by stimulating the growth and activation of mast cells and eosinophils, as well as differentiating B cells into IgE secreting cells (Ekkens et al., 2003). Finally, activated Th17 cells produce IL-17A (also referred to as IL-17), IL-21, IL-1β, IL-6 and TNF-α, and play important roles in the clearance of certain microbes, particularly extracellular bacteria and fungi, by mediating intense chemotaxis of neutrophils to sites of inflammation (Andoh et al., 2001; Jovanovic et al., 1998; Matsuzaki and Umemura, 2007; Gutcher and Becher, 2007).

Tight regulation of effector T cell responses is required for effective control of infections and avoidance of immunopathological diseases (Costantino et al., 2008). In this scenario, aberrant Th1 and, mainly, Th17 cell responses play critical roles in triggering organ-specific autoimmunity (Miossec, 2009), whereas Th2 cells are pivotal in allergy and asthma (Makani et al., 2008). Many works have demonstrated that fine immuneregulation is mainly performed by a pool of regulatory T cells (as revised by Vignali et al., 2008).

A number of T cells with regulatory functions have been described in literature. These include IL-10-secreting T-regulatory 1 (Tr1) cells (Maynard et al., 2007), transforming growth factor (TGF)-β-secreting Th3 cells (Kitani et al., 2003) and, more recently, $CD4^+$ $CD25^{high}$ $Foxp3^+$ Treg cells (Shevach, 2009). These regulatory T cells suppress immune responses by several mechanisms, such as the production of anti-inflammatory cytokines, mainly IL-10 and TGF-β. These cytokines are pivotal to control immune responses by inhibiting not only the development of Th1, Th2, and Th17 cells, but also their effector functions (Belkaid, 2007; Li and Flavell, 2008; McGeachy et al., 2005).

Anxiety has been clearly linked to a greater susceptibility to infectious diseases. In addition, it is also known to contribute to the pathogenesis of many inflammatory/autoimmune diseases (Gill et al., 2009; Wessa and Rohleder, 2007; Lemieux et al., 2008; Wilson et al., 1999; Boscarino and Chang, 1999; Cohen and Schwartz, 1999; Affleck et al., 1997). Therefore, taking into count the high heterogeneity of T cells subsets, the aim of the present work was to explore the T cell profile following *in vitro* non-specific activation in a group of patients with generalized anxiety disorder and to investigate the impact of glucocorticoid on this profile. This kind of study is very important for the understanding of the specific mechanisms by which chronic stress damages immune functionality and how it favors the occurrence of infectious and autoimmune/inflammatory diseases.

2. Materials and methods

2.1. Participants

For our study 20 individuals with generalized anxiety disorder (8 male, 12 female; mean age = 29.4 years, SD = 12.7, range 17–42) and 20 healthy subjects without any detectable psychiatric disorder matched by age (mean age = 29.7 years, SD = 10.3, range 19–40), gender, racial background and annual income were recruited to participate this study. We used the Spielberger State (STAIS) and Trait (STAIT) Anxiety Inventory (Kohn et al., 2008), Hamilton Depression Rating scale (HAMD) and Hamilton Anxiety Rating scale (HAMA) to measure mood states in all subjects (Hamilton, 1959). Individuals with a present, past and family history of mental disorders were assessed through of the Semi structured Interview for the DSMIII-R (SCID) (Spitzer et al., 1992). According to the HAMA, we included only the patients with moderate–severe symptoms of anxiety.

Subjects were excluded if they reported intake of any medication with immune-modulating effects, such as glucocorticoids, had acute or chronic organic illnesses, or met criteria for additional mental disorders other than anxiety or stress-related affective disorders. Anxiety disorders often co-exist with other mental disorders, particularly clinical depression, which may occur in as many as 60% of people with anxiety. In our study, 05/20 among anxiety patients met the DSM-IV criteria for current major depressive episode, however, they were not excluded because no significant difference was observed between them and the other non-depressive anxious individuals concerning the results from immune function analysis. Although 14/20 anxious patients reported past intake of psychotropic medication (12 anxiolytics and 2 antidepressants), all participants were completely medication-free for at least six months prior to blood sampling. Of note, none of the women were pregnant or lactating. Finally, the Survey of Immunological and General Health (SIGH) was used as a self-report measure of physical health (Kang et al., 1991). The SIGH also includes questions on age, weight, and demographic background and has been successfully used in a wide variety of studies (Kang et al., 1991; Lemieux and Coe, 1995; Strauman et al., 1993, 2004). Body Mass Index (BMI), that might affect immune function, was calculated as weight in kilograms divided by the square of height in meters. For our study, all subjects had BMIs ranging from 18 to 30.

