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Biomarkers and Applications

John AB et al. Biomark Applic 2017: 101.

Research Article

Use of MDDS core, a Biomarker Panel and Algorithm, for the Detection of Co Morbid Major Depressive Disorder in Patients with Chronic Intractable Pain

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Citation: John Ab, Linda Mt, Forest St (2017) Use Of MDDS core, A Biomarker Panel And Algorithm, For The Detection of Co Morbid Major Depressive Disorder In Patients With Chronic Intractable Pain. Biomark Applic 2017: 101.

Received Date: 2 January, 2017; **Accepted Date:** 13 February, 2017; **Published Date:** 20 February, 2017

Abstract

Purpose: The presence of co morbid MDD has a direct impact on chronic pain, being associated with increases in pain intensity, co morbidities and the frequency of suicide. A multi-Analyte blood test for MDD, which monitors the inflammatory, metabolic, neurotrophic and HPA axis pathways altered in MDDS, was applied to CIP patients to aid in determination of treatment for co morbid MDD as opposed to general mood change.

Methods: The study groups analyzed included: (a) 93 patients diagnosed with CIP from the overact Intractable Pain Clinic, and (b) 86 healthy non-CIP subjects. Each subject had a blood sample drawn for Quantization by immunoassay of 9 serum biomarkers (Alpha-1 Antitrypsin, Apo lipoprotein C3, Brain Derived Neurotrophic Factor, Cortisol, Epidermal Growth Factor, M eloperoxidase, Prolactin, Resist in, and soluble TNF Receptor II (sTNFR2). MDDS core™ was calculated using a proprietary algorithm and the probability of MDD scored on a scale of 1-9.

Results: Approximately half of the CIP patients (53.2%) had MDDS cores ≥ 5 (mean 8.39; median 9) indicative of a high probability for unipolar depression. The CIP patients with MDDS cores < 5 had a mean of 1.68 and a median score of 1.00. Similarly, the non-pain subjects had a mean MDDS core of 1.97 with a median score of 1.00. Hyper mapping indicated that in CIP populations, data for the high and low MDDS core populations indicate that the largest difference between the CIP populations and non-pain subjects resides in the inflammatory and the stress (HPA axis) pathways.

Conclusions: Our results indicate that MDDS core could segregate CIP patients into two major groups. This study also suggests that it is possible to identify CIP patients with an increased risk of co morbid MDD.

Keywords: Biomarker Panel ; Chronic Intractable Pain (CIP); Diagnostic Test; Major Depressive Disorder

Introduction

Depressed mood states are common within the physically ill population, with prevalence rates between 12 and 30% [1,2]. Specifically, the presence of co morbid major depressive disorder (MDD; unipolar depression) has a direct impact on chronic pain, associated with higher pain intensity and more co morbidity particularly in elderly patients [3,4]. Current or traditional diagnostic systems have failed to adequately assess true MDD in patients with chronic pain. In particular, it is difficult to apply assessment tools to segregate MDD from the demoralization or other changes in mood inherent in chronic pain states [5,6]. Depression scales and/or clinical evaluations of depression are not routinely used in chronic pain patient care. Faced with the serious problem of suicide, which is significantly higher in pain patients with co morbid chronic pain and MDD [7-10], a convenient and objective test for

depression diagnosis in chronic pain patients is needed.

Patients with Chronic Intractable Pain (CIP) all have incurable, extremely painful conditions. These patients may be non-functional, bedridden or housebound and often suffer from fatigue, insomnia, along with depression and anxiety. Objective assessment invariably reveals intermittently elevated blood pressure and pulse rate and abnormal concentrations of serum cortisol and other adrenal hormones, indicating the presence of a severe and extended stress state [11-13]. In clinical practice, it is useful to consider CIP a central pain syndrome when affected individuals have multifocal pain combined with other somatic symptoms indicated above. CIP, like other neuropathic pain states, may be directly caused by eliciting neuro inflammation which leads to damage or dysfunction of the central nervous system. To this point, animal studies demonstrated that activated microglia and astrocytes can produce cytokines such as TNF α and IL1β, which are thought to play an essential role in the pathogenesis of chronic pain [14,15]. Recently Loggia et al [16] using PET scan data has found direct evidence of neuroinflammation in key regions of the brains of patients with chronic pain. Similarly, there is evidence of the involvement of glia and microglial cell activation in the neuropathology of mood disorders [17-20].In addition, it has been suggested that the expression, secretion and/or actions of neurotrophin (NTs) at the synapse to modify synaptic transmission and connectivity [21-23].

