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Cancer & Blood Diseases Institute

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Wiskott Aldrich Syndrome diagnosis through WAS protein evaluation

Wiskott-Alrich syndrome (WAS) is a rare X-linked recessive immunodeficiency that is traditionally characterized by the triad of thrombocytopenia, eczema, and recurrent infections. Patients are also prone to autoimmunity and malignancies. The *WAS* gene encodes for the conveniently named WAS protein (WASP) and is expressed by hematopoietic cells. WASP has been documented to be important in various processes related to cell signaling and the regulation of actin polymerization.

Although the current gold standard for WASP diagnosis is genetic sequencing of *WAS*, the evaluation of WASP expression level in lymphocytes has been used as a quicker and more efficient way of screening for WAS. However, data has been lacking regarding the clinical diagnostic accuracy of this method. To address this, Dr. Sam Chiang retrospectively extracted and evaluated results from individuals for whom both WASP staining and *WAS* sequencing had been performed by the Diagnostic Immunology Laboratory and Human Genetics Laboratory between June 2010 and October 2016. A total of 49 samples with genetically confirmed *WAS* mutations and 58 samples with no apparent mutation in *WAS* were collected. From these 107 samples, analyses of diagnostic accuracy were performed.

Flow cytometry Method

Fresh whole blood samples from patients and controls were stained and analyzed via flow cytometry. Lymphocytes were first gated by CD45 and side scatter (Figure 1A). The mean channel fluorescence (MCF) intensity of WASP gated from total lymphocytes was then normalized against a control donor run on the same day to obtain a WASP expression ratio. From this cohort, a wide range of staining intensities was observed, ranging from 0.13 to 1.3 (Figure 1B). The use of a ratio readout allows for data comparison between samples assayed over different time periods and different laboratories.

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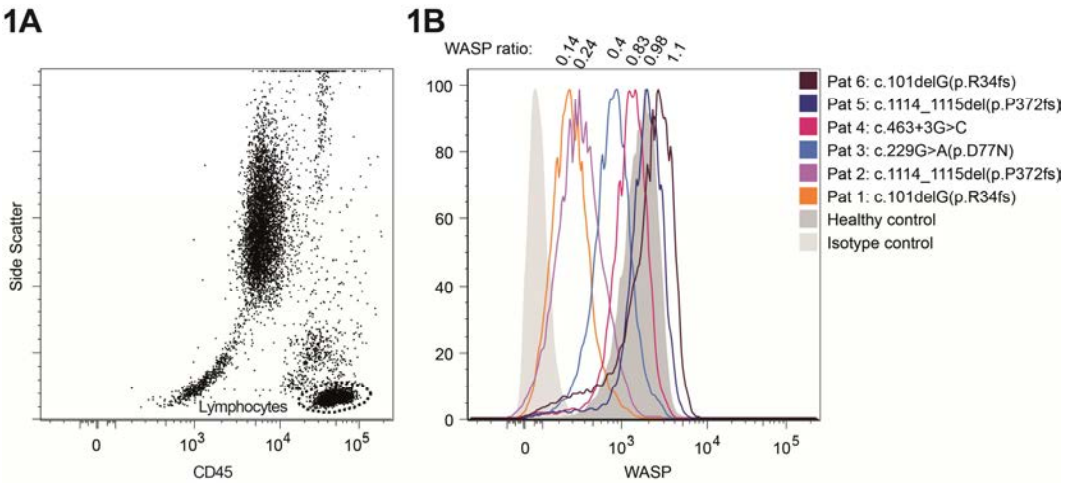
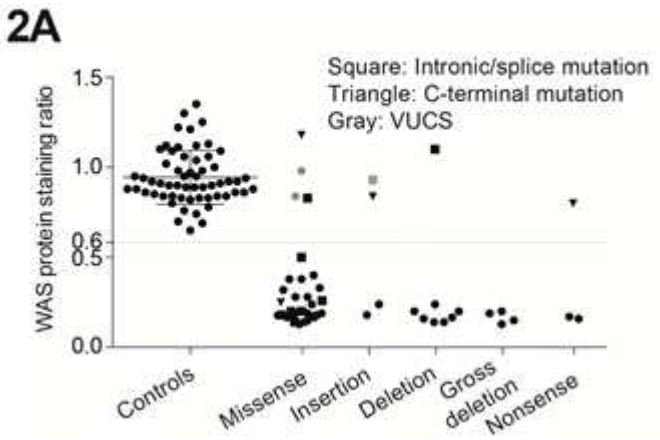