After a complete description of the study to the participants, written informed consent was obtained for each individual. The study was approved by the Ethical Committee for Research on Human Subjects of the Federal University of the State of Rio de Janeiro (UNIRIO).

2.2. Peripheral blood mononuclear cell cultures and stimulus

For our study, 20 mL of peripheral blood was collected between 10 and 11 a.m. in heparin treated tubes (BD Vacutainer, Franklin Lakes, NY). The peripheral blood mononuclear cells (PBMC) were obtained by centrifugation on Ficoll–Hypaque density gradients. The viable PBMC, evaluated by trypan blue exclusion, were adjusted to 1×10^6/mL and cultured either in a 96-well flat-bottomed microplates with 200 μL of complete RPMI 1640 medium or in a 24-well flat-bottomed microplates with 1 mL of complete RPMI 1640 medium. The RPMI 1640 was considered complete when it was added with 2 mM of L-glutamine (GIBCO, Carlsbad, CA, USA), 10% of FCS, 20 U/mL of penicillin, 20 μg/mL of streptomycin and 20 mM of HEPES buffer. In order to induce T cell polyclonal activation, whole PBMC cultures were maintained for 3 days with phytohemaglutinin (PHA, 1 μg/mL), which corresponds to the period of maximal T cell activation. To evaluate the effect of glucocorticoid, dexamethasone (DEX) (Sigma Chemicals, St Loius, MD), at a concentration of 10^{-6} M, the highest dose detected *in vivo* in stress situations (Franchimont et al., 2000; Agarwal and Marshall, 1998), was added to some wells at the beginning of cell cultures. Of note, this high DEX concentration did not induce cell death (data not shown). All cells were cultured at 37 °C in a humidified 5% CO_2 incubator.

2.3. MTT assay

The 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide (MTT) assay was performed as previously described (Roehm et al., 1991). Briefly, 3 days after beginning of the PBMC cultures, activated or not with PHA (1 μg/mL), 40 μL of 5 mg/mL MTT were added to each well in a 96-well flat-bottomed microtiter plates and additionally incubated for 2 h at 37 °C in a humidified 5% CO_2 incubator. The reaction was revealed after dissolution of the formozan crystals with acidified isopropanol (150 μL/well). The resulting purple solution was spectrophotometrically measured at 570 nm in microplate reader (Dynex technologies, Chantilly, VA). A blank with propanol alone was measured

and subtracted from all values. The results are presented as mean ± SD of optical density (D.O.).

2.4. Cell counts

In order to perform the cell counts, PMBC (1×10^6/mL) were seeded in a 24-well flat-bottomed microplates and stimulated or not with PHA (1 μg/mL) for 3 days. At the end of culture time, the number of viable cells in PBMC cultures was evaluated by trypan blue exclusion by using a hemocytometer. The number of viable (unstained) cells was counted and the values were expressed as mean ± SD.

2.5. Cytokine determination

In order to determine the *in vitro* cytokine contents, the supernatants were collected 3 days after the stimulation of PBMC with polyclonal T cell activator (PHA, 1 μg/mL), and submitted to cytokine measurement by OptEIA ELISA kits (BD, Pharmigen, San Diego, CA), according to manufacturer's protocol. Briefly, each ELISA was performed using pairs of mAbs directed to human IL-2, IL-1β, IL-6, IL-10, IL-4, TNF-α, IFN-γ, TGF-β, and IL-17. The reaction was revealed with streptavidin-horseradish peroxidase, using 3,3′,5,5′-tetramethylbenzidine (TMB) as substrate. Recombinant human cytokine ranging from 10–500 pg/mL were used to construct standard curves.

2.6. Statistical analysis

The nonparametric Mann–Whitney U test was applied to determine whether the two groups were statistically different for each given variable. The Student's t-test was applied to verify if a determined variable was statistically different among the subjects from the same group. The significance in all experiments was defined as $p<0.05$.