The MDD score™ biomarker panel and algorithms were initially developed to aid in the diagnosis of Major Depressive Disorder (MDD). Target biomarkers were selected based on their role in biological systems with known involvement in the pathophysiology of depression including: neurotrophic, hypothalamic-pituitary-adrenal, metabolic and inflammatory pathways [24,25].Given the similarity in the mechanistic pathways involved in both chronic pain and major depressive disorder [26,27] we applied the MDDS core biomarker panel to a series of patients with CIP.

Methods

Patient Population

The study group consisted of 94 (94) patients from the Veract Intractable Pain Clinic diagnosed with Centralized Intractable Pain (CIP), with a mean age of 46.6±1.2 (range from 24-72 years). Fifty-nine (~63%) were female and 35 (~37%) were male. The average BMI for females was 26.5±7.8 (range from 13.3-48.7).The average BMI for males was 27.9±4.2 (range from 19.6-40.9).

There were no specific exclusion criteria for pain patients based upon gender or other co morbidities; patients 18 years and younger were excluded. All medications were allowed, while they represent potential confounders, they were substantially diverse and often used in combination and essential for the patient's well-being.

Healthy Subjects without Chronic Pain

Samples from healthy normal subjects (non-pain) were obtained from the Brain Institute of the University of Utah (n=9) Caritas St. Elisabeth Medical Center (Boston MA) (n=3). These 12 patients were selected based upon clinical interviews at both academic sites. A series of serum samples from normal subjects (n=31) with no history of neurologic disease, mood disorders or chronic pain were obtained from a commercial source (Precision Med, San Diego CA). In addition, we procured serum samples prospectively from healthy volunteers in both San Diego, CA (n=9) and Durham, NC (n=35). Non-pain subjects from local sources were excluded if they were taking drugs for chronic pain (e.g. opioids, NSAIDS, antidepressants). The quality of life in the normal volunteer population was assessed using the PHQ-9 [28] patients with PHQ-9 scores >5 were excluded. Female subjects without chronic pain (n=40) had an average BMI of 25.0±6.4 as compared to males (n=46) who had an average BMI of 27.7±4.8.

Sample Collection and Handling

Each study subject provided a blood sample, which was processed to collect serum. The sites prepared serum under standardized conditions. Briefly, blood was allowed to clot for 30 minutes, centrifuged 10 minutes at 1300 x g (RCF: Relative Centrifugal Force) to collect serum that was a liquated within 30 minutes of centrifugation. Serum samples were promptly shipped at 4 °C (or frozen at ─80 °C until ready for shipment on dry ice) to the Ridge Diagnostics CLIA Laboratory (Research Triangle Park, NC). The date and time of the blood draw was recorded by the study site for each sample, along with subject gender, height, and weight. All samples were identified by a sequentially applied accession number which blinded the technician doing testing to the source of the sample and patient identifiers.

Serum Biomarker Assays

Serum levels of the 9 biomarkers in the MDDS core panel, (Alpha1 Antitrypsin [A1AT], A polipoprotein C3[ApoC3], Brain Derived Neurotrophic factor [BDNF], Cortisol, Epidermal Growth Factor [EGF], My eloperoxidase [MPO], Prolactin [PRL], Resist in [RETN], and soluble Tumor Necrosis Factor alpha receptor type II [s TNFRII]), were measured by individual quantitative immunoassays. Standard curves for calibrating the quantity of each biomarker were generated by dilutions of each purified protein. The range of the standards for each assay was based upon the Ridge

CLIA regulated laboratory's analysis of multiple samples to establish the standard distribution surrounding the concentrations found in the serum of male and female subjects. Both A1AT and ApoC3 concentration was measured by an analytically validated immune turbid metric assay developed at the Ridge Laboratory. BDNF and s TNFRII concentrations were determined using Quantizing human ELISA kits from R&D Systems (Minneapolis, MN), EGF and RETN concentrations were determined using reagents from R&D Systems in a validated immunoassay developed at the Ridge Laboratory, MPO was measured by a human serum ELISA kit obtained from ALPCO Immunoassays (Salem, NH), prolactin in serum was measured using a human serum ELISA obtained from Mono bind (Lake Forest, CA), and cortisol in serum was quantified using a competition ELISA (Mono bind)).

As previously reported a proprietary algorithm was used to generate an MDDS core (range 1-9) using serum biomarker concentration.

