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Depression and Tumor Burden in Pancreatic Cancer

Scientific Abstract
Pancreatic cancer patients frequently present with a history of depression; often new-onset depressive symptoms precede the diagnosis of cancer. These patterns of psychiatric symptoms, arising prior to cancer diagnosis, suggest that the pathophysiology of depression in pancreatic cancer may result from biological changes, such as alterations in cytokine levels, that are induced by the presence of the tumor itself. One proposed mechanism for cytokine-induced depression involves alterations in tryptophan catabolism as mediated by the enzyme IDO. Tryptophan is required for the production of the neurotransmitter serotonin; decreased serotonin levels are thought to contribute to depressive symptoms. Tryptophan is also the substrate for neurotoxic metabolites such as quinolinic acid, produced via the kynurenine pathway. Current pancreatic cancer research suggests that tumor-expressed IDO facilitates the creation of an immunotolerant environment, permissive to tumor growth and metastasis. Another effect of increased IDO expression is decreased production of serotonin and increased production of kynurenines, including quinolinic acid; both decreases in serotonin production and increased production of neurotoxic metabolites could cause depressive symptoms. Our study will explore the correlation between patient's mood symptoms, as assessed by questionnaire, and levels of serum IDO activity, before and after surgery, ie. where the patient's tumor burden is reduced through resection. We will also study the correlation between tumor burden (tumor stage and percent positive lymph nodes) and IDO activity level.

Lay Abstract
Pancreatic cancer patients often have symptoms of depression before they are actually diagnosed with cancer. Alterations in levels of certain chemicals in the blood, such as the enzyme IDO, can cause symptoms of depression. IDO from a tumor can also hamper the body's natural defenses against cancer, allowing the tumor to grow and multiply faster. Our study will explore the correlation between patient's mood symptoms and IDO activity. Mood symptoms will be assessed by questionnaires before and after surgery. IDO activity will be calculated from samples of peripheral venous blood, obtained before and after surgery. We will also study the correlation between tumor size and IDO activity.

Study Purpose and Rationale
The literature frequently describes patients with pancreatic adenocarcinoma who present with a history of depression; often new-onset depressive symptoms precede the diagnosis of cancer [1]. A number of case reports from the 1920s to the present have noted this association; several larger studies have also explored the topic more rigorously. A 1967 series of 46 patients with pancreatic cancer found that 76% had depressive symptoms - approximately half of the depressed patients experienced the onset of depression prior to the diagnosis of cancer [2]. Later studies have confirmed this pattern of psychiatric symptoms, even when comparing pancreatic cancer with other abdominal neoplasms. A 1981 series of 107 patients with pancreatic cancer and 111 patients with gastric cancer found that pancreatic cancer patients had significantly higher levels of depression than gastric cancer patients [3]. These patterns of psychiatric symptoms arise prior to cancer diagnosis, and are presumably not an intellectual response to a disease with a poor prognosis. Such findings suggest that the pathophysiology of depression in pancreatic cancer may result from biological changes,

such as alterations in cytokine levels, that are induced by the presence of the tumor itself [1].

Cytokine -induced depression has been reported in patients treated with interferon for hepatitis C and cancer [4]. Depression has also been associated with elevated levels of pro-inflammatory cytokines in patients with chronic medical conditions such as rheumatoid arthritis, cancer and cardiovascular disease [5]. However, the exact mechanism of cytokine effect on the central nervous system has not been fully elucidated. One would assume that any proposed mechanism for depression would involve the central nervous system. However, cytokines are relatively large molecules that cannot cross the blood-brain barrier. Several theories have been proposed; some propose that cytokines may reach the brain via active transport, or by diffusing through sites where the blood-brain barrier is “leaky” or deficient. It has also been suggested that cytokines may act on vagal afferents [4]. One of the more promising theories explores the impact of cytokines on the regulation of serotonin production.

Serotonergic and noradrenergic neurotransmission has been the target of antidepressant drug development for many years. The majority of first-line antidepressant drugs used today are selective serotonin repute inhibitors (SSRIs), which inhibit the reuptake of serotonin in the CNS and thereby increase the stimulation of serotonin receptors. Serotonin is synthesized from the essential amino acid tryptophan. However, tryptophan is also the substrate in the kynurenine pathway which eventually produces NAD+ as well as quinolinic acid products which are potentially neurotoxic. Interferon gamma (IFN-gamma), a proinflammatory cytokine, upregulates the expression of the enzyme IDO, which catalyzes the conversion of tryptophan to kynurenine. Thus, in the presence of IFN-gamma, and subsequent overexpression of IDO, tryptophan is shunted away from serotonin production toward the kynurenine pathway, leading to both lower serotonin production, and increased levels of neurotoxic products of kynurenine metabolism, both of which could contribute to symptoms of depression [6, 7]. Of interest, serotonin is a precursor to melatonin, a hormone which may reduce cancer-related cachexia[8]. Meanwhile, IDO expression and the kynurenine shunt have also been implicated in tumor-induced immunomodulation.