3. Results

3.1. Impact of anxiety on proliferation of peripheral blood cell cultures in response to T cell polyclonal activator in the presence or absence of glucocorticoid

The first immune event measure in our study was T cell proliferation induced by the polyclonal activator phytohemagglutinin (PHA) by using MTT assay. As shown in Fig. 1A, the extent of T cell proliferation in response to PHA was statistically lower in cell cultures from anxious individuals as compared with control group. Interestingly, the level of MTT reduction was significantly higher in non-stimulated cultures (medium) from the anxious group than in control cultures.

Although the level of MTT reduction is classically used as an indirect marker of the number of viable (living) cells, we also directly quantified the number of viable cells at the end of cultures (3 days) by trypan blue exclusion. Taking into account that the number of PBMC at the beginning of the cultures was 1×10^6/mL, we did not observe any detectable cell proliferation in the non-activated cell cultures from the anxious group at direct cell counting (Fig. 1B). Nevertheless, as observed by MTT test, we observed a lower cell proliferation following PHA-activation in cell cultures from anxious individuals (Fig. 1A).

Concerning the impact of glucocorticoid (GC) on the level of cell activation, we observed that, although the DEX had reduced the level of spontaneous cell activation in cultures derived from anxious group, it did not reduce significantly the level of T cell proliferation in response to PHA. On the other hand, at the same dose, the GC successfully inhibited in approximately 48% the level of T cell proliferation in activated cell culture from the control group.

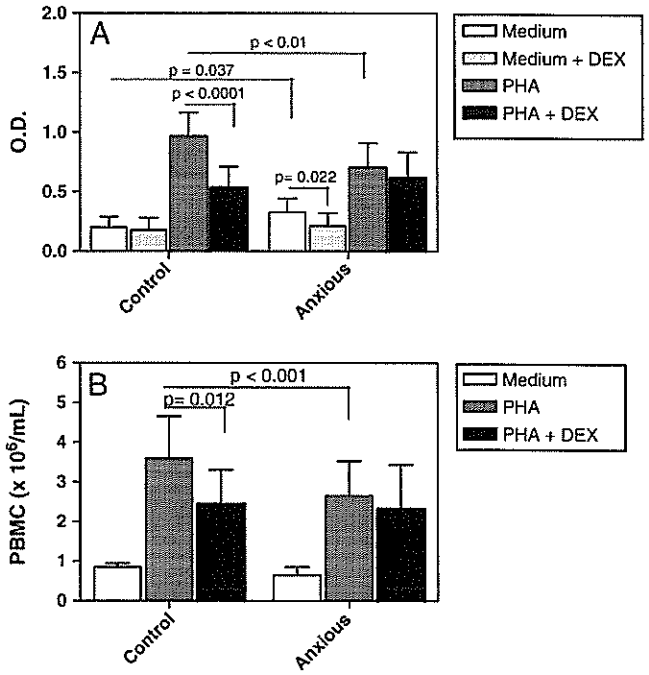

Fig. 1. Proliferative response of T cells from anxious and non-anxious subjects. PBMC (1×10^6/mL) purified from anxious (n = 20) and non-anxious (control, n = 20) individuals were kept in culture with only medium or with PHA (1 μg/mL) for 3 days. The impact of glucocorticoid on T cell proliferation in both cell cultures (control and anxious) was evaluated following addition of stress-related dose (1×10^{-6} M) of dexamethasone (DEX) at beginning of the cell cultures. In A, the proliferation was determined by the level of MTT reduction, while in B, it was evaluated by direct cell count in trypan blue. The *p* values are indicated at the figure.