Biomarker Hyper mapping

This approach involves the construction of a multi analyze hyper map versus analyzing single markers either alone or in groups. Using clusters of biomarkers reflective of different physiologic parameters (e.g. HPA axis vs. Metabolic vs. Inflammatory biomarkers), the patient's biomarker responses are mapped onto a multi-dimensional hyperspace [29]. Distinct coefficients are used to create the hyperspace vectors for subsets of patients and age matched normal subjects. The inflammation pathway vector is a compilation of the weighted transformed values of three biomarkers: A1AT, MPO and s TNFRII. Our calculated HPA axis (our stress response system) vector is composed of the biomarkers: cortisol and EGF. Metabolic pathway vectors are the weighted transformed values of three biomarkers: PRL, RETN and ApoC3. The neurotrophic pathway is represented by the weighted transformed value for BDNF. In the example shown in figure 1below, in this example the x, y, and z axes had the values 2, 3, 5. Thus this patient's location in 3D hyperspace (P) would be described by the hyperspace vector values 2, 3, 5. As an alternative to the hyperspace map, a comparison between pathways can be performed by calculating the pathway value for each patient within a test group and then use a box-whisker plot to compare groups. A histogram can also be developed, after determining the mean of each test group.

Results

MDDS cores for CIP Patients

Based upon previous data [24,25], a score of within the range of 5 - 9 indicates that the patient has an approximately 90% likelihood of having MDD. Forty-eight percent of the patients had an MDDS core below 5 and based upon earlier findings has approximately 95% likelihood of not having MDD. Figure 1: below shows a histogram of the MDDS cores obtained for the 94 CIP patients. Fifty-two percent of the patients had MDDS cores ≥ 5 (red bars).

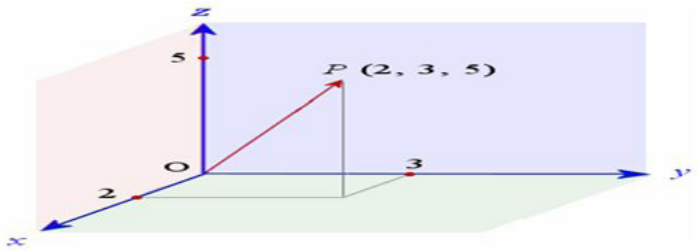

Figure 1: Example of a 3-dimentional hyperspace map a hyper map of patient P is shown with the coordinates 2, 3, 5 for the x, y and z axes respectively.

The histogram above indicates a bimodal distribution with approximately half of the CIP patients having MDDS cores >5. Sensitivity and Specificity of the test from controlled studies of healthy subjects and MDD patients were 94% and 92% respectively [24,25]. The sensitivity and specificity of MDDS core for the CIP population is presently unknown. Figure 2: Histogram of the MDDS cores of a series of 94 CIP patients. A group of 9 biomarkers was used in a combination algorithm to calculate the MDDS core for each patient

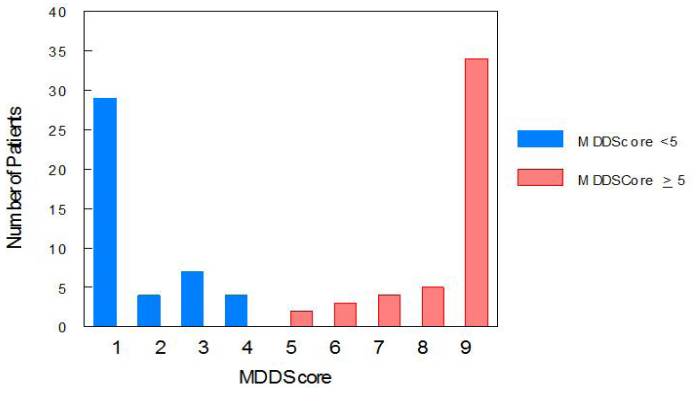

Figure 2: Histogram of the MDDS cores of a series of 94 CIP patients.

The CIP patients with an MDDS core ≥ 5 were comprised of 34 females and 16 males, while the patients with the MDDS cores of less than 5 had 25 females and 19 males. While the males were nearly identical with regards to BMI (average BMI 27.5); the females with the MDDS core ≥ 5 had a mean of 28.0 ± 8.4 while their <5 comparators had an average of 24.4 ± 8.4 (p=0.07).

Hyperspace Mapping of CIP Patients:

Figure 3 shows 3-dimensional hyperspace mapping (hyper maps) of the CIP patients segregated by whether they had a low or high probability of major depression using the MDDS core test. As would be expected we observed a wide distribution of patients within each subset based upon the diversity of individual patients and biomarker expression. Using the hyper

Mapping technique we were also able to characterize a vector for each biological pathway wherein its magnitude reflects the comparative activation.

