Tuberculosis in Non-HIV Infected Immunosuppressed Hosts

TNFα inhibitors and beyond

David E. Griffith, MD
Assistant Medical Director
Heartland National TB Center
Professor of Medicine
University of Texas Health Science Center, Tyler, TX
US Reported Infections Associated With Biologic Drugs

- Salmonellosis
- Coccidioidomycosis
- Blastomycosis
- Legionellosis
- Listeriosis
- Parasitic Infections
- Aspergillosis
- CMV
- Severe Pneumococcal Disease
- Histoplasmosis
- Invasive *Staphylococcus aureus*
- TB/NTM

CMV, cytomegalovirus; NTM, nontuberculous mycobacteria; TB, tuberculosis.

Tumor Necrosis Factor-α (TNF-α)

- Expressed by activated macrophages, T and B lymphocytes
- Biological effects numerous
  - Granuloma formation and maintenance
  - Macrophages activation to ingest and kill pathogens
- Soluble and transmembrane forms
  - Bind p55 and p75 receptors
  - p55/TNF-α interaction critical for granuloma formation
Overexpression of TNF-α

- Inflammation and tissue destruction
- Immune-mediated inflammatory diseases (IMID)
  - Rheumatoid arthritis, inflammatory bowel disease, psoriasis, ankylosing spondylitis, others
- Rewards of TNF-α blockade
  - Highly successful in treatment of these conditions
IMID Biologic Therapies

• TNF-α inhibition
  – Infliximab, adalimumab, golimumab, certolizumab (monoclonal antibodies)
  – Etanercept (soluble p75 receptor)

• Newly approved
  – CD4 co-stimulation modulator: abatacept
  – B-cell (CD20+) antibody: rituximab
  – Anti-IL-6 receptor antibody: tocilizumab
  – Anti-IL12/IL23 antibody: ustekinumab
  – Anti Cd2 binder on T cells: alefacept
TNFI Risk of TB: Monoclonal Antibody RX vs Soluble TNF Receptor RX

(Tubach et al. Arthritis Rheum 2009; 60: 1884)

• FrenchTB surveillance study: 69 TB cases over 3 years
• Standardize Incidence ratio (SIR) compared to background French population
  - Adalimumab 29.3 (20.3-42.4)
  - Infliximab 18.6 (13.4-25.8)
  - Etanercept 1.8 (0.7-4.3)
• Case-control with etanercept as reference
  - Adalimumab OR 17.1 (3.6-80.6)
  - Infliximab OR 13.3 (2.6-69.0)
TNFI Risk of TB: Monoclonal Antibody RX vs Soluble TNF Receptor RX

- British Soc fro Rheum Biologics Registry: 10,712 TNFI RX’d RA patients c/w 3232 RA patients
- 40 TB cases all in the TNFI cohort
- TB case rates:
  - Adalimumab (ADA): 144/100,000 person-years
  - Infliximab (INF): 136/100,000 person-years
  - Etanercept (ETA): 39/100,000 person-years
- “The rate of TB in patients with RA treated with anti-TNF RX was 3-4 fold higher in patines receiving INF and ADA than those receiving ETA.”
UK Biologic Registry: Background UK rate 12-14/100,000

| Table 2 | Numbers and rates of incident tuberculosis, switchers included |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Number of patients ever received the drug | DMARD (n = 3232) | All anti-TNF (n = 10,712) | ETA (n = 5521) | INF (n = 3718) | ADA (n = 4857) |
| On drug* | 7345 | 28,447 | 12,744 | 8,069 | 7,634 |
| Person-years | 0 | 27 | 5 | 11 | 11 |
| Cases of TB | 0 | 95 (63 to 138) | 39 (13 to 92) | 136 (68 to 244) | 144 (72 to 258) |
| Rate/100,000 person-years (95% CI) | 0 | Referent | 3.1 (1.0 to 9.5) | 4.2 (1.4 to 12.4) |
US Population-based Data

• Kaiser-Permanente Northern CA 2000-2008,
  – Anti-TNF users (n=8,418)

<table>
<thead>
<tr>
<th></th>
<th>Anti-TNF</th>
<th>ETN</th>
<th>INF</th>
<th>ADA</th>
</tr>
</thead>
<tbody>
<tr>
<td>*TB</td>
<td>49</td>
<td>17</td>
<td>83</td>
<td>61</td>
</tr>
<tr>
<td>*NTM</td>
<td>74</td>
<td>35</td>
<td>116</td>
<td>122</td>
</tr>
</tbody>
</table>

*Case rates per 100,000 pt/years; Note all 95% CIs overlap. No statistical differences between groups.

