From the Clinical Laboratories of the
Cancer & Blood Diseases Institute

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Featured Assays

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FEATURED ASSAYS

IL-18

IL-18: What Is It?

IL-18 (also known as Interferon-gamma Inducing Factor or IGIF) is an important pro-inflammatory cytokine that is involved in a variety of immune processes associated with infection, inflammation, and autoimmunity. IL-18 is synthesized as a precursor molecule (pro-IL-18) in cells such as dendritic cells, macrophages, and intestinal epithelial cells. When activated, IL-1b converting enzyme (caspase-1) cleaves this precursor allowing it to be released from the cell. In combination with IL-12, IL-18 has been shown to act on T helper (Th1) cells to induce their production of Interferon-gamma (IFN-g). [1] IL-18 enhancement of Th1 cytokine (IL-2, GM-CSF, and IFN-g) production, FAS ligand expression, and IL-2R alpha chain expression has also been reported in Th1 cells. [2] IL-18 binding protein (IL-18BP) is a naturally occurring antagonist of IL-18. IL-18BP can downregulate TH1 responses by binding to IL-18, thus reducing the production of IFN-g.

IL-18: How Do We Measure It?

The Diagnostic Immunology Laboratory offers IL-18 testing. The IL-18 Immunoassay performed in our laboratory is a solid-phase ELISA (enzyme-linked immunosorbent assay) that employs the quantitative sandwich enzyme immunoassay technique. Specimen requirements are whole blood collected in an SST or red top tube. The tube may be spun once the clot has formed and preferably two serum aliquots of 0.3 ml placed at -20° C. When ready to ship, aliquots should be packaged on dry ice in a Styrofoam packing container for delivery the next day. Alternatively, we can accept unspun samples if they are received in our laboratory within 24 hours of collection and are kept at room temperature.

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IL-18: Why Measure It?

Measurement of IL-18 can aid clinicians in the diagnosis and monitoring of several immunological disorders. High levels of IL-18 are observed in patients with certain immune deficiencies and autoinflammatory diseases that involve activation or dysregulation of inflammasomes, such as XIAP deficiency and mutations of NLRC4. [3, 4, 5] These patients have persistently high levels of circulating total and also free IL-18 which contributes to disease pathogenesis (see Figure 1). Notably, a clinical trial is open to study the efficacy of a recombinant human IL-18 binding protein, Tadekinig alfa, in treating patients with XIAP and NLRC4 mutations.
(https://clinicaltrials.gov/ct2/show/NCT03113760).

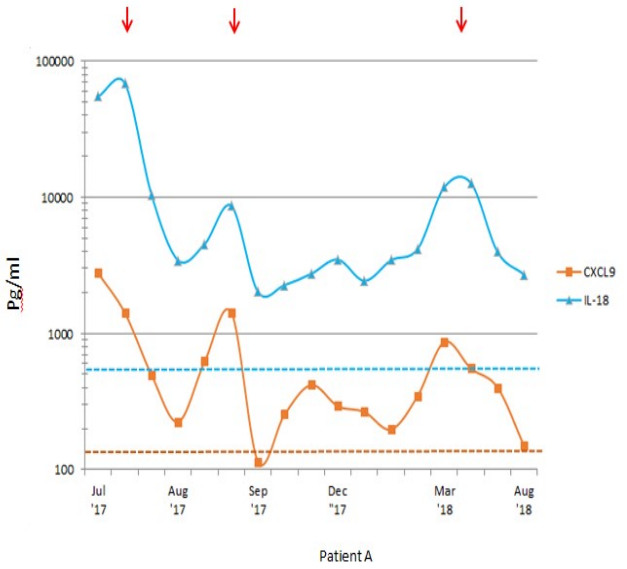

Figure 1. Patient A presented to CCHMC with XIAP deficiency. IL-18 and CXCL9 (see discussion of CXCL9 on the next page) levels were used to monitor disease activity over the course of about 12 months. During this time the patient did experience disease flares indicated by the red arrows. The patient was treated and CXCL9 levels approached reference levels (dashed lines) during times of HLH remission. The IL-18, however, remained elevated even after recovery, indicating baseline ongoing inflammasome activity.

High IL-18 levels are also observed in patients with systemic-onset juvenile arthritis (sJIA) and may be used to help differentiate sJIA from other pyretic diseases. Studies have shown that IL-18 concentrations have a much higher predictive value than other serological biomarkers when trying to distinguish sJIA from diseases such as acute lymphoblastic leukemia, severe infections, Kawasaki disease and JIA, since the clinical symptoms of sJIA are often found in all these diseases. [6, 7] IL-18 can be used to help differentiate the diagnosis and prevent delay in treatment. Once the diagnosis has been made, it can be a beneficial marker to track disease activity and severity during management (see Figure 2).