IDO is expressed in a number of tumors, including pancreatic adenocarcinoma [9,10]. The exact mechanism of IDO -induced immunosuppression remains controversial; some propose that enhanced IDO expression results in immune tolerance to tumor antigens through modifications of T cell tryptophan catabolism [9,11,12]. Experimentally, IDO inhibition with the false metabolite 1-methyl-d-tryptophan (D-1MT) has been shown to limit tumor growth; use of D-1MT is currently being evaluated in two NCI-sponsored phase I trials [12]. In an immunocompetent state, the body mounts an inflammatory T cell response against tumor antigens. However, this inflammatory response exerts a selection pressure in favor of tumor cells which express IDO and are therefore capable of inducing T cell toleragenicity in the local microenvironment, or in tumor- draining lymph nodes [11]. We hypothesize that, in pancreatic cancer, a side effect of enhanced IDO expression is reduced production of serotonin and increased production of neurotoxic metabolites, both of which could result in symptoms of depression.

To date, we know of no published data exploring the impact of IDO and tryptophan metabolism on psychological morbidity in pancreatic cancer patients. However, a 2002 study of quality of life in patients with metastatic colorectal cancer found a significant correlation between serum tryptophan level and quality of life; this correlation was not

observed in healthy controls, or in colorectal cancer patients without metastatic disease. Serum tryptophan in cancer patients was significantly reduced and the serum kynurenine/tryptophan ratio was significantly increased in cancer patients relative to controls. Both of these biochemical findings could be explained by enhanced IDO activity, which would be expected in a tumor that had already gained the capacity to avoid local immune surveillance and metastasize [13].

The information provided by this study could help further clarify the mechanism of depression in pancreatic cancer patients and help to guide treatment; likewise, examining the use of IDO as a potential biomarker of tumor burden could provide valuable information for preoperative staging and for measuring the success of therapeutic interventions.

2. Study Design and Statistical Procedures
Statistics:
This is a pilot study and only very limited historical data regarding differences between groups in this population is available. Prior studies utilizing the Beck Depression Inventory (BDI) amoung cancer patients have found standard deviations ranging from approximately SD=5 – 10 [14-15].
The power calculations in this section demonstrate the effect (change in depression score= delta BDI) required to achieve power=0.80 at alpha=0.05 for our patient sample of n=40 for a range of possible SD values of BDI values . Paired t test is used, with the group 1 representing patients prior to surgery and group 2 representing the same group after surgery.

	SD=5	SD=10	SD=15	SD=20
required effect (delta BDI) to achieve power=0.80	2.3	4.4	6.8	9.1

Data will be collected into an Excel spreadsheet. To protect patient confidentiality, the data will be deidentified after the collection process, prior to analysis. Descriptive statistics (frequencies, percentages for categorical data, medians, and interquartile ranges) for all psychological, demographic, quality of life, histopathological and biochemical will be determined. Independent t-test or Mann-Whitney testing will be used for comparisons involving continuous data. Univariate and multivariate analysis will be used to determine if IDO activity level correlates with psychological morbidity quality of life and tumor burden, using parametric methods if the data conform to normality assumptions, or non-parametric ones if they do not.

3. Study Procedures_
We plan to enroll 40 patients with pancreatic adenocarcinoma over a 12 month period. We will recruit patients with resectable disease at Pancreas Center visits prior to surgery. We plan to complete recruitment within 12 months.

Access to the patient population will be achieved through the physicians at the Pancreas Center at Columbia University Medical Center. Drs. Chabot, Allendorf, Schrope and Stevens, working with Nicole Goetz, Maureen Morrison and Ashley Ray who are all members of the Center, will provide preliminary contact. The physicians will contact eligible patients either in person or by telephone and inform them about the study. If they are willing, the patients will be contacted by recruitment staff and will be offered to participate.

Three questionnaires and a demographic data sheet, which have been described below, will be provided at enrollment and at a regular medical follow-up visit 6 weeks after surgery.

Upon enrollment and on 6 week post-operative visit, 25ml of blood will be collected from each patient by venipuncture, for a total of no more than 50ml of blood in an 8 week period.

Tumor burden will be determined from surgical pathology reports; specifically, tumor burden will be assessed by tumor stage (T stage) and percent of tumor-positive lymph nodes in the surgical resection specimen. Aside from the patients' regular medical care, no additional procedures will be performed.