3.2. Impact of anxiety on cytokine profile in cell cultures in response to T cell polyclonal activator

The type of acquired immune response is mainly determined by the cytokine network produced by activated T cells. Therefore, we decided to evaluate the cytokine profile of the PHA-activated or none activated cell cultures from the two experimental groups. Of note, no detectable spontaneous release of any cytokine was observed (data not shown). As demonstrated in Fig. 2, the production of the classical Th1 cytokines, IL-2 and IFN-γ, after T cell activation was significantly higher in control cell cultures than cultures derived from anxious group. Concerning the determination of the Th2 cytokines, IL-4 and IL-5, our results demonstrated that their release was also significantly higher in cell

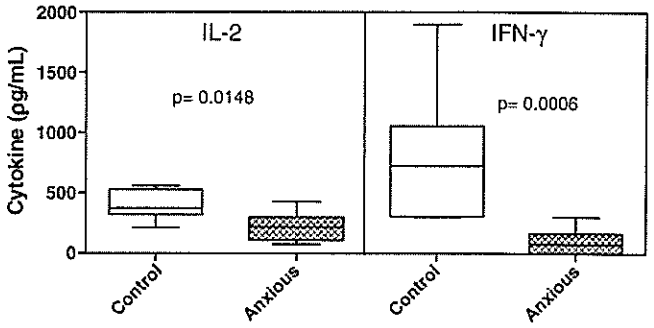

Fig. 2. The *in vitro* Th1 cytokines in anxious individuals. The polyclonal T cell activator (PHA, 1 μg/mL) was added to PBMC (1×10^6/mL) cultures obtained from anxious (n = 20) and non-anxious (control, n = 20) individuals and their supernatants were collected after 3 days. IL-2 and IFN-γ were evaluated by ELISA. In the figure, the horizontal bars within boxes correspond to the median; box limits correspond to 25th and 75th percentiles and vertical lines indicate the range. The mean values of control and anxious groups were compared and the *p* values are indicated at the figure.

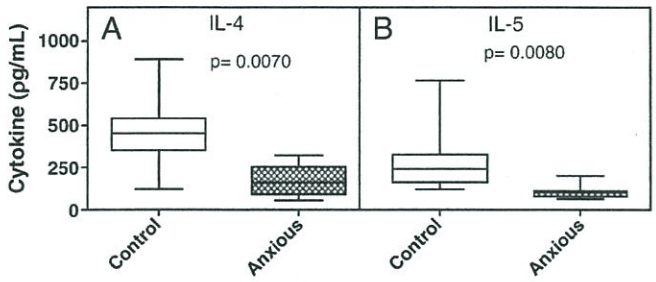

Fig. 3. The impact of anxiety on *in vitro* Th2. The polyclonal T cell activator (PHA, 1 μg/mL) was added to PBMC (1×10^6/mL) cultures obtained from anxious (n = 20) and non-anxious (control, n = 20) individuals and their supernatants were collected after 3 days. In A, IL-4 and in B, IL-5 cytokines were evaluated by ELISA. In the figure, the horizontal bars within boxes correspond to the median; box limits correspond to 25th and 75th percentiles and vertical lines indicate the range. The mean values of control and anxious groups were compared and the *p* values are indicated at the figure.

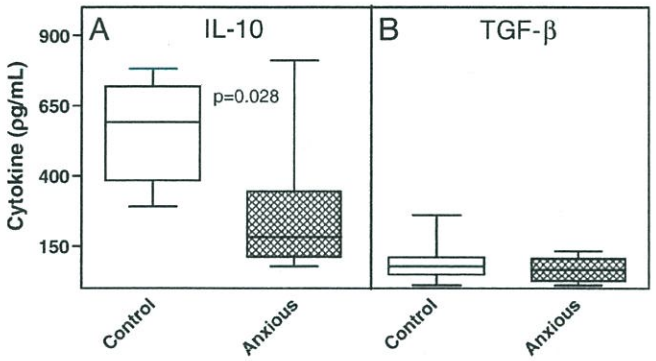

Fig. 4. The impact of anxiety on *in vitro* Tr-1 and Th3 cytokines. The polyclonal T cell activator (PHA, 1 μg/mL) was added to PBMC (1×10^6/mL) cultures obtained from anxious (n = 20) and non-anxious (control, n = 20) individuals and their supernatants were collected after 3 days. In A, IL-10 and in B, TFG-β cytokines were evaluated by ELISA. In the figure, the horizontal bars within boxes correspond to the median; box limits correspond to 25th and 75th percentiles and vertical lines indicate the range. The mean values of control and anxious groups were compared and the *p* values are indicated at the figure.

cultures from control group after PHA addition (Fig. 3). Among the anti-inflammatory cytokines, the IL-10, whose production among human T cells is restricted to the Tr-1 phenotype, was also significantly lower in cell cultures from the anxious group (Fig. 4A). TGF-β, a cytokine produced mainly by activated Th3 cells, was low and did not reach any statistical difference between two groups (Fig. 4B). Finally, we performed the measurement of the cytokines related to Th17 phenotype, particularly the IL-17 cytokine. As demonstrated in the Fig. 5, the production of TNF-α and, mainly, IL-17 were significantly higher in the anxious group. Although no significant difference was observed, we noted a clear tendency towards increased IL-1β and IL-6 cytokine production from activated cells cultures derived from the anxious group (Fig. 5).

