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Pharmacologic blockade of tumor necrosis factor-alpha and its effect on the performance of the T-SPOT.*TB* assay in the diagnosis of latent tuberculosis infection among patients with rheumatoid arthritis and inflammatory bowel disease

1. Study Purpose and Rationale

Tumor necrosis factor-alpha (TNF-α), a proinflammatory cytokine, plays a pathogenic role in chronic immune-mediated diseases such as rheumatoid arthritis (RA), psoriasis, and the inflammatory bowel diseases (IBD), Crohn's disease and ulcerative colitis. Blockers of TNF-α, which include the monoclonal antibodies infliximab and adalimumab and the soluble TNF-α receptor etanercept, have become a mainstay of treatment in these diseases. TNF-α also plays an important role in the formulation of granulomas and protect against mycobacterial infection. As such, these TNF-α blockers, and infliximab in particular, have been associated with increased risk of active tuberculosis. Therefore, prior to commencing this regimen, clinicians screen for latent tuberculosis infection (LTBI) with tuberculin skin testing (TST), and treat this infection if present.

Several studies have shown that the new T-cell based assays for diagnosing LTBI (QuantiFERON and T-SPOT.*TB*) have higher specificity and better correlation with exposure to active tuberculosis than the TST. The T-SPOT.*TB* assay incubates lymphocytes with tuberculosis antigens and measures interferon gamma (IFN-γ) release from T-cells as spot-forming cells (SFCs). In addition to a nil control, lymphocytes are stimulated with the mitogen phytohaemagglutinin (PHA) as a positive control to determine whether a negative result is a true negative or a false one due to a lack of functioning T-cells. An indeterminate result is reported when fewer than 20 SFCs are detected in the PHA-stimulated control wells.

While these new diagnostic modalities have shown promise in immunocompetent subjects, there is very limited data on the accuracy of these assays in patients maintained on TNF-α blocker therapy. There is reason to suspect that inhibition of TNF-α may interfere with IFN-γ release assays. Saliu, et al., found that the addition of infliximab and adalimumab (but not etanercept) caused a concentration-dependent decrease in IFN-γ levels of whole blood cultures incubated with tuberculosis antigens.(1) If this is indeed the case, pharmacologic blockade of TNF-α would cause a decline in IFN-γ release by PHA-stimulated T lymphocytes, thus increasing the number of indeterminate results among patients maintained on TNF-α blockade.

As therapy with TNF-α blockade has been associated with an increased risk of active TB, it is crucial to understand the accuracy of the tests being used to diagnose infection in this patient population. The blood-based assays are gaining popularity based on studies

showing their increased specificity in diagnosing LTBI; however, if these tests are less accurate in certain circumstances due to an increased number of indeterminate results, the applicability of these assays may be limited.

2. Study Design and Statistical Procedures

Participants in this study will consist of three groups:

(i) **Control Group** – 100 patients with rheumatoid arthritis or inflammatory bowel disease (Crohn's disease or ulcerative colitis) *not* maintained on a TNF-α blocker currently or in the past, and not exposed to one of the following immunosuppressive drugs in the past 6 months: systemic corticosteroids, budesonide, methotrexate, leflunomide, mycophenolate mofetil, azathioprine, 6-mercaptopurine, cyclosporine, cyclophosphamide, rituximab, abatacept, and anakinra.

(ii) **Immunomodulator group** - 100 patients with rheumatoid arthritis or inflammatory bowel disease *not* maintained on a TNF-α blocker currently or in the past, but exposed to one of the following immunosuppressive drugs in the past six months: systemic corticosteroids, budesonide, methotrexate, leflunomide, mycophenolate mofetil, azathioprine, 6-mercaptopurine, cyclosporine, cyclophosphamide, rituximab, abatacept, and anakinra.

(iii) **TNF-α blocker Group** – 100 patients with rheumatoid arthritis or inflammatory bowel disease currently maintained on a TNF-α blocker.

We will obtain one blood sample from each patient. There will be three possible results – positive, negative and indeterminate. Using less than 20 spot forming cells in the positive control well as the definition of an indeterminate result, we will compare the difference in the rates of indeterminate results in both groups.

As this is a pilot study designed to detect large differences, we will recruit 100 patients in each group. With this sample size, assuming a 5% indeterminate rate in the control group (based on prior published rates), an indeterminate rate of 19% in the immunosuppression group and 38% in the TNF-α blocker group would demonstrate a statistically significant stepwise difference between the three groups, using an α of 5% and 80% power.

Using a chi-square analysis to compare the difference between groups, a sample size of 72 in each group will show a significant difference of 20% between groups. A larger sample size of 220 in each group will detect a difference of 10% between the groups and a sample size of 100 will detect a 15% difference.

3. Study Procedures

We will identify study participants by approaching rheumatologists and gastroenterologists and asking them to recommend potential participants from their patient panels. These physicians will ascertain whether or not the patients are willing to discuss the study with investigators. These patients will then be approached at the physician's office, clinic or the infusion center (where patients receive a three-hour-long infusion of infliximab). Participants will be given an explanation of the risks and benefits of participation and be asked to sign an informed consent form. The investigator will conduct an interview, acquiring information regarding the patient's tuberculosis risk factors as well as medical history and medication regimen.