KPNC background rates: TB = 2.8/100,000 and NTM = 4.1/100,000
More TB Risk with Monoclonals?

- Drug mechanisms differ
- Greater TNF-α binding
  - Transmembrane and soluble TNF-α
  - Forms stable complex
- Interferon-gamma down-regulation
- Differential granuloma penetration
Interferon-γ Downregulation

Saliu et al. JID 2006
Granuloma Penetration

A. Survival of acutely infected mice

B. Bacterial burden in the lungs

C. Survival of chronically infected mice

D. Bacterial burden in lungs
Granuloma Penetration

- Acute TB infection (mouse)
  - Large bacillary load and death
  - No difference between anti-TNFs
- Chronic TB infection (mouse)
  - Monoclonal antibodies = death (1 month)
  - Etanercept = 60% alive at 6 months
  - Lung path: etanercept with less penetration of granulomas

Plessner et al. JID 2007
# LTBI Screening

Table 1. Summary of selected recommendations for tuberculosis screening prior to anti-TNF therapy

<table>
<thead>
<tr>
<th>Agency/Region</th>
<th>Year</th>
<th>Regional BCG use</th>
<th>Regional TB prevalence (cases/100,000)</th>
<th>Risk assessment</th>
<th>Initial screening test</th>
<th>Chest radiograph</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>BTS(^{26})</td>
<td>2005</td>
<td>Yes</td>
<td>Low (12)</td>
<td>Yes</td>
<td>None(^{c})</td>
<td>Yes(^{y})</td>
<td>Empiric INH for those from highly prevalent regions</td>
</tr>
<tr>
<td>Switzerland(^{37})</td>
<td>2008</td>
<td>Yes</td>
<td>Low (4.9)</td>
<td>Yes</td>
<td>IGRA</td>
<td>Yes(^{y})</td>
<td></td>
</tr>
<tr>
<td>France(^{39})</td>
<td>2006</td>
<td>Yes</td>
<td>Low (6.2)</td>
<td>Yes</td>
<td>IGRA</td>
<td>Yes(^{y})</td>
<td></td>
</tr>
<tr>
<td>Spanish(^{11})</td>
<td>2004</td>
<td>Yes</td>
<td>Low (17)</td>
<td>Yes</td>
<td>TST (two-step)</td>
<td>Yes(^{y})</td>
<td></td>
</tr>
<tr>
<td>Germany(^{38})</td>
<td>2009</td>
<td>Yes</td>
<td>Low (5.1)</td>
<td>Yes</td>
<td>IGRA</td>
<td>Yes(^{y})</td>
<td></td>
</tr>
<tr>
<td>ACR(^{28})</td>
<td>2008</td>
<td>No</td>
<td>Low (4.8)</td>
<td>Yes</td>
<td>TST</td>
<td>Yes(^{+})</td>
<td></td>
</tr>
<tr>
<td>CDC(^{27})</td>
<td>2005</td>
<td>No</td>
<td>Low (4.8)</td>
<td>Yes</td>
<td>TST</td>
<td>Yes(^{+})</td>
<td></td>
</tr>
<tr>
<td>Canada(^{29})</td>
<td>2008</td>
<td>No</td>
<td>Low (5)</td>
<td>Yes</td>
<td>TST</td>
<td>Not specified</td>
<td>IGRA in those with negative TSTs but risk factors</td>
</tr>
</tbody>
</table>