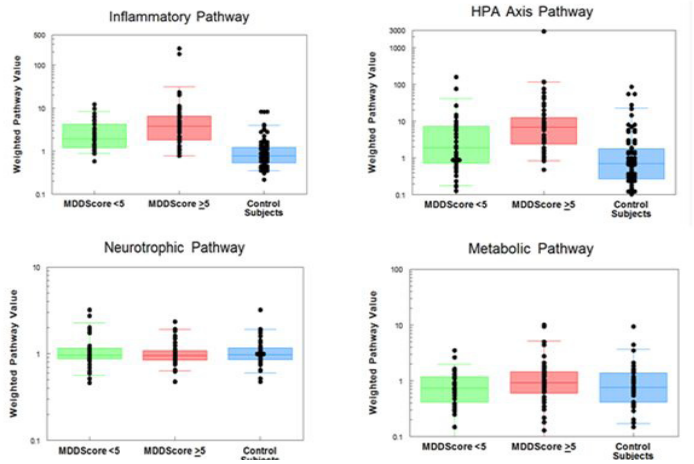

Figure 3: Hyper maps of CIP patient and normal populations Using clusters of biomarkers reflective of different physiologic parameters (e.g. HPA axis vs. neurotrophin vs. inflammatory markers), the patient's biomarker responses are mapped onto a three-dimensional hyperspace. Visual inspection of the hyper map display can reveal distinctions between individual patients. 3 A is a hyper map of CIP patients and normal subjects using the neurotrophin vector as the y-axis. 3 B is the same patients sorted by MDDS core with the neurotrophin pathway vector as the y-axis. Panel 3C shows the same CIP patients using the metabolic pathway vector as the y-axis.

The comparison between the CIP patients with high and low MDDS core patients is shown in comparison to a series of healthy subjects without chronic pain. Figure 4

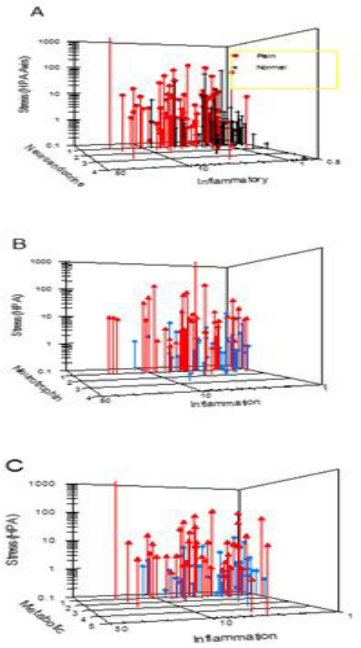

Figure 4: Box Whisker plots of weighted pathway values for individual CIP patients with MDDS cores <5 or ≥5 and control subjects. Mean values for the Inflammatory pathway was 8.4 ± 30.7 vs 13.2 ± 41.6 for <5 and ≥5 respectively Similarly the HPA axis vectors were 1.7 ± 2.4 and 72.2 ± 397.8.* The neurotrophin pathway vectors were 1.1 ± 0.5 and 1.0 ± 0.4 while the metabolic pathway vectors were 1.2 ± 1.5 and 1.4 ± 2 for CIP patients with MDDS cores of <5 and ≥5 respectively. * The high standard deviation is due to one patient with a high HPA value (2780).

Pathway Vector Histogram of CIP Patients and Non-Pain Subjects

Figure 5 is a histogram which shows the individual pathway vector values calculated based upon the mean values determined for each test group as shown in figure 4. CIP patients with MDDS cores greater or equal to 5 had increased in the pathway vector values for both inflammation and the HPA axis our central stress response system.

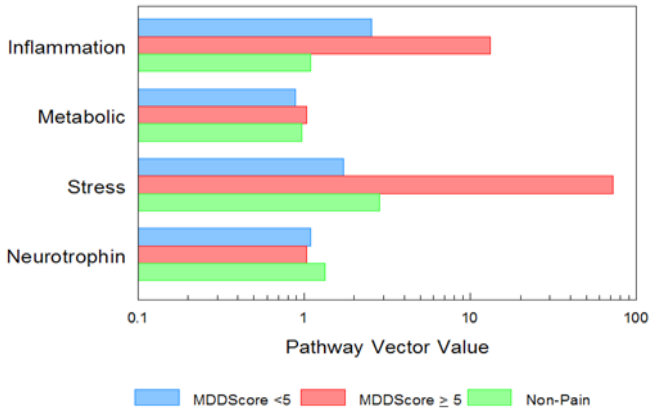

Figure 5: Pathway Vector Histogram Biomarker data was obtained for each patient and the vectors are the sums of the transformed biomarker data for non-pain subjects and the MDDS core <5 and ≥ 5 populations of CIP patients. Defined and validated coefficients were used to create the hyperspace vector values for each of the four pathways. The CIP patient's pathway vectors were compared to a series of healthy subjects (n=86) without chronic pain.