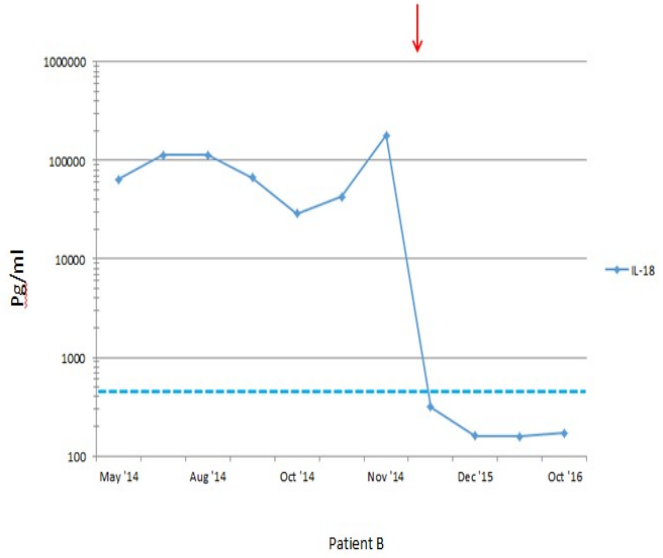

Figure 2. Patient B presented to CCHMC with a diagnosis of sJIA and macrophage activation syndrome (MAS). IL-18 was used to monitor the patient during attempted disease control with biologics over the course of several months. These treatments were unsuccessful and plans were made to prepare the patient for an allogenic hematopoietic stem cell transplant (HSCT). The HSCT was performed in December 2014 (indicated by the red arrow). Subsequently, IL-18 levels approached reference levels and remained there (dashed line).

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High levels can additionally be observed in patients with active hemophagocytic lymphohistiocytosis (HLH), with levels of IL-18 usually returning to normal once HLH is in remission (except in patients with XIAP deficiency or NLRC4 mutations). Notably, the IL-18:CXCL9 ratio may help differentiate patients with sJIA and MAS (Macrophage Activation Syndrome) from patients with familial HLH. (8)

Lastly, IL-18 is also considered to be a secondary diagnostic criterion for autoimmune lymphoproliferative syndrome (ALPS).(9)

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CXCL9

Measurement of CXCL9: What Does It Mean?

Chemokines are types of cytokines that cause various cells to move (chemokinesis), usually towards inflammation. There are numerous chemokines that act in the immune system, with a confusing array of receptors. CXCL9 is a chemokine, previously known as 'monokine induced by interferon gamma' (MIG), which binds the chemokine receptor CXCR3 along with two other chemokines, CXCL10 and CXCL11.[1,2] These 3 chemokines have a number of roles, but their main function is to attract T cells into inflamed tissues. As its prior name suggests, CXCL9 is secreted by monocytes and macrophages in response to another cytokine, interferon gamma (IFN-γ).

The CXCL9 Immunoassay performed in our laboratory is a solid-phase ELISA that employs the quantitative sandwich enzyme immunoassay technique. Specimen requirements are whole blood collected in an EDTA tube that has been spun within 8 hours of collection. Divide all available plasma into 0.5 ml aliquots. Three aliquots of 0.5 ml of plasma is preferred, one is acceptable. Freeze immediately at -20° C or below and ship to our laboratory on dry ice.

CXCL9: How Do We Measure It?

CXCL9 is unique because its release is triggered almost entirely by IFN-γ (IFN-α and TNF-α have a slight effect on it in some contexts).[1,2] Because of this specificity, it is an excellent biomarker for the action of IFN-γ. While IFN-γ can be measured directly in plasma, measurement of CXCL9 in experimental contexts appears to be a much more sensitive measure of IFN-γ activity. One possible explanation of this is the fact that IFN-γ is secreted by lymphocytes in a very directional fashion (a 'private message' for the interacting cell), while CXCL9 is secreted by mononuclear cells in all directions (a 'public broadcast' calling other cells towards inflammation).[3]

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What is known about CXCL9 in disease?

CXCL9 may be helpful when diagnosing inflammatory conditions where IFN-γ is important. Specifically, IFN-γ appears to play a significant role in hemophagocytic lymphohistiocytosis (HLH) and macrophage activation syndrome (MAS). Elevated CXCL9 is seen in experimental models of these diseases.[4 5] In patients, it is reported to be elevated in lymphoma-associated HLH[6] and MAS[7], while our unpublished observations indicate that it is elevated in essentially all patients with HLH. Though these observations suggest that CXCL9 is very sensitive for detecting HLH/ MAS, it is not likely to be entirely specific for these conditions, as IFN-γ is involved in many immune responses.

CXCL9 may also be useful for assessing response to IFN-γ blocking therapies. An investigational agent which blocks IFN-γ (emapalumab) is currently being studied as treatment for HLH and MAS, and JAK inhibitors (blocking IFN-γ signaling) have been suggested as experimental therapy for these disorders. In both cases CXCL9 may be a useful marker of IFN-γ blockade in patients and may prove to be a valuable part of routine therapeutic monitoring.

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The Newsletter is edited by Rebecca Marsh, MD

BULLETIN BOARD

Our laboratory recently completed an 8-week course using hands-on exercises, team-based projects and 1-on-1 coaching to deepen each participant's ability to solve problems and improve operational performance. The course was based on the lean tools & methods used by Toyota and top performing healthcare organizations to identify non-value added work and lead improvements in safety, quality, productivity, lead time, and cost effectiveness. Cincinnati Children's Hospital is committed to empowering our frontline staff to drive change to eliminate non-value added work and improve our customer (you and your patients) satisfaction!

In our project, we tackled our specimen intake process. Receiving over 100 tests a day from our external clients is no small feat! By reviewing this process using our Lean methodology, we were able to reduce our specimen processing time by 37% without affecting quality. This allows us to get back to being available for our customers sooner, expedite initiation of sample testing, and reduce over-processing. We want you to know that we are committed to changing the outcome and improving our services. This was a great introductory project that allowed us to become familiar with these tools that we can then carry forward to other improvements within the laboratory.

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