Oxford Immunotec will provide the required consumables (Becton Dickinson Cell Preparation Tubes (CPT) and venipuncture system) and the T-SPOT.*TB* assay kits. Patients will have a blood sample taken into two 8mL CPT tubes. Samples will be processed the same day using the standard operating procedure for the T-SPOT.*TB* assay as provided. Spot counts will be recorded. The assay cutoff criteria as stated in the pack leaflet will be applied. Assay result and questionnaire data will be entered into a clinical database.

All patient information will be collected and stored using a unique patient code. Patient information will be stored in a locked cabinet and computer analysis will be done using coded information. Only the principal investigator and co-investigators will have access to this information.

4. Study Drugs or Devices

This study will not expose patients to any new drugs or devices; subjects will receive their regular medications as instructed by their own physicians.

5. Study Questionnaires

There will be one questionnaire to be completed by the investigator who will interview each subject. The enrollment questionnaire will gather information regarding patient demographics, tuberculosis exposure history, occupational history, medical conditions and current medications (see Attachment #1)

6. Study Subjects

Group 1 (Control Group) – 100 subjects
Inclusion criteria:
Persons 18 years of age and older
Persons with diagnosed rheumatoid arthritis, Crohn's disease or ulcerative colitis

Exclusion criteria:
Refusal of consent
Age younger than 18 years
Known infection with human immunodeficiency virus (HIV)
Use of cancer chemotherapy in the past six months
Use of one of the following immunomodulating agents in the past six months: systemic corticosteroids, budesonide, methotrexate, leflunomide, mycophenolate mofetil, azathioprine, 6-mercaptopurine, cyclosporine, cyclophosphamide, rituximab, abatacept, and anakinra.
Persons who have ever received TNF-α blocker therapy

Group 2 (Immunomodulator Group) – 100 subjects
Inclusion criteria:
Persons 18 years of age and older
Persons with diagnosed rheumatoid arthritis, Crohn's disease or ulcerative colitis
Use of one of the following immunomodulating agents in the past six months: systemic corticosteroids, budesonide, methotrexate, leflunomide, mycophenolate mofetil, azathioprine, 6-mercaptopurine, cyclosporine, cyclophosphamide, rituximab, abatacept, and anakinra.

Exclusion criteria
Refusal of consent
Age younger than 18 years
Known infection with HIV
Use of cancer chemotherapy in the past six months
Persons who have ever received TNF-α blocker therapy

Group 3 (TNF-α Blockade Group) – 100 subjects
Inclusion criteria:
Persons 18 years of age and older
Persons with diagnosed rheumatoid arthritis, Crohn's disease or ulcerative colitis

Persons currently on a scheduled TNF-α blocker regimen having received at least 2 doses, with the most recent dose within the past 8 weeks.

Exclusion criteria
Refusal of consent
Age younger than 18 years
Known infection with HIV
Use of cancer chemotherapy in the past six months

7. Recruitment

We will identify study participants by approaching rheumatologists and gastroenterologists and asking them to recommend potential participants from their patient panels. These physicians will ascertain whether or not the patients are willing to discuss the study with investigators. These patients will then be approached at the clinic, the physician's office, or the infusion center where they receive their three-hour-long administration of infliximab. An email will be sent to attendings and fellows (see attachment #2)

8. Confidentiality of Study Data

All patient information will be collected and stored using a unique patient code. Patient information will be stored in a locked cabinet and computer analysis will be done using coded information. Only the principal investigator and co-investigators will have access to this information.

9. Potential Risks

There are limited risks to being in this study. As a result of having blood drawn, subjects may experience pain, bruising, dizziness, or fainting. Blood will be drawn in a sterile fashion to minimize risk of infection.

There is a small risk that patient information could be released unintentionally to unauthorized personnel. As noted above, patient data will be stored in a locked cabinet, and only the principal investigator and co-investigators will have access to this information.

10. Potential Benefits

There is no benefit to the individual subject. The results of this study overall will benefit society by yielding a greater understanding of the new blood-based assays for LTBI and their applicability to immunosuppressed patients, particularly those maintained on TNF-α

blocker therapy.

11. Alternatives

N/A

12. References

1. Efthimiou P et al. Quantiferon TB gold test: The new standard for screening of latent tuberculosis in patients with rheumatoid arthritis? Ann Rheum Dis 2006

2. Ewer K, et al. Comparison of T-cell based assay with tuberculin skin test for diagnosis of *Mycobacterium tuberculosis* infection in a school tuberculosis outbreak. Lancet 2003;361:1168-1173

3. Hamdi H, Mariette X, Godot V, et al. Inhibition of anti-tuberculosis T-lymphocyte function with tumour necrosis factor antagonists. Arthritis Res Ther 2006;8(4):R114.