Discussion

MDDS core is a blood test which provides a score based upon the serum levels of biomarkers within the inflammatory, HPA axis, metabolic and neurotrophin pathways [24,25]. Our results indicate that the MDDS core test could segregate seemingly CIP patients into two groups. Approximately half of the seriously ill chronic pain patients in our study had MDDS cores ≥ 5 consistent with unipolar depression. This distribution is similar to controlled studies in the prevalence of depression in the general and primary care populations [30-32]. In particular, an estimated 30-54% of clinic-based chronic pain patients suffer from clinically established MDD at any given point [33] which is remarkable when these rates are compared with the prevalence of MDD in the general population. Kessler and colleagues [34] reported that the lifetime prevalence was 16.2% and 6.6% for 12-month prevalence in a large US population study.

We used hyper mapping to identify biological pathways that discriminated the two populations identified by MDDS core. While the technology uses the quantitation of each biomarker within the four pathways (Inflammatory, HPA, metabolic and neurotrophin), data collected on hundreds of samples resulted in pathway-specific weighted coefficients. These coefficients reflect the relative contribution of each of the markers to the magnitude of the vector in one direction. It is the plot of three pathway vectors that determines the position of an individual patient in space which can be useful in determining the course of treatment. By way of example, a high inflammatory vector may indicate the need to treat for residual inflammation. As we continue to collect treatment outcome data on individual patients, we will eventually be able to use biomarker technology to better classify patients and select the appropriate treatment regimen.

Figure 5 summarized the pathway vector data for the high and low MDDS core populations and normal subjects. It is clear from the figure that the largest difference between the two populations resides in the inflammatory and the stress pathways. Normal subjects clearly had lower levels of inflammation than either of the CIP populations. The inflammatory pathway includes A1AT, MPO, and sTNFR2 biomarkers, as well as a gender contribution, of chronic as opposed to acute inflammation. The HPA axis biomarkers include the well-known "stress hormone" cortisol and the growth factor EGF, which is an activator of the HPA axis. One interesting observation was that the CIP population with an MDDS core less than 5 had a lower level of HPA activation than normal subjects. However, this patient population is treated with multiple therapeutics including chronic opioids which can influence the levels of hormones such as cortisol [11,35-37] and those within the metabolic group (e.g. prolactin and resist in [38,39].Regarding the neuro trophic pathway there was no significant difference in BDNF levels in the MDDS core group with <5 (26,502 ± 7118 pg/m L) or those CIP patients with a score of ≥ 5 (27584 ± 7144 pg/m L; p value = 0.44).

One major caveat is that this CIP patient population is composed of heavily-treated patients, whose regimens included commonly used analgesics, antidepressants and hormones. It is also true that the methodology used in this study measured levels at a single time point during treatment. Other studies are in progress to chart changes over time in patients treated with opioids and other drugs used in the treatment of chronic pain. Nonetheless these data suggest that MDDS core may be able to identify patients with a higher probability of co morbid MDD and the additional suffering that ensues. MDDS core and hyper mapping technology may also provide insight into the sub-classification of pain patients particularly those patients with residual inflammation and hormone abnormalities. In addition, we know that antidepressants are not mood elevators and can have significant side effects; one question we want to answer is whether MDDS core may be used to determine whether an individual patient may profit by inclusion of an antidepressant in their treatment regimen.

Lastly, in a large and diverse population of older adults with arthritis (mostly osteoarthritis) and co morbid depression, benefits of improved depression care extended beyond reduced depressive symptoms and included decreased pain as well as improved func-

tional status and quality of life [39]. In treating chronic pain the primary goals are to reduce or relieve pain and improve the patient's ability to return to normal daily function. Clearly the identification of co morbid pain and depression can provide a more direct path to the appropriate treatment.

Acknowledgement

We would like to acknowledge the support of Ridge Diagnostics who provided partial funding for this study. In addition, former colleagues at Ridge Diagnostics, who provided valuable discussions and critique. We acknowledge the excellent laboratory assistance of Ms. Laurie Rotchford MLS who performed most of the assays reported in this study.

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