3.3. Impact of glucocorticoid on cytokine profile in cell cultures in response to T cell polyclonal activator

Finally, our next step was to evaluate the impact of stress-related doses of GC on the different sets of cytokines produced in control and anxious groups. As shown in Fig. 6, while GC was able to attenuate the release of IFN-γ, IL-4, and IL-17 from PHA-activated cell cultures from control group, it did not significantly alter the production of these cytokines by the anxious group. Concerning IL-10, GC tended to up-regulate its production only in control cultures.

4. Discussion

Anxiety disorders, such as generalized anxiety disorder (GAD), are associated with an enhanced susceptibility to various somatic diseases. In this study, we observed important immune dysregulations in these individuals that can have implications for human health.

The first immune event analyzed was the level of T cell proliferation following non-specific stimulation with a T cell mitogen, by using the MTT assay. Interestingly, we detected a significant MTT reaction in non-activated cell cultures from the anxious group. However, a direct cell count in these cultures demonstrated an absence of cell proliferation. Therefore, although the extension of MTT reaction is traditionally related to the number of viable cells (Roehm et al., 1991), in anxious individuals, this measurement was overestimated by the high basal activated state of cells from these subjects. MTT values are due to mitochondrial amount/activity, which would reflect elevated monocytes percentage, known to have more mitochondrial than lymphocytes. Nevertheless, in our cohort, no statistical difference was observed concerning the proportion of T cells ($CD3^+$), B cells ($CD19^+$), and monocytes ($CD14^+$) in the initial PBMC isolates (data not shown). The reason for this higher background activation status may be related to endogenous release of higher levels of neurotransmitters documented among anxious individuals, such as substance P (Tuluc et al., 2009). On the other hand, cell cultures from GAD individuals responded less to PHA. This lower T cell proliferative response in GAD individuals could be related to activation-induced cell death following excessive stimulation. At the moment, we are dedicating our efforts to investigate this issue.

The kind of acquired immune response is strongly determined by the cytokine network produced by activated T cells. The production of both Th1 and Th2 cytokines was significantly lower in activated cell cultures from anxious individuals, as compared with control ones. The

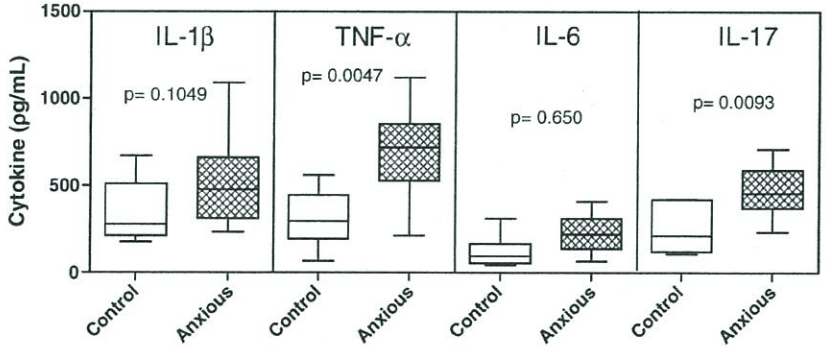

Fig. 5. The *in vitro* Th17 cytokines in anxious individuals. The polyclonal T cell activator (PHA, 1 μg/mL) was added to PBMC (1×10^6/mL) cultures obtained from anxious (n = 20) and non-anxious (control, n = 20) individuals and their supernatants were collected after 3 days. IL-1β, TNF-α, IL-6, and IL-17 cytokines were evaluated by ELISA. In the figure, the horizontal bars within boxes correspond to the median; box limits correspond to 25th and 75th percentiles and vertical lines indicate the range. The mean values of control and anxious groups were compared and the *p* values are indicated at the figure.

