Blood test for asthma

By Lauren Martz, Staff Writer

Asthma is notoriously difficult to accurately diagnose because its symptoms can be transient and common tests are susceptible to user error. A **University of Wisconsin–Madison** team believes that it can improve the process by using a microfluidic device that analyzes the mobility of inflammatory cells contained in a single drop of blood.¹

Salus Discovery LLC has exclusively licensed the technology and is seeking development partners.

Asthma is diagnosed using a combination of qualitative and quantitative assessments. Qualitative measures typically include surveys of patient symptoms and medical history, whereas quantitative measures include spirometry, which can measure lung function and airway constriction, and the nitric oxide test, which relies on elevated nitric oxide in the breath as a marker of airway inflammation.

Because quantitative tests require the presence of symptoms at the time of the test, they can sometimes report false-negative results. In addition, compliance for airway tests can be difficult in young children.

Shawn Aaron, a professor in the Department of Medicine at the **University of Ottawa**, told *SciBX* that current asthma diagnostic methods cause both under- and overdiagnosis. About 30% of treated patients do not actually have asthma, and the diagnostic criteria also miss a significant proportion of actual patients.

There are other well-known indicators of asthma, but they are difficult to measure noninvasively. These include an increase in numbers of immune cells, such as eosinophils and neutrophils, in bronchoalveolar lavage samples.^{2,3}

Researchers have also shown that inflammatory cells from patients with asthma can behave differently than cells isolated from healthy individuals.

David Beebe and colleagues at the University of Wisconsin–Madison hypothesized that in addition to increases in the levels of inflammatory cells in the lungs of patients with asthma, the ability of these cells to migrate may also be affected. Thus, the team sought to adapt a microfluidic assay that it had previously developed to study neutrophil function for use with samples from patients with asthma.⁴

Beebe is associate chair of research and faculty development and a professor in the Department of Biomedical Engineering at the University of Wisconsin–Madison.

The kit-on-a-lid-assays (KOALA) platform consists of self-contained

microfluidic chambers containing prepared reagents and microchannels that can be used to measure cell functions such as migration and chemotaxis.⁵

To adapt the device, they designed chambers with P selectin (SELP; CD62P)-coated polystyrene, which can bind neutrophils and allows the cells to be purified from whole-blood samples with a simple wash step. Once the neutrophils are purified, a hydrogel chemoattractant lid is applied to the chamber, and chemotaxis of the cells toward the lid is measured with tracking software.

The researchers first tested whether the device can help discriminate between asthma and nonasthmatic allergic rhinitis, two conditions with similar clinical presentation. In blood samples from 23 mildly asthmatic patients, neutrophil movement velocity toward chemoattractants including IL-8 (CXCL8) and formyl-methionyl-leucine-phenylalanine was slower than that in samples from 11 nonasthmatics with allergic rhinitis. Importantly, most of the patients with asthma were not symptomatic at the time of the test, suggesting that the test may help overcome the issue of false negatives caused by transient symptoms.

Using a cutoff velocity of 1.545 $\mu\text{m}/\text{min}$, the microfluidic device accurately identified 22 of 23 asthmatic subjects and 8 of 11 nonasthmatic controls, for a sensitivity of 96% and specificity of 73%.

The team also compared the accuracy of its test to the published accuracy of existing diagnostics, including the nitric oxide test. The researchers found that the new method had the best sensitivity of any test but was usually less specific.

Data were published in the *Proceedings of the National Academy of Sciences*.

Diagnostic applications

Although the University of Wisconsin–Madison team found that the device can discriminate between patients with asthma and allergic rhinitis, a key next step for the team is to determine whether the device can also differentiate between asthma and other inflammatory diseases, such as chronic obstructive pulmonary disease (COPD).

“The next steps are to test broader patient populations, including some with inflammatory disorders that may affect neutrophil function, and see how the results hold up,” Beebe told *SciBX*.

He added that compared with existing diagnostic tests, the microfluidic test has the potential to be more accurate in these populations because it directly measures the function of inflammatory cells involved in the pathology. Other indicators of asthma measure indirect effects of the inflammation.

The team is in discussions with potential industry and academic collaborators to gain access to the clinical samples required for the testing and expand into indications beyond asthma.

Prajak Barde, associate director of clinical research at **Rhizen Pharmaceuticals S.A.**, said that testing the device in patients with COPD would be particularly valuable in determining its diagnostic utility.

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—Prajak Barde,
Rhizen Pharmaceuticals S.A.

Aaron agreed. “Clinically, it is very important to be able to differentiate between asthma and COPD because the diseases present similarly but are treated very differently,” he said. “Asthma is currently discriminated from COPD based on an evaluation of the clinical history of the patient, because a long history of smoking is often associated with COPD, and based on how they respond to bronchodilators. Problems with lung function are often reversible with bronchodilators and/or inhaled steroids in asthma patients, while patients with COPD continue to exhibit chronic airflow obstruction that does not resolve despite intensive treatments.”

Rhizen has asthma therapeutics in preclinical testing including the dual phosphoinositide 3-kinase- δ (PI3K δ) and PI3K γ inhibitor RP6503 and the calcium release-activated calcium channel (CRAC) inhibitor RP3128.

Barde noted that the lack of easy and reliable confirmatory quantitative tests can lead to overdiagnosis of asthma. “Recent studies indicate that many of the diagnoses of asthma in the primary care setting rely solely on clinical evaluation or response to treatment and may lead to misdiagnosis,” he said. “Inevitably, any misdiagnosed cases lead to overtreatment or inappropriate treatments and increased risk of side effects in the absence of any pharmacological benefit.”

He added that a potential disadvantage of the test is its low specificity but said it will be important to determine whether the specificity can be improved in studies in larger populations.

Aaron also wanted to see the test used in more patients. He added that a specificity of 73% would lead to a lot of false positives and therefore would not resolve the issue of unnecessary treatments for nonasthmatics, but he was optimistic the specificity could be improved.

“This test was only used in a limited number of patients, and the researchers seem to have chosen a relatively arbitrary cutoff point based on the velocity that gave the best sensitivity and specificity in the data that they have. The researchers need to conduct a larger validation study, which could lead to a new cutoff point that provides improved specificity.”

Salus discovery

Although the researchers are moving forward with the asthma diagnostic indication, Salus’ initial plans for commercial development are in the

R&D tool space, in which products can be brought to market quickly.

Beebe, who is also the founder of Salus, said that the company has a suite of technologies for next-generation sample preparation and cell-based assays. “These technologies simplify bioassays by making them smaller, cheaper and better.”

He added that Salus’ initial focus is on developing products for sample preparation for researchers based on a separate technology platform, and the first product is scheduled for launch by the end of this year.

“The initial products and markets for the KOALA technology, upon which the asthma assay was built, will likely be in the R&D market, not the diagnostic, where KOALA can greatly simplify cell-based assays. We are actively seeking commercial partners for this application of KOALA now,” Beebe said. He added that validating the asthma diagnostic will require more patient studies as a first step.

The **Wisconsin Alumni Research Foundation**, which handles IP for the University of Wisconsin–Madison, has filed several patent applications covering the technology. The IP is exclusively licensed to Salus, and the company is seeking partnerships to develop bioassays including the asthma diagnostic assay.

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