American College of Rheumatology (ACR); British Thoracic Society (BTS); National Institute for Health and Clinical Excellence (NICE); US Centers for Disease Control or Prevention (CDC).
<table>
<thead>
<tr>
<th>Table 1. Risk factors for prior tuberculosis exposure [15,30].</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Known prior exposure to active tuberculosis case</td>
</tr>
<tr>
<td>• Birth or extended residence in a country where tuberculosis is prevalent. This includes most countries in Latin America, Asia, the Caribbean, Eastern Europe, Africa, and Russia</td>
</tr>
<tr>
<td>• History of living or working within congregate settings where tuberculosis is more common including the following:</td>
</tr>
<tr>
<td>• Jail or prison</td>
</tr>
<tr>
<td>• Homeless shelters</td>
</tr>
<tr>
<td>• Healthcare centers that treat tuberculosis patients</td>
</tr>
<tr>
<td>• History suggestive of prior LTBI diagnosis including the following:</td>
</tr>
<tr>
<td>• Prior positive screening tests (TST, IGRA)</td>
</tr>
<tr>
<td>• Chest radiographic findings (i.e. fibronodular opacities) associated with prior tuberculosis</td>
</tr>
</tbody>
</table>

**LTBI:** latent tuberculosis infection; **IGRA:** interferon-γ-release assay; **TST:** tuberculin skin test.
Interferon-gamma Release Assays (IGRAs)
IGRAs in Anti-TNF Candidates

- **Greater specificity for tuberculosis than TST**
  - Does not cross-react with BCG or most environmental mycobacteria

- **Relative sensitivity with TST for LTBI?**

- **Matulis et al, 2007**¹
  - Patients with inflammatory rheumatic conditions treated with anti-TNF or non-biologic treated (n = 126)
  - 12% QFT positive vs 40% TST positive
  - QFT-IT more closely associated with LTBI risk factors than TST

¹P < .05 for comparisons.

BCG, bacille Calmette-Guérin; RA, rheumatoid arthritis.
Relative Sensitivity of IGRA

- Case-control study, Peru
- 80% BCG use in both groups
- High prednisone use among RA group

<table>
<thead>
<tr>
<th></th>
<th>RA (n = 101)</th>
<th>Controls (n = 93)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TST+</td>
<td>27 (27%)</td>
<td>61 (66%)</td>
</tr>
<tr>
<td>QFT-IT+</td>
<td>45 (45%)</td>
<td>55 (59%)</td>
</tr>
</tbody>
</table>

IGRAs in the Immunocompromised

• Anergy with TST and IGRAs
  – IGRAs less affected by prednisone?
  – False negative with IGRA in patients already receiving anti-TNF therapy

• Indeterminate results
  – QFT-IT and T-SPOT.TB in 2-5%

• LTBI sensitivity
  – QFT-IT similar to T.SPOT.TB (and probably similar to or greater than TST)

LTBI Treatment

- Begin treatment before starting anti-TNF therapy
  - 9 months isoniazid (INH) preferred in US
  - 4 months rifampin is alternative
- Start INH 1 month prior to anti-TNF initiation
  - 83% reduction in INF-associated cases in Spain\(^1\)
  - Ensure INH compliance and tolerance
- Liver function testing
  - Many patients taking MTX

MTX, methotrexate.
Screening with other Biologics?

• Rituximab
  – CD20+ B-cell antibody
  – Depletes peripheral B cells for 6-12 months
  – No TB in RA clinical trials or in lymphoma use
  – B cell importance to granuloma/survival in murine model of TB*

• EIN Survey
  – 8 TB/NTM cases with rituximab
  – All cases also on prednisone

Abatacept

- Murine chronic TB not affected by abatacept*
  - Mortality, T cell, B cell, INF-γ production in lung, and bacillary load
- No human cases reported
- Tuberculosis risk unknown
  - Screened in clinical trials
  - Should screen in practice

*Bigbee et al. Arth Rheum 2007
Tocilizumab

- IL-6 receptor inhibitor
- Did not screen for LTBI in clinical trials
- 10 cases TB
  - 5 pulmonary
  - Rate = 100/100,000 pt/yrs
- Should we be screening?
  - Yes. On FDA label
What is the TNFI Bottom Line?

- Official ATS TNFI/TB Guidelines
  - “Due out anytime” (5/12)
  - “I don’t think the guidelines will ever come out” (6/12)
- Ersatz (sort of) “ATS” Suggestions
  - Consensus of “some guys” with a little insider knowledge
- *European TBNET Consensus Statement 2010
  - “The risk of tuberculosis related to TNF antagonist therapies”
What is the TNFI Bottom Line?

