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Study of the differential effects of minimally invasive and open colorectal resection on post-operative immune function by evaluating the circulating levels of plasma proteins and cytokines.

Purpose and Rationale of study:

Previous investigators have been able to clearly describe how surgical trauma is associated with substantial alterations in the immune system (specifically towards suppression) and concomitant immune response. Such studies of immune function in this setting have outlined the type, number and operative status of the various immune cell subsets. In cancer patients, it would not be unrealistic to envisage how this state of relative immuno-suppression could create a tumor-permissive environment. One that could accelerate the growth of residual primary cancer cells and facilitate metastasis.

Studies in humans have shown a laparotomy related decrease in the plasma levels of Insulin like Growth Factor binding protein 3 (IGFBP-3), a tumor inhibitor protein. This is believed to account in some part for the observed increased mitogenicity of post-laparotomy plasma when it was added to in-vitro cultures of the human colon cancer cell line HT-29 (1). Post-operative day 1 plasma was found to stimulate in vitro tumor growth to a significantly greater extent than preoperative plasma from the same patients. The duration and magnitude of this effect correlated directly with incision length ($r = 0.58$ and $P < 0.01$). The addition of exogenous IGFBP-3 to the POD1 plasma lowered the tumor cell proliferation rates to levels observed in the pre-operative plasma samples. Subsequently, the addition of IGFBP-3 antibody to preoperative plasma result in an increase in its tumor stimulatory effect parallel to that evident with post-operative day 1 plasma.

Laparoscopic-assisted procedures were by and large associated with smaller decreases in plasma IGFBP-3 levels and more importantly no significant change in the in-vitro cancer stimulating capacity of post-operative plasma. It is believed that IGFBP-3 acts to contain tumor growth by directly inducing apoptosis and also by binding to and thus limiting the circulating levels of Insulin-like Growth factor 1 (IGF-1), a protein known to promote tumor growth.

Other studies have corroborated the above observation, one such study in mice showed that full sham laparotomy when compared to carbon dioxide (CO_2) insufflation, was associated with a higher rate of *in-vivo* tumor cell (C-26 colon adenocarcinoma) proliferation and a lower apoptotic rate on post-operative day 14 (2). Similar results were appreciated in other studies that compared open vs. closed bowel resection (3-5).

The primary purpose of this study is to gather peri-operative plasma from patients undergoing major open and minimally invasive procedures with the aim of investigating and better understanding the effects of both surgical intervention types on post-operative immune function. Micro array analyses on circulating lymphocytes will be

performed as well as a validation ELISA assay on expression levels of 5 corresponding plasma proteins. Thus far, micro array techniques have not been utilized to study the host immune response to surgical insult. Ultimately, we hope to be able to correlate the presence and extent of these peri-operative alterations in plasma markers with immediate and long-term outcome in the respective patients. Our assertion is that the bulk of the immunologic alterations that occur after surgery are not diagnosis related; as a result this study will strive to include patients with both benign and malignant indications for surgery.

Study Hypothesis:

Open and laparoscopic surgical methods are associated with significant alterations in circulating levels of plasma immuno-proteins, with the alteration seen in laparoscopic-assisted procedures occurring to a lesser extent.

Study Design:

This will be a prospective, non-randomized multi-center trial. Adult patients (18years of age or older) with benign conditions (polyp, diverticulitis, rectal prolapse, e.t.c.) or colorectal cancer who are about to undergo elective open (via traditional large incision methods) or minimally invasive (laparoscopic-assisted) colorectal resection at a participating institution will be eligible for inclusion into this study. It is anticipated that patient enrollment will take between 18 and 24 months to complete; hopefully with comparable overall numbers for open and closed surgical patients upon ending. In an effort to obtain comparable patient numbers, the Columbia group has established collaboration with the Cleveland Clinic Foundation in Cleveland, Ohio. We are in the process of setting up similar collaborative relationships with the Ferguson Clinic in Vermont and Fletcher Allen Center in Michigan.

Patients who undergo minimally invasive resection that are converted to open procedures will, for statistical purposes, be kept in the minimally invasive group because of the "intention to treat" principle. Accompanying post-operative blood samples will still be drawn at the appropriate time-points regardless of whether conversion was necessary.

Statistical analysis will be performed by comparing pre- and post-operative results from each patient in the open and minimally invasive study arms, using a one sample t-test. Since we expect to investigate the plasma levels of 5 novel parameters (of which not all of their identity is known) and because the decrease or increase of each parameter will be different, it is impossible to provide a power calculation for each parameter at this time. However, one plasma protein that is expected to be included in our battery of markers is Clusterin; a sulfated glycoprotein that is almost universally expressed in body fluids and participates in a wide breadth of physiological processes like DNA repair and lipid transport. However, there is no precedent in the literature comparing pre- and post-operative plasma levels of this peptide in patients with benign or malignant conditions. Using an unpaired t-test with a power of 0.8 and an alpha of 0.05, power analysis demonstrated a need for 45 patients in each study arm to able to detect an effect size of at least 25 μg/ml between the laparoscopic and open group. The population standard deviation utilized in this calculation is 42 μg/ml respectively; as reported for fasting males in the Morrissey et. al. paper (6)

Study Procedure:

A total of 4 blood samples (ideally) will be taken:

1. Pre-operative (mandatory and defined as any time prior to induction of anesthesia)
2. Post-operative day 1 (mandatory)
3. Post-operative day 3 (mandatory)
4. Any time point between post operative day 7-14 during their initial follow- up visit (if possible).