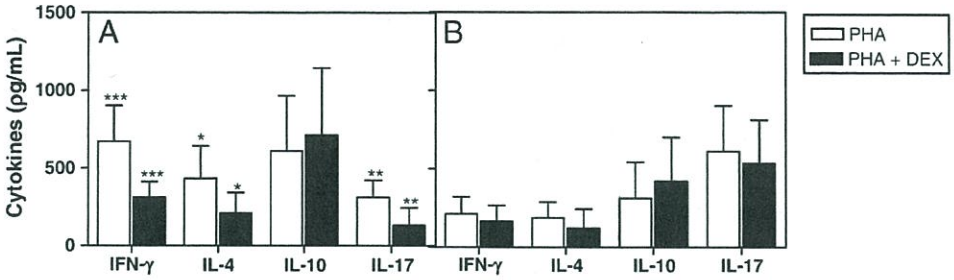

Fig. 6. The impact of glucocorticoid on *in vitro* cytokines in anxious individuals. The polyclonally-activated (PHA, 1 μg/mL) PBMC (1×10^6/mL) obtained from anxious (n = 20) and non-anxious (control, n = 20) individuals were maintained for 3 days with or without stress-related dose of dexamethasone (DEX, 1×10^{-6} M). After 3 days, the supernatants were collected and IFN-γ, IL-4, IL-10 and IL-17 cytokines were evaluated by ELISA. At the figure, the *, **, and *** indicate *p* values <0.05, <0.01, and <0.001 respectively. The absence of * indicates no significant difference.

Th1 cytokines IL-2 and IFN-γ are pivotal in the immune response against intracellular pathogens by supporting effector functions and maintenance of memory $CD8^+$ and B lymphocytes (Gutcher and Becher, 2007). Thus, Th1 phenotype deficiency results not only in greater susceptibility to infectious diseases but also favors the development of malignancies (Takkouche et al., 2001; Aviles et al., 2004; Cohen et al., 1999; Ben-Eliyahu, 2003). On the other hand, Th2 cytokines are involved in both protection against gastrointestinal nematodes and allergic disorders (Romagnani, 1997). In order to avoid recruiting individuals with high tendency to develop Th2 pattern, we excluded from the study all subjects with chronic atopic disorders, like asthma and atopic dermatitis. Our results indicate that chronic anxiety-related stress damages both Th1 and Th2 responses. There are some mechanisms by which Th1 and Th2 responses could be attenuated in anxious subjects. Firstly, concerning Th1 cells, some literature data demonstrated a high susceptibility of Th1 lymphocytes to activation-induced cell death through CD95 (Fas)/CD95L engagement (Akkoc et al., 2008). Additionally, during persistent inflammation, IFN-γ and TNF-α induce indoleamine 2,3 dioxygenase expression, a DC enzyme that degrades tryptophan amino acid in L-formylkynurenine, decreasing tryptophan availability that is pivotal to Th1 function (O'Connor et al., 2009; Dai and Dai, 2008). Finally, in chronically stressed patients the HPA axis is often hyperactivated, resulting primarily in the release of high levels of CRH and cortisol (Holsboer, 2000; Arborelius et al., 1999). While CRH inhibits Th1 (Poliak et al., 1997), the cortisol reduces both Th1 and Th2 responses (Ashwell et al., 2000). In our anxious group, however, we do not believe in an involvement of cortisol in the reduction of Th1 and Th2 responses, because we observed a clear proinflammatory profile in this group, particularly with the overproduction of Th17 cytokines, which would also be inhibited by cortisol. Our results demonstrate a higher IL-17 and TNF-α release in activated cell cultures from anxious individuals. Although IL-6 and IL-1β production were not significantly different between the two groups, these two cytokines are mainly produced by Th17 cells following phagocyte or T cell activation in the presence of pathogen-associated molecular patterns (PAMPs), such as LPS from bacteria (van de Veerdonk et al., 2009). At the moment, we are evaluating the response of PBMC cultures from anxious individuals to bacterial and fungal antigens. To our knowledge, this is the first report that documented an enhanced Th17 phenotype in anxious individuals. Of note, we also assessed the kinetics of cytokine release 24 and 48 h after PHA addition, and Th17-related cytokines were always dominant in the cell cultures from GAD individuals (data not shown). The polarized development of Th17, Th1 and Th2 cells depends on counterregulatory effects on each other. In our system, the damage of both Th1 and Th2, phenotypes in GAD patients could be related to the inhibitory effects of Th17 cytokines (Toh et al., 2009; Newcomb et al., 2009).