- What is the preferred test for LTBI in patients who will receive TNFI, TST or IGRA?
  - In general IGRA preferred, see algorithm. Important to get tested regardless of method
  - * No clear advantage of T-spot over QFT
  - *2-step TST not recommended (↑ Sens, ↓ Spec)

- * In general, IGRAs are superior to the TST in immunocompromised individuals. In addition, mitogen controls in the IGRAs give an advantage over the TST at they can be sued ro discrimination between anergy and true negative antigen specific immune responses. However, the only evidence available is that a (+) result in a 2-step TST predicts the development of TB in individuals undergoing TNFI.”
What is the TNFI Bottom Line?

- * Screening for LTBI and preventive chemotherapy against TB should not be different for patients with different underlying disease (RA, psoriasis, IBD) who are candidates for TNFI.

- If TST and IGRA results are discordant, do you give priority to the + test?
  - If the patient has risk factors, YES, give priority to the + test.
  - If no risk factors or BCG to explain the discordance, repeat IGRA at least once.
  - *A history of significant past exposure or untreated TB is an indication for LTBI RX even with (-) LTBI tests.
What is the TNFI Bottom Line?

- What about BCG vaccinated individuals who are TST + but IGRA negative?
  - Specificity of IGRA takes precedence, but caveat doctor.

- What is the recommended duration of LTBI therapy before starting TNFI?
  - At least one month (Spanish study). The real answer may be “before” TNFI. Important to make sure the patient has actually started the LTBI therapy as recommended
  - * An “induction period” of 4 weeks is considered safe by most experts to start TNFI after the induction of LTBI RX
What is the TNFI Bottom Line?

- Is there a preferred LTBI treatment regimen for TNFI patients?
  - Short answer: NO
  - No data on new 3IR therapy

- For TST/IGRA (-) patients, should testing be repeated during TNFI therapy?
  - Not unless patients live in endemic area or are re-exposed (FDA label suggest “periodic” retesting, ? not endorsed by CDC)
  - No other immunocompromised settings in the U.S. where periodic repeat testing recommended
What is the TNFI Bottom Line?

• What about patients with a history of prior TB disease or LTBI therapy who will take TNFI?
  – No therapy if documentation for prior therapy available or if history compelling (judgment call)
  – Default is retreat

• What are recommendations for monitoring patients on TNFI and LTBI therapy?
  – Usual monitoring for hepatotoxicity
  – Monitoring for progression to active disease
What is the TNFI Bottom Line?

• If a patient develops TB while on TNFI and TNFI stopped, how long on TB therapy before restarting TNFI?
  – Do not restart TNFI until the patient is on a regimen known to be effective and has had a “good” clinical response (?2 months)
  – * Expert opinion suggests initiating RX with TNFI when a full course of anti-TB RX has been completed

• Are standard short course TB regimens appropriate for TB patients on TNFI?
  – Presumably yes, but little data. Need to consider the high incidence of extra-pulmonary/disseminated disease. In practice probably frequent 9-12 mos RX.
What is the TNFI Bottom Line?

• Is there a recommendation for restarting a new TNFI if TB occurs during TNFI therapy?
  – Not much data, consider etanercept given evidence of lower risk vs monoclonals
  – Consider alternative agent: abatacept or rituximab

• Is the clinical presentation of active TB different for TNFI patients vs immune competent patients?
  – High percentage of extrapulmonary and disseminated disease so similar clinical presentations to other immune compromised patients.
What is the TNFI Bottom Line?

- Are there significant drug-drug interactions of TNFI with rifamycins? Is rifabutin preferable to rifampin?
  - Short answers no and no. Problems arise with medications for other comorbidities
  - INH and MTX concern for hepatotoxicity
- Are these TNFI “recommendations” unique for TB or can they be extrapolated to other granulomatous disease OI (NTM, fungi, etc.)?
  - Yes: TB basically the only OI associated with TNFI that is preventable with screening
Using both TST and IGRA maximizes sensitivity. In the presence of risk factors, any positive result is considered infected. In the absence of risk factors, all positives are considered infected except in cases of TST+/IGRA−, where history of BCG vaccination is considered. This method maximizes the predictive value of the screening tests according to a patient’s a priori likelihood of exposure.
Nontuberculous (NTM) Disease