15cc of blood will be taken at each sampling point; 10ml will be collected in heparin containing tubes and 5ml in EDTA-containing test tubes. Blood will be drawn and captured in the usual sterile fashion from an arm vein. All steps will be taken to ensure minimal patient discomfort. Within 6 hours of collection, plasma and cell isolates will be processed (according to the appropriate protocol) from all patient blood samples obtained at all time points. These samples of plasma and cell isolates will be promptly de-identified, labeled with a unique marker and kept in a -80C freezer.

T cells and erythrocyte depleted total blood cells will be isolated as described in protocol 1 and kept at -80C until their delivery to the Columbia study center. RNA will be extracted by the staff at the Columbia study center using the TRIzole extraction method. Quality of RNA was ensured prior to labeling by analyzing 20-50ng of each sample using a Bioanalyzer 2100. Only samples with a 28S/18S ribosomal peak ratio of 1.8-2 will be considered suitable for labeling and use. 2 micrograms of total RNA will then be used for cDNA synthesis via the Superscript double-stranded cDNA synthesis kit (Invitrogen). 10 micrograms of labeled and fragmented cRNA (obtained by in-vitro transcription of the generated DNA) will be hybridized to the Human Genome U133A GeneChip (Affymetrix) at 45C for 16hr. To look for genes that are differentially expressed the linear model "LIMMA" from Bioconductor micro array analysis will be used.

Micro array validation studies will be carried out via commercially available ELISA kits on thawed pre-operative and post-operative plasma samples. Assays will be conducted according to the manufacturer's instructions .The evaluations will be performed using an ELISA plate reader (Bio-Tek) and concentrations calculated via bundled software (ELx 800). The intra-assay precision will be evaluated by testing 2 different samples of known concentration 5 times on one plate. The inter-assay precision will also be evaluated by testing 2 samples on known concentration in 5 different assays.

Study Drugs:

No drugs

Medical Devices:

No devices

Study questionnaire:

The study co-coordinator, RN or a designated person will obtain and record the following information regarding each patient enrolled in the study:

1) Standard demographic and medical history (name, age, gender, ethnicity, BMI, past surgical history, past medical history, allergies, current medications)
2) Indication for surgery
3) Planned surgical procedure
4) Actual procedure performed
5) For laparoscopic resections, was conversion to open performed
6) Length of operation
7) Length of longest incision (in cm)
8) Pathology results
9) Post-operative complications (wound infection, anastamotic leak, hernia formation)
10) Length of stay

Study Subjects:

Inclusion criteria are patients above 18 years of age who undergo elective open or laparoscopic colorectal resection. Exclusion criteria include a recent history of blood transfusion, steroid or other immunosuppressive drug use. Patients who have received radiotherapy or chemotherapy within 2 months prior to the surgery date will also be excluded. Patients who have undergone major surgery within the month prior to surgery will not be eligible for entry into this study. Pregnant women, psychiatric patients, minors, prisoners and patients undergoing emergency operations are ineligible for entry into this study.

Patients will not be excluded if they do not speak or understand English; as an appropriate translator will be used to present and fully explain the study. The pre-operative result of each patient will serve as their own baseline value against which the post-operative results will be compared.

Patient recruitment:

Potential patients will be identified by a participating surgeon, who will initiate contact and ascertain their willingness to discuss a study with the research team before said patient grants audience to the study personnel. This is in accordance with hospital policy directing patient enrollment in research studies

Confidentiality of Study data:

All study data will be kept coded without personal identifiers and maintained in a secure location accessible only to investigators to ensure maximum confidentiality.

Location of Study:

This will be part of a multi center trial to be conducted at various institutions whose respective IRB approved this trial. CPMC will be one of the sites. In each hospital, blood samples will be obtained in the doctor's private office or clinic (pre-operative samples) as well as in the hospital itself (post-operative samples). The blood samples

will be processed by appropriate blood processing protocol and transiently stored at each hospital or university before being shipped to Columbia University College of Physicians and Surgeons where all study tests involving the samples will be carried out.

Potential risks and benefits:

Enrolled patients will not receive any benefit from their participation in this study. The possible risks to enrolled patients from their participation in this trial are few. There are minimal risks involved with venipuncture and blood collection including a hematoma at site of phlebotomy. Rarely, a localized wound might ensue around the venipuncuture site; should this scenario arise, it will be addressed with antibiotics and warm compresses.

Alternative Therapies:

Not applicable

Compensation and costs to subjects:

No compensation will be provided to study subjects. There will also be no added cost to the patient or their insurance company as a result of their participation in this trial.

Radiation or Radioactive substances:

Not applicable

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