Chronic psychological stress, including anxiety disorders, has been associated with exacerbations of autoimmune disease (Kimerling, 2004; Fortune et al., 2005; Arican et al., 2005; Matusevicius et al., 1999; Kurasawa et al., 2000), such as psoriasis, multiple sclerosis (MS), and rheumatoid arthritis (RA) (Affleck et al. 1997, Kimerling, 2004; Fortune et al., 2005; Arican et al., 2005; Matusevicius et al., 1999; Kurasawa et al., 2000). In humans, there is a considerable body of evidence suggesting that enhanced Th17 response is involved in the aetiopathogenesis of inflammatory and autoimmune diseases. In RA, for example, IL-17 can activate and enhance all mechanisms of tissue injury by up-regulating and/or synergizing with local inflammatory mediators such as IL-6 (Katz et al., 2001; Chabaud et al., 1998), IL-1β and TNF-α (LeGrand et al., 2001; Katz et al., 2001; Chabaud et al., 1998), and pro-oxidants such as nitric oxide (Lubberts et al., 2000), as well as by stimulating the production of extracellular matrix metalloproteinases that causes tissue damage (Jovanovic et al., 2000, 2001; Wright et al., 2004). Furthermore, this Th phenotype has been also implicated in the genesis of delayed type hypersensitivity responses (DTH), and work by Altemus et al. (2003) demonstrated that individuals with post-traumatic stress disorder (PTSD) evidence stronger DTH responses.

In agreement with our results, it is well established that anxiety is related to increased circulating levels of C-reactive protein, IL-6 and TNF-α, IL-1β and IL-8 (O'Donovan et al., 2010; von Känel et al., 2010; Gill et al., 2009; Wessa and Rohleder, 2007; Lemieux et al., 2008; Wilson et al., 1999; Boscarino and Chang, 1999; Cohen and Schwartz, 1999; Affleck et al., 1997; von Känel et al., 2007; Muller and Schwarz, 2002; Sutherland et al., 2003; Gill et al., 2008). The excessive inflammation in anxious individuals might be a consequence of an insufficient regulation of the immune function. In PTSD, for example, the HPA axis is often dysregulated, resulting in altered cortisol levels and activity (De Kloet et al., 2006).

Glucocorticoid (GC) hormones, particularly cortisol, can suppress inflammatory cytokine production by downregulating the activity of the transcription factor nuclear factor kappa B (NFκB) (De Bosscher et al., 2000). NFκB is a potent activator of proinflammatory gene transcription, such as for IL-1β, TNF-α, IL-6, and IL-12 (Pahl, 1999). It is believed that, after long periods of exposure to stress-related hormones, glucocorticoid resistance arises, primarily through a reduction of both sensitivity and expression of GC receptors. This GC resistance compromises the physiological regulation of inflammatory responses by HPA axis, leading to a high basal immune activation state (De Kloet et al., 2007; Gotovac et al., 2003). Indeed, in our cohort, although we did not measure the endogenous cortisol release, we observed a clear GC resistance following T cell activation with PHA.

An exhaustion-induced damage of the intrinsic regulatory mechanisms of the immune system might also enhance the immune dysregulation in chronic stress. In this context, we demonstrated a lower IL-10 production following T cell activation in the anxious group. Multiple studies *in vivo* have shown that the release of IL-10 by Tr-1 cells constitutes an important mechanism of control of inflammatory responses (Belkaid, 2007; McGeachy et al., 2005). Although we did not

