BTK Protein Expression by Flow Cytometry

Indications for Ordering

Initial screening for suspected primary agammaglobulinemia

Test Description

Whole blood specimen is stained with CD14 and CD20 to identify monocytes and B cells, respectively
- Followed by intracellular staining with fluorescently labeled anti-Bruce tyrosine kinase (BTK) and isotype control antibodies
  - Flow cytometry is used to detect BTK protein expression in monocytes and B cells

Tests to Consider

Primary test
Bruton Tyrosine Kinase (BTK) Protein Expression by Flow Cytometry 2012002
- Preferred test for initial screening for individuals with clinical suspicion of X-linked agammaglobulinemia/hyposgamaglobulinemia

Related tests
Initial screening for immunodeficiency
- CBC with Platelet Count and Automated Differential 0040003
- Immunoglobulins (IgA, IgG, IgM), Quantitative 0050630
- Lymphocyte Subset Panel 7 – Congenital Immunodeficiencies 0095899
- Lymphocyte Antigen and Mitogen Proliferation Panel with Cytokine Response 2013117
- Familial Mutation, Targeted Sequencing 2001961
  - Useful when a pathogenic familial variant identifiable by sequencing is known

Confirm suspected agammaglobulinemia
Primary Antibody Deficiency Panel, Sequencing (35 Genes) and Deletion/Duplication (26 Genes) 2011156
- Preferred genetic test for individual with clinical phenotype of primary antibody deficiency (eg, common variable immunodeficiency)

Disease Overview

Prevalence
- X-linked agammaglobulinemia (XLA) – ~1/250,000-700,000

Age of onset – usually within the first 2 years of life

Symptoms
- Recurrent or chronic infections
  - Encapsulated pyogenic bacteria
  - Enteroviruses
- Neutropenia
- Pneumonia/empyema
- Gastroenteritis
- Otitis media
- Meningitis
- Sepsis

Diagnostic issues
- BTK gene mutations are responsible for XLA
- Deficiency or partial deficiency of BTK protein expression is surrogate marker for lack of functioning BTK gene
- Screening for BTK protein expression is recommended prior to genetic testing for phenotype/genotype correlations

Pathophysiology
- Most mutations of BTK gene result in absence of BTK protein, which is essential for maturation of B cells
- Absence or lack of functional BTK protein leads to a lack of immunoglobulins
  - Causes increased susceptibility to infections
  - Occurs almost exclusively in males

Genetics
- Gene – BTK
- Inheritance – X-linked
- Mutations – ~600 known mutations in BTK gene cause XLA

Test Interpretation

Results
- Normal – suggests the presence of BTK protein
- Absent – consistent with XLA in males
- Reduced – suggests XLA in males
- Mosaic – two populations (one with normal BTK expression and one with abnormal BTK expression)
  - Suggests carrier status for XLA in females
Limitations

Normal expression of BTK protein
- Occurs in 20-30% of patients with XLA due to truncated or inactive BTK protein with abnormal function
  - Genetic analysis is recommended
- Does not exclude mutations or defective protein function
- Does not rule out XLA in males
- Does not rule out XLA carrier status in females