Blood samples will be processed and frozen at -70° until recruitment is completed. High performance liquid chromatography, using previously described protocols will be utilized to determine levels of products of tryptophan catabolism, including tryptophan, 5-HTP, 5-HT, kynurenine and quinolinic acid [14]. IDO enzyme activity will be determined by the Michaelis-Menten equation as applied to substrate tryptophan and products 5-HTP and kynurenine. Enzyme-linked immunosorbent assay (ELISA) will be carried out on all samples to assess levels of neopterin, a marker of cellular immune system activation.

Clinical information, including operative procedure performed and standard pre-and post-operative laboratory data will be obtained from the patient's medical record.

4. Study Drugs or Devices_Not applicable.

5. Study Instruments
1) Demographics and general health questionnaire
2) FACT-PA
3) Beck Depression Inventory
4) Beck Anxiety Inventory

The listed psychiatric instruments have all been validated and have been used among cancer patients [16-18].

Demographic and general health questionnaire:
A general epidemiological and general health questionnaire, including age, race/ethnicity, socioeconomic status and medical history.

FACT-PA
The FACT-PA is a self-administered generic and pancreatic disease-specific health status survey in which participants are scored against age- and gender-matched controls. The scaled measures are transformed to a 0-100 scale with zero representing the worst quality of life and 100 representing the best.

Beck Depression Inventory
The Beck Depression Inventory is a self-administered measure of depressive symptoms that has been previously used in a cancer populations. The instrument is scored on a 0-63 scale with higher scores indicating more severe depression.

Beck Anxiety Inventory

The Beck Anxiety Inventory is a self-administered measure of anxiety symptoms that has been previously used in a cancer populations. The instrument is scored on a 0-63 scale with higher scores indicating more severe anxiety.

6. Study Subjects_
Inclusion criteria:
1. 18 years of age and older.
2. Surgery planned with preoperative diagnosis of pancreatic adenocarcinoma.

Exclusion criteria:
1. Patient reports treatment within the past month with antidepressant medication, including SSRI, SNRI, TCAs
2. History of auto-immune diseases such as SLE, multiple sclerosis, rheumatoid arthritis, polymyalgia rheumatica, temporal arteritis
3. History of congestive heart failure
4. History of recent stroke
5. History of HIV/AIDS
6. Patient has undergone other major surgery in the past month.

This study is not an evaluation of therapy. Total questionnaire time is approximately 1 hour.

7. Recruitment
Patients will be enrolled by their physicians at the Pancreas center during their Center visits or by telephone. Respondents will be asked to participate using standard informed consent processes. It will be emphasized that the decision to participate in the study will in no way affect medical care or other services and participation is completely voluntary.

8. Informed Consent Process_
Subjects will be recruited through Columbia-Presbyterian Medical center faculty practices. A study investigator will obtain informed consent prior to enrollment, using Columbia University Medical Center IRB approval consent forms, which will be signed to indicate the participant's consent.

9. Confidentiality of Study Data_
Study participants will be assigned a unique study number. Once the data has been collected, it will be entered into an electronic database on a secure HIPPA compliant server. Once completed, the dataset will be stripped of all identifiying information including patient name, date of birth and medical record number. The deidentified dataset will be used for the investigation.

10. Privacy Protections_
All study data will be de-identified and coded to maintain confidentiality of subjects. Data will be stored in a password-protected spreadsheet or in a locked file cabinet in The Pancreas Center, available only to investigators. We will collect the minimum amount of information needed for the purposes of this study. Only the investigators in this study will have access to this data, and all of the information gathered will be used for this study only. The information will not be given to other investigators, institutions or agencies.

11. Potential Risks_
The only risks associated with this study are the potential for distress that may arise

when addressing questions of depression and anxiety, as well as the slight potential risks associated with obtaining blood by venipuncture. Another small potential risk is the release of the patient's private health information. Efforts to prevent such an occurrence are outlined above.

There will be no alteration of patient treatment. Specifically, patients who are found to be depressed by their physician will be advised to seek psychiatric care and medication if necessary, regardless of their study participation. Patients who choose to begin treatment with antidepressants after enrollment will not be dropped from the study. Efforts will be made to obtain a second set of questionnaires and bloods prior to the intiation of antidepressant therapy. The second set of questionnaires and the next set of bloods will not be obtained if the patient has already been taking antidepressant drugs. However, the pre-operative, pre-antidepressant data obtained upon enrollment from such patients will be included in the final analysis.

13. Potential Benefits_
Participants will not receive direct benefit from taking part in this research study. However, the information collected from this research may help others in the future.