explore the contribution of $CD4^{+}CD25^{high}Foxp3^{+}$ Treg cells, study by Sommershof et al. (2009) demonstrated that PTSD patients have a reduced proportion of these regulatory T cells. IL-2 is essential not only for Treg cell homeostasis *in vivo* (Burchill et al., 2007; Davidson et al., 2007), but also for their efficient suppressor function (Horwitz et al., 2008). Thus, in our study, the IL-2 deficiency detected *in vitro* in anxious individuals can compromise the regulatory function of these Treg cells. Furthermore, it has been suggested that IL-6, IL-1β, and TNF-α produced by APC as a result of Toll-like receptor engagement, not only blocks $CD4^{+}CD25^{+}$ Treg cell-suppressive activity (Pasare and Medzhitov, 2003, Sutmuller et al., 2006), but may convert them into proinflammatory cells. Indeed, it was recently reported that Treg cells can be converted to IL-17-expressing cells in an appropriate proinflammatory cytokine environment (Yang et al., 2008; Osorio et al., 2008). Furthermore, the presence of these proinflammatory cytokines can, in turn, potentiate both anxiety, by acting on Central Nervous System (Dantzer, 2001; Goehler et al. 2007; Sternberg, 2006; Dantzer, 2001; Goehler et al., 2007; Bonaccorso et al., 2001), and cortisol resistance, by reducing both number and sensitivity of glucocorticoid receptors (Pace et al., 2007).

In summary, our results indicate that the profoundly altered composition of the peripheral T cell compartment might cause a state of compromised immune responsiveness, which may explain why anxious patients show an increased susceptibility to infections, and inflammatory and autoimmune diseases.

Conflict of interest

All authors declare that there are no conflicts of interest.

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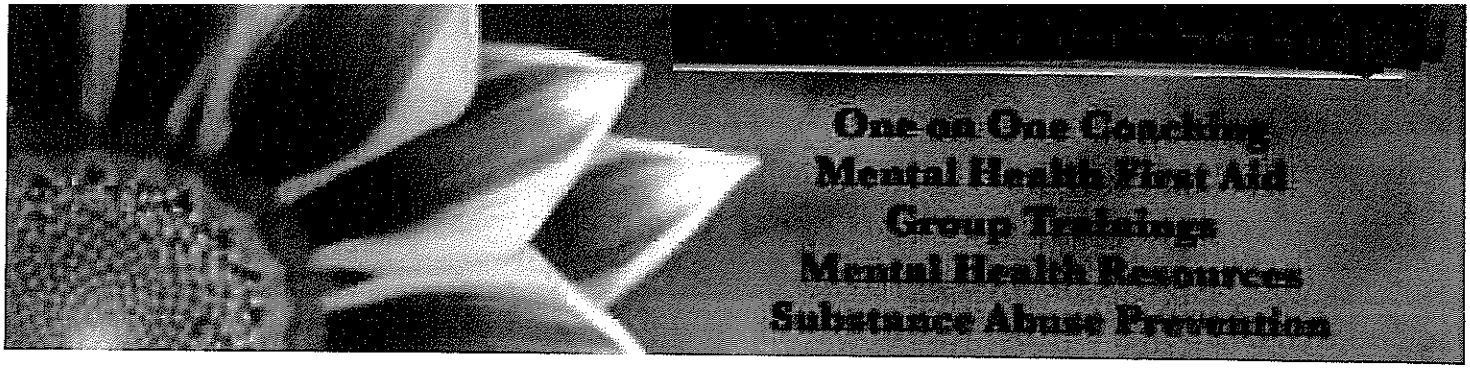

Aloha,

My name is [redacted] and I am a state Licensed CSAC as well as an MRAS and CPS, currently residing in the State of Hawaii.

I am sending this letter as a letter of support to include Generalized Anxiety Disorder to the list of medical conditions with which medical marijuana can be utilized for treatment.

In my over ten years of working in the field of Behavioral Health, I can personally attest to the fact that using marijuana in a medicinal fashion (tincture, oils, flower) is as effective, if not more so in the treatment of GAD in comparison with conventional medicines. Relief from the cortisol/stress induced hormones is more immediate and longer lasting with medical marijuana which in turn, results in an individual's ability to return to a state of calm and continue their daily activities.

Also, side effects which keep individuals from using psychotropic medications such as, sexual side effects and brain fog from SSRI's as well as exhaustion and dangerous withdrawal effects with Benzodiazepines, are not present with medical marijuana when used appropriately.

It is my professional opinion, based on over a decade worth of experience and research, that GAD should be included on the list of allowable illnesses with which medical marijuana can legally be used for treatment.

Please feel free to contact me for further information.

[redacted] CSAC, CPS, MRAS

Website - www[redacted].com, Email - [redacted] Phone - [redacted]