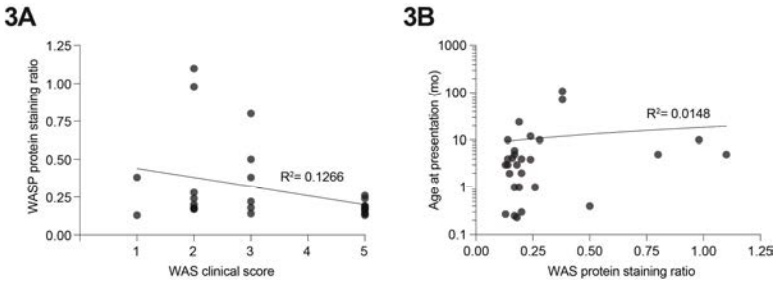
Cohort Evaluation

A total of 49 patients with genetic abnormalities and 58 with normal *WAS* sequence were included for analysis. All 58 patients with normal *WAS* sequence displayed WASP expression at levels comparable to healthy controls (mean staining ratio=0.94, SD=0.15) while 41 of 49 patients with a genetic abnormality were found with reduced staining ratios (mean staining ratio=0.34, SD=0.28). The cohort was further divided into mutation categories of missense, insertion, deletion, gross deletion, and nonsense (Figure 2A). Three individuals were noted to carry variants of unknown clinical significance (VUCS).



Comparison to clinical WAS score

The WAS score for disease severity stratification was compared against WASP stain index. Scores were available for 28 confirmed WAS patients and was found to poorly correlate to the WASP staining ratio (Figure 3A) or age at presentation (Figure 3B).

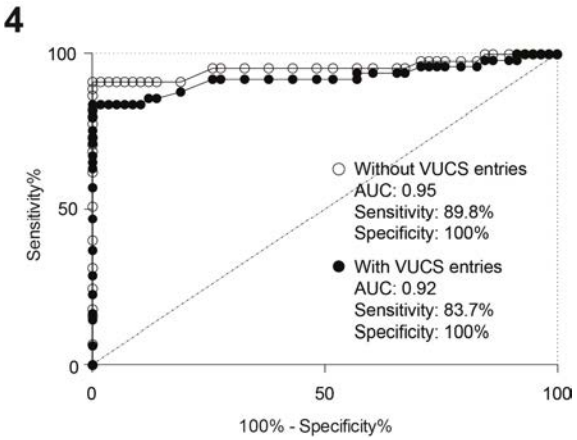


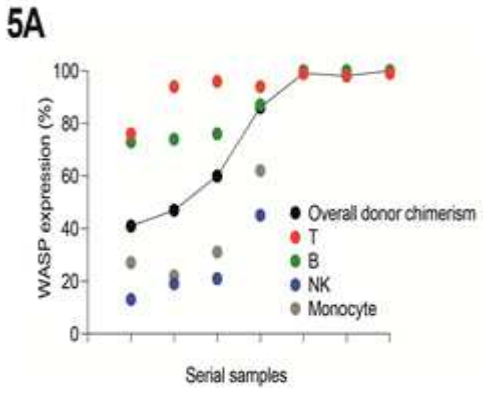
WASP assay accuracy

Staining for WASP could clearly identify the majority of WAS patients as confirmed through the receiver operating characteristic (ROC) curve analysis (Figure 4). A cutoff staining ratio of 0.60 returned a sensitivity and specificity of 82.6% and 100%, respectively. The exclusion of three VUCS entries improved the sensitivity and specificity to 89.1% and 100%, respectively.

Chimerism monitoring

Evaluation of lineage-specific donor chimerism in myeloid and lymphocyte subset populations can also be performed following allogeneic hematopoietic cell transplantation by incorporating surface markers to the assay (available to order as WASP Transplant Monitor on the DIL Requisition Form). An example is shown in Figure 5.





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Conclusion

The WASP staining ratio is a simple flow cytometry based assay with up to 95% accuracy and is useful for the rapid screening diagnosis of WAS. In this cohort, all patients clearly deficient in WASP expression carried *WAS* mutations. However, a portion of patients with mutations, including VUCS, expressed normal WASP levels, indicating that genetic sequencing should also be performed in patients suspected to have WAS.

The Diagnostic Immunology Laboratory accepts daily requests for WASP evaluation with a turnaround time of 2 days. The Diagnostic Immunology Laboratory is CAP accredited. Visit our website or call us for further information. https://www.cincinnatichildrens.org/service/c/cancer-blood/hcp/clinical-laboratories/diagnostic-lab

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S100A8/A9 and S100A12 Assays to Aid in the Management of Autoinflammatory Syndromes

The calcium-binding proteins S100A8 (alias: MRP8), S100A9 (alias: MRP14) and S100A12 (aliases: Calgranulin C, EN-RAGE) are typically secreted during activation of neutrophils and monocytes. S100A8/A9 form a complex (alias: Calprotectin) that can serve as an endogenous TLR agonist and trigger TLR4 signaling pathways [1] leading to production of proinflammatory cytokines including IL1-β. S100A12 can also activate human monocytes via Toll-like receptor 4 [2, 3].