4. Piana F, et al. Use of a T-cell based test for detection of tuberculosis infection among immunocompromised patients. Eur Respir J 2006;28:31-34

5. Rangaka MX, Wilkinson KA, Seldon R, et al. The Effect of HIV-1 Infection on T cell Based and Skin Test Detection of Tuberculosis Infection. Am J Respir Crit Care Med 2006.

6. Saliu OY, Sofer C, Stein DS, Schwander SK, Wallis RS. Tumor-necrosis-factor blockers: differential effects on mycobacterial immunity. J Infect Dis 2006;194(4):486-92.

7. Winthrop K. Risk and prevention of tuberculosis and other serious opportunistic infections associated with the inhibition of tumor necrosis factor. Nature 2006;2(11): 602-603

Attachment #1 – Enrollment Form

Participant ID: _____
Date of enrollment: _____
Date of specimen collection: _____

Eligibility Checklist
__Informed consent complete
__Age 18 or older
__Diagnosis of RA or IBD
__On maintenance TNF blocker currently or never on TNF blocker
__Not HIV positive
__No history of cancer chemotherapy in the past 6 months

Demographics

Gender: ___Male ___Female
Age (years): ___

Ethnicity:
___ White (non-Hispanic) ___Asian
___ Black (non-Hispanic) ___Hispanic
___Mid-East/Indian ___Other:_____

Residence:
Current country of residence: _____
Years in residence: ___

Country of birth: _____

Years in residence: ___

Spent 3 or more years in an undeveloped country? ___ Yes ___No
Name of country: _____ Dates of residence: _____
Name of country: _____ Dates of residence: _____
Name of country: _____ Dates of residence: _____
Name of country: _____ Dates of residence: _____

Tuberculosis History:
Previous vaccination for TB (BCG vaccine): ___Yes ___No ___Unknown
Vaccination scar? ___Yes ___No ___Unknown

Previous TST? ___Yes (year)_____ ___No ___Unknown
Previous TST result __Pos _____mm ___Neg ___Unknown
Multiple previous TST's? __Yes ___No ___Unknown

Previous LTBI ___Yes (date)_____ ___No
Treatment: (medications/dates)_____/_____
Previous active tuberculosis: ___Yes (date)_____ ___No
___Pulmonary ___Extrapulmonary
Treatment: (medications/dates)_____/_____

Occupation history:
___ Healthcare _____(job title, dates)
___ Nursing home_____(dates)
___ Homeless shelter_____(dates)
___ Drug rehab facility_____(dates)
___ Prison system_____(dates)
___ Military_____(dates)
___Other _____

Medical Conditions:

___Crohn's Disease ___Ulcerative colitis ___Indeterminate IBD
___Rheumatoid arthritis ___Other_____

Year of diagnosis: _____

___Asthma
___Diabetes
___Leukemia/lymphoma
___Solid organ malignancy (specify)_____
___Chronic liver disease (specify)_____
___Chronic kidney disease

Medications

___Anti-TNF therapy (dates):
- ___Infliximab (Remicade) _____
- ___Etanercept (Enbrel) _____
- ___Adalimumab (Humira) ____
- ___Other _____

___ Corticosteroids
- Name/dose/dates: _____/_____/_____
  - _____/_____/_____
  - _____/_____/_____
  - _____/_____/_____
  - _____/_____/_____

___Budesonide (Entocort) (dose/dates) _____/_____
___Methotrexate (Trexall) (dose/dates) _____/_____
___Mycophenolate mofetil (Cellcept) (dose/dates) _____/_____
___Cyclophosphamide (Cytoxan) (dose/dates) _____/_____
___Hydroxychloroquine (Plaquenil) (dose/dates) _____/_____
___Leflunomide (Arava) (dose/dates) _____/_____
___Anakinra (dose/dates) _____/_____
___6-mercaptopurine (Purinethol) (dose/dates) _____/_____
___Azathioprine (Imuran)(dose/dates) _____/_____
___5-aminosalicylates (name/dose/dates): _____/_____/_____
___Cyclosporine (Neoral/Sandimmune/Gengraf) (dose/dates) _____/_____
___Rituximab (Rituxan) (dose/dates) _____/_____
___Abatacept (Orencia) (dose/dates) _____/_____

Group 1 __1 (control) __2 (immunosuppression) __3 (TNF-blocker)

Attachment #2 - Email to be sent to gastroenterology and rheumatology fellows:

A CALL FOR REFERRALS

We are launching a clinical study that aims to measure the utility of an investigational blood-based assay for the identification of latent tuberculosis infection (LTBI) in patients with rheumatoid arthritis (RA) and inflammatory bowel disease (IBD).

Study representatives will be present at rheumatology gastroenterology clinic in the Vanderbilt Clinic room 240 on Wednesday and Thursday afternoons**. If you have a patient with RA or IBD, this patient may be eligible for our study.**

Inclusion criteria:

- Age 18 years or older
- Have a diagnosis of RA or IBD (Crohn's disease or ulcerative colitis)
- HIV-negative.
- No history of cancer chemotherapy in the past six months

The entire process (from screening to the blood draw) will take approximately 15 to 30 minutes. Subjects will be reimbursed with a $10 MetroCard®

Please consider referring your patients for enrollment in this study.