14. Alternatives_
The alternative is not to participate in this study.

References
1. AI, Austin CP. Psychopathology of pancreatic cancer. A psychobiologic probe. Psychosomatics. 1993 May-Jun;34(3):208-21.
2. Fras I, Litin EM, Pearson JS. Comparison of psychiatric symptoms in carcinoma of the pancreas with those in some other intra-abdominal neoplasms. Am J Psychiatry. 1967 Jun;123(12):1553-62.
3. Holland JC, Korzun AH, Tross S, Silberfarb P, Perry M, Comis R, Oster M.Comparative psychological disturbance in patients with pancreatic and gastric cancer. Am J Psychiatry. 1986 Aug;143(8):982-6.
4. Wichers M, Maes M. The psychoneuroimmuno-pathophysiology of cytokine-induced depression in humans. Int J Neuropsychopharmacol. 2002 Dec;5(4):375-88.
5. Irwin MR, Miller AH. Depressive disorders and immunity: 20 years of progress and discovery. Brain Behav Immun. 2007 May;21(4):374-83.
6. Russo S, Kema IP, Fokkema MR, Boon JC, Willemse PH, de Vries EG, den Boer JA, Korf J. Tryptophan as a link between psychopathology and somatic states.Psychosom Med. 2003 Jul-Aug;65(4):665-71.
7. Müller N, Schwarz MJ. The immune-mediated alteration of serotonin and glutamate: towards an integrated view of depression. Mol Psychiatry. 2007 Nov;12(11):988-1000
8.Lissoni P. Is there a role for melatonin in supportive care? Support Care Cancer. 2002 Mar;10(2):110-6. Epub 2001 Nov 13.
9. Witkiewicz A, Williams TK, Cozzitorto J, Durkan B, Showalter SL, Yeo CJ, Brody JR. Expression of indoleamine 2,3-dioxygenase in metastatic pancreatic ductal adenocarcinoma recruits regulatory T cells to avoid immune detection. J Am CollSurg. 2008 May;206(5):849-54; discussion 854-6. Epub 2008 Mar 4.
10. Witkiewicz AK, Costantino CL, Metz R, Muller AJ, Prendergast GC, Yeo CJ, Brody JR. Genotyping and expression analysis of IDO2 in human pancreatic cancer: a novel, active target. J Am Coll Surg. 2009 May;208(5):781-7; discussion 787-9.
11. Katz JB, Muller AJ, Prendergast GC. Indoleamine 2,3-dioxygenase in T-cell tolerance and tumoral immune escape. Immunol Rev. 2008 Apr;222:206-21.

12. Löb S, Königsrainer A, Rammensee HG, Opelz G, Terness P. Inhibitors of indoleamine-2,3-dioxygenase for cancer therapy: can we see the wood for the trees? Nat Rev Cancer. 2009 Jun;9(6):445-52.
13. Huang A, Fuchs D, Widner B, Glover C, Henderson DC, Allen-Mersh TG. Serum tryptophan decrease correlates with immune activation and impaired quality of life in colorectal cancer. Br J Cancer. 2002 Jun 5;86(11):1691-6.
14 Laich A, Neurauter G, Widner B, Fuchs D. More rapid method for simultaneous measurement of tryptophan and kynurenine by HPLC. Clin Chem. 2002 Mar;48(3):579-81.
14. Wedding U, Koch A, Röhrig B, Pientka L, Sauer H, Höffken K, Maurer I. Depression and functional impairment independently contribute to decreased quality of life in cancer patients prior to chemotherapy.Acta Oncol. 2008;47(1):56-62.
15. Ellis J, Lin J, Walsh A, Lo C, Shepherd FA, Moore M, Li M, Gagliese L, Zimmermann C, Rodin G. Predictors of referral for specialized psychosocial oncology care in patients with metastatic cancer: the contributions of age, distress, and marital status. J Clin Oncol. 2009 Feb 10;27(5):699-705.
16. Kelsen DP, Portenoy RK, Thaler HT, Niedzwiecki D, Passik SD, Tao Y, Banks W, Brennan MF, Foley KM. Pain and depression in patients with newly diagnosed pancreas cancer. J Clin Oncol. 1995 Mar;13(3):748-55
17. Hopko DR, Bell JL, Armento ME, Robertson SM, Hunt MK, Wolf NJ, Mullane C. The phenomenology and screening of clinical depression in cancer patients. J Psychosoc Oncol. 2008;26(1):31-51.
18. Cella DF, Tulsky DS, Gray G, Sarafian B, Linn E, Bonomi A, Silberman M, Yellen SB, Winicour P, Brannon J, et al. The Functional Assessment of Cancer Therapy scale: development and validation of the general measure. J Clin Oncol. 1993 Mar;11(3):570-9.