High levels of S100A8/A9 and S100A12 are characteristic of active systemic juvenile idiopathic arthritis (sJIA) and may distinguish sJIA from other febrile illnesses, including systemic infection, various forms of leukemia, and Kawasaki disease [4, 5]. S100A8/A9 serum concentrations correlate closely in response to drug treatment and disease activity and therefore might be an additional measurement for monitoring anti-inflammatory treatment of individual patients with sJIA. During clinically inactive disease, S100A8/A9 serum concentrations are reported to be one of the first predictive biomarkers indicating subclinical disease activity. In sJIA, serum levels of S100A8/A9 above 740 ng/ml predicted disease flares with 92% sensitivity and 88% specificity [6]. Low S100A8/A9 levels indicate that it is relatively unlikely that subclinical disease activity is present at the time the test is performed [7].

Highly elevated S100 proteins are also a feature that may be shared with other autoinflammatory disorders (AIFD). Along with use as biomarkers in autoimmune forms of juvenile idiopathic arthritis, S100 assays are increasingly used in evaluation of patients with AIFD.

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In contrast to autoimmune diseases, characterized by high-titer autoantibodies and self-reactive T cells, AIFD are characterized by dysfunction in the innate immune system. Clinically, patients with AIFD experience seemingly random or unprovoked episodes of systemic inflammation, which can be persistent, recurrent, or sporadic. The best characterized monogenic AIFD are the hereditary periodic fever syndromes such as Familial Mediterranean Fever (FMF) or the Cyropyrin-Associated Periodic Syndromes (CAPS) [8].

S100 alarmin proteins, produced largely by innate immune cells such as monocytes, macrophages, and neutrophils, are useful biomarkers for AIFD diagnosis and disease activity. The systemic subtype of JIA (sJIA) is considered to be largely an AIFD. In our sJIA cohort we have found that S100A8/A9 levels are significantly higher in sJIA patients with active systemic disease than those without active systemic features (inactive). See Figure 1.

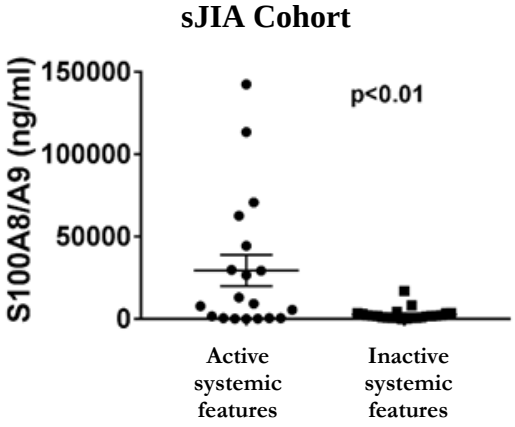

Figure 1. A CCHMC sJIA cohort showing levels of S100 A8/A9 in patients with active systemic features vesus patients with inactive systemic features.

S100 proteins can also be helpful in evaluation of patients with suspected AIFD. Patient A presented as a teenager with intermittent episodes of low-grade fever, rash, arthralgia, chest and abdominal pain. The patient had been managed with NSAIDs, steroids, and colchicine, but continued to have episodes. They were admitted to CCHMC during a severe episode with features of macrophage activation syndrome. At that time the patient was found to have elevated S100A8/A9, and genetic testing suggested AIFD Nod2-Associated Autoinflammatory Disorder (NAID). The patient was started on IL-1 blockade with canakinumab, had complete resolution of the inflammatory episode, and had S100A8/A9 levels that returned to normal. See Figure 2.

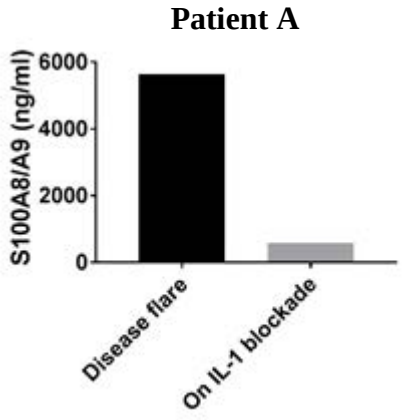

Figure 2. Patient A showing S100A8/A9 levels at the time of disease flare and after 7 weeks of treatment with canakinumab, an Interleukin-1β blocker.

The S100A8/A9 and S100A12 Immunoassays performed in our laboratory are both a solid-phase ELISA that employs the quantitative sandwich enzyme immunoassay technique. Specimen requirements are whole blood collected in an SST tube that has been spun within 4 hours of collection. Two aliquots of 0.3 ml of serum are preferred **for each assay**. Freeze immediately at -20° C or below and ship to our laboratory on dry ice.

The Diagnostic Immunology Laboratory accepts daily requests for S100A12 or S100A8/A9. Visit our website or call us for further information.
https://www.cincinnatichildrens.org/service/c/cancer-blood/hcp/clinical-laboratories/diagnostic-lab

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Content

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Spring Meeting

Please stop by and say "hello" in May at the American Society of Pediatric Hematology/Oncology (ASPHO) meeting in Pittsburg this year. We look forward to seeing you in person and meeting new faces, as well! We will be there as the Cincinnati Children's Clinical Laboratories.

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