• Environmental mycobacteria
  – Lung, skin/soft tissue, disseminated disease
  – *M. avium, M. kansasii, M. chelonae, M. abscessus*

• Surveyed IDSA Emerging Infectious Network (EIN)
  – ¼ of US infectious disease specialists

• Reported 1,876 TB or NTM cases
  – 49 (2.6%) associated with biologics
  – 32 cases NTM vs 17 TB
  – *Mycobacterium avium* complex most common (n = 16)

EIN, Emerging Infections Network; IDSA, infectious Diseases Society of America.
## Anti-TNF associated TB and NTM

<table>
<thead>
<tr>
<th></th>
<th>Crude incidence rate (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tuberculosis</td>
</tr>
<tr>
<td>General KPNC population</td>
<td>2.8 (2.6-3.0)</td>
</tr>
<tr>
<td>General KPNC population, ≥50 years</td>
<td>5.2 (4.7-5.8)</td>
</tr>
<tr>
<td>Unexposed RA population</td>
<td>8.7 (5.3-13.2)</td>
</tr>
<tr>
<td>RA Anti-TNF users</td>
<td>56 (24-111)</td>
</tr>
</tbody>
</table>

* *Rate per 100,000 patient years (95% confidence interval)*
*Unexposed anti-TNF therapy*
Resumption of biologic therapy after treatment

<table>
<thead>
<tr>
<th>Infecting organism</th>
<th>Resumption during or after infection treatment?</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB</td>
<td>Yes</td>
</tr>
<tr>
<td>NTM</td>
<td>?</td>
</tr>
<tr>
<td>Histo</td>
<td>Yes</td>
</tr>
<tr>
<td>Cocci</td>
<td>?</td>
</tr>
<tr>
<td>Blasto</td>
<td>?</td>
</tr>
</tbody>
</table>
Prednisone and Tuberculosis

- Risk of reactivation TB poorly defined
  - Based on anecdotal reports from 1950-70s
- CDC 2000 TB statement
  - >15mg/day for one month or more
  - Dose shown to suppress tuberculin skin test reactivity
- No observational or prospective data to support
- Retrospective studies in low incidence areas unable to demonstrate any risk of TB
Prednisone and Tuberculosis

- General Practice Research Database, UK
- TB cases 1990-2001 and controls†
- Current glucocorticoid use *OR 4.9 (2.9-8.3)
- ≤15mg/day *OR 2.8 (1.0-7.9)
- >15mg/day *OR 7.7 (2.8-21.4)

— Causal versus severity of underlying disease

*Adjusted for smoking, BMI, lung disease, diabetes, anti-rheumatic therapy, other TB risk factors

†Controls matched for age, sex, residence, time clinically followed
TB in Solid Organ Transplant (SOT) & Hematopoietic Stem Cell Transplant (HSCT) Recipients

• TB more frequent than in the general population
  – 20-74 X in SOT
  – 2X in HSCT
  – SOT and HSCT TB rate highly linked with TB endemnicity
  – TB generally early in SOT and relatively late in HSCT

• TB more often fatal than in the general population
  – 31% in SOT
  – 50% in HSCT
TB in Solid Organ Transplant (SOT) & Hematopoietic Stem Cell Transplant (HSCT) Recipients

- Infection with MTB relevant in 4 scenarios
  - LTBI in candidate recipient
  - LTBI in living or deceased donor
  - De novo exposure post-transplantation
  - Active TB patient urgently needs transplant

- Detection of TB infection/disease not always possible, depends on:
  - Reliable LTBI detection in recipients & donors
  - Awareness of post transplant exposure
TB in Solid Organ Transplant (SOT) & Hematopoietic Stem Cell Transplant (HSCT) Recipients

- Rate of post transplant TB dependent on the organ transplanted (highest for lung)
- Other risks for post-transplant TB: lymphocyte depleting antibodies, enhanced immune suppression, CRI or hemodialysis, DM, hep C infection in kidney transplant recipients, chronic liver disease, (+) TST or IGRA
- Extrapulmonary and disseminated disease common
- If the a priori risk of MTB infection is high, therapeutic decisions may have to be made without evidence
TB in Solid Organ Transplant (SOT) & Hematopoietic Stem Cell Transplant (HSCT) Recipients

- In general, the same LTBI and TB disease treatment regimens are recommended for SOT and HSCT patients as for other TB patients.
- LTBI RX need not be completed before transplant.
- Rifamycin-free TB treatment regimens are an important option to avoid interaction with immune suppressive drugs to reduce graft rejection.
- “Liver friendly” regimens frequently necessary.
TB in Solid Organ Transplant (SOT) & Hematopoietic Stem Cell Transplant (HSCT) Recipients

- The risk of TB in transplant candidates and recipients: A TBTNTE consensus statement. ERJ, April 10, 2012


Risk factors associated with TB

- Under-nutrition, vitamin deficiencies, overcrowded living conditions, genetic susceptibility, sex, age: ?X
- HIV 20-37X (#1 risk factor LTBI to active disease)
- Diabetes 3X
- Smoking 2X
- Indoor air pollution ?2X
- Silicosis 3X
- Alcohol 3X
- End-stage renal disease 10X
- Malignancy ?X
- Corticosteroid therapy 2X
- TNF alpha inhibitor ?X (>risk TNF antibodies vs soluble TNF receptor)
### Common Risk Factors for Increased Likelihood of Progression from LTBI to Active TB

*(Horsburgh & Rubin, NEJM 2011; 364: 1441)*

<table>
<thead>
<tr>
<th>RISK FACTOR</th>
<th>RELATIVE RISK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advanced, untreated HIV</td>
<td>9.9</td>
</tr>
<tr>
<td>Close Contact</td>
<td>6.1</td>
</tr>
<tr>
<td>Abnormal CXR</td>
<td>5.2</td>
</tr>
<tr>
<td>Prednisone ≥ 15 mg/day</td>
<td>2.8</td>
</tr>
<tr>
<td>Chronic Renal Failure</td>
<td>2.4</td>
</tr>
<tr>
<td>TNFI RX</td>
<td>2.0</td>
</tr>
<tr>
<td>Poorly Controlled DM</td>
<td>1.7</td>
</tr>
<tr>
<td>Weight ≥ 10% below normal</td>
<td>1.6</td>
</tr>
<tr>
<td>Smoking</td>
<td>1.5</td>
</tr>
</tbody>
</table>
TB and Diabetes

- Diabetes associated with a relative risk (RR) for TB of 2-3
  - Jeon et al. PloS Medicine 2008, 5; e152 (13 obs studies)
  - Baker et al. CID 2012, 54; 818 (Taiwan)
  - Dobler et al. BMJ Open 2012, 2; e000666 (Australia)

- “Dysglycemia” may be associated with increased risk for developing TB
  - Baker et al vs Leegaard et al Diabetes Care 2011, 34; 2530

- Cost-effectiveness of screening for LTBI in diabetics questioned
  - Dobler et al, Linas et al AJRCCM 2011, 184; 501
Careful history for TB risk factors

Place TST\textsuperscript{*} and perform IGRA\textsuperscript{*}

TB risk factors present (exposure likely)

TST - / IGRA -

Presume uninfected
NOTE: If patient is immunosuppressed and false negative results more likely, consider re-testing with TST and IGRA

TST + or IGRA +

Diagnose TB infection
Proceed to rule out active TB\textsuperscript{§}

IGRA +

TST + / IGRA -

No BCG History

Diagnose TB infection
Proceed to rule out active TB\textsuperscript{§}

Presume uninfected

No TB risk factors (exposure unlikely)

TST - / IGRA -

BCG History

Presume uninfected

Using both TST and IGRA maximizes sensitivity. In the presence of risk factors, any positive result is considered infected. In the absence of risk factors, all positives are considered infected except in cases of TST +/IGRA -, where history of BCG vaccination is considered. This method maximizes the predictive value of the screening tests according to a patient's a priori likelihood of exposure.

\textsuperscript{*}Tuberculin Skin Test

\textsuperscript{§}Quantiferon\textsuperscript{®} Gold In-Tube (Cellestis, Australia)

\textsuperscript{TM}T-SPOT.TB\textsuperscript{®} (Oxford Immunotec, UK)

\textsuperscript{§}Chest Radiograph and collection of respiratory samples, if indicated