Study finds promising biomarker for cellular rejection after organ transplant

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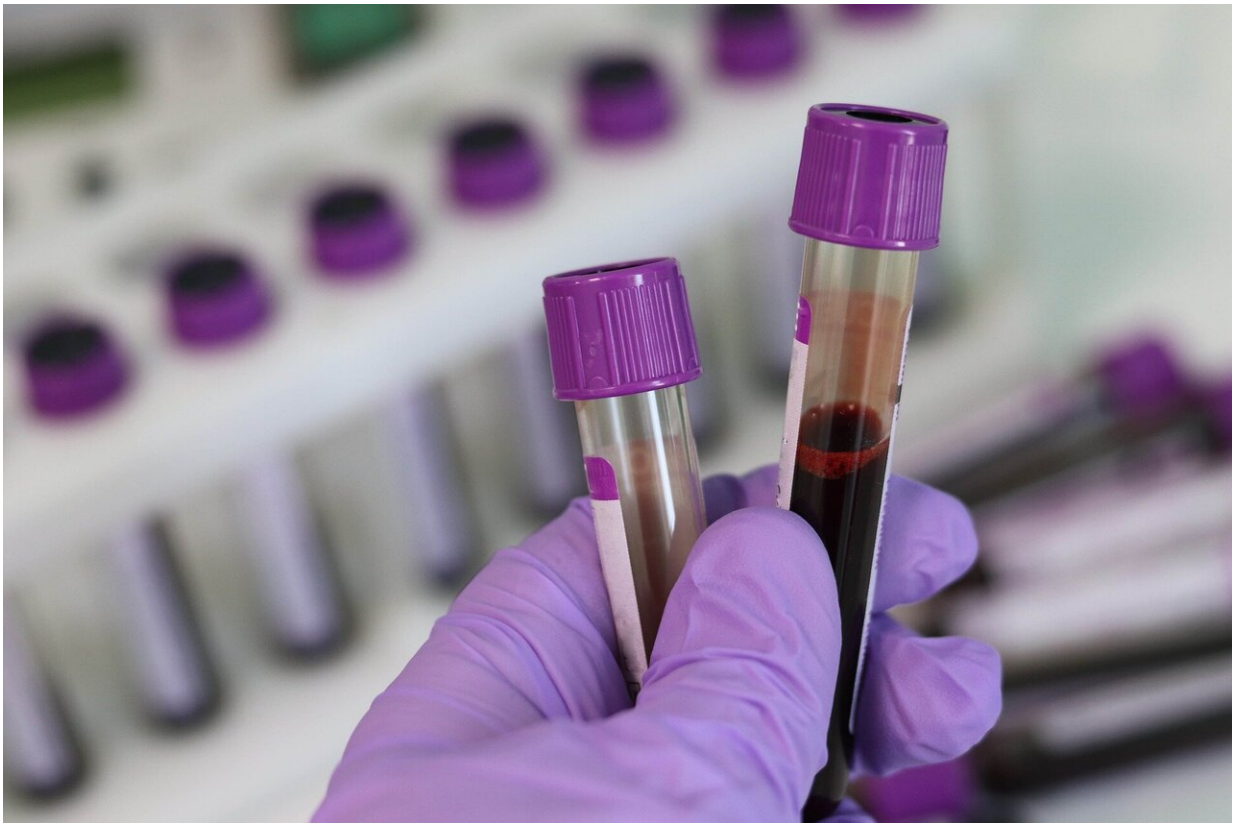

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Today, patients who receive an organ transplant need repeated surgical biopsies to test for acute cellular rejection (ACR) throughout their lifetimes. But a blood test for ACR could be on the horizon following

the discovery of a promising biomarker.

ACR occurs when a patient's immune cells, known as T cells, begin attacking the transplanted organ. However, when looking at T cells in blood samples, researchers have not been able to identify any notable differences that arise during organ rejection.

Now, a Yale team has found that exosomes from T cells are significantly altered during ACR. Exosomes are extracellular vesicles released by cells that allow them to communicate with each other. The researchers published their findings in the March 2024 issue of the *American Journal of Transplantation*.

"We've developed a novel biomarker platform that shows promise to reliably detect rejection through a blood sample," says Prashanth Vallabhajosyula, MD, MS, associate professor of surgery (cardiac) and the study's principal investigator. "As we continue to do more studies in the clinical setting, we hope this biomarker platform will eventually replace surgical biopsy."

Organ rejection is only detectable through invasive biopsies

To protect our body from pathogens, our immune system is able to distinguish self from non-self. As a result, transplanted organs are under constant risk of rejection by the recipient's immune system. This process is mediated by two types of immune cells—T cells and B cells. T cells are responsible for the most common kind of rejection, which clinicians call acute cellular rejection, or ACR.

To prevent this, recipients of an organ transplant must remain on immunosuppressive medications for the rest of their lives. However,

even when these patients are on immunosuppressants, breakthrough cases of rejection can still occur. As a result, organ transplant patients need to be under long-term surveillance by their doctors.

Surgical biopsies allow clinicians to physically examine tissues for signs of ACR. These examinations are essential for guiding appropriate patient care. If the biopsy detects rejection, clinicians will immediately escalate the patient's immunosuppression therapy. Then, the patient will need subsequent biopsies to ensure that the increased treatment is working.

As a cardiac surgeon, Vallabhajosyula has seen firsthand that, while these biopsies are a crucial part of patients' care, they can also significantly hinder quality of life. Biopsies can be associated with serious complications, Vallabhajosyula says. Biopsies of the heart, for example, can lead to complications such as arrythmias, cardiac tamponade, and heart valve injury.

"In the case of the heart, if rejection happens it can cause damage to the heart and affect its function. In some cases this could be a matter of life or death," he says. "So for the past 60 plus years that we've been doing heart transplants, there has been a huge push in the cardiac community to develop a blood test to replace biopsies."

T cell exosomes are a potential biomarker for ACR

In his lab, Vallabhajosyula's team studies exosomes, which carry and transport materials as cargoes, including proteins and RNAs that play functional roles in communications between cells. "Exosomes are ubiquitous—they're everywhere in all biological fluids," he says. "We believe cells communicate with one another through these little packets of information."

Parent cells can modify the content carried by their exosomes. While ACR doesn't alter T cells themselves in the blood, Vallabhajosyula's team wondered if circulating T cell exosomes and their cargoes would be altered with ACR. "Exosomes have been extensively studied in the context of cancer diagnostics, but very little has been done in the field of transplantation diagnostics," he says.

However, there is a significant challenge to studying T cell exosomes: A sample of blood contains exosomes from every cell type in the body. "There's too much noise in the blood," Vallabhajosyula explains. To overcome this, his lab has developed a way to enrich T cell exosomes in biological samples to give his team an unparalleled look into how their cargo changes during ACR.

ACR significantly alters T cell exosome cargoes

In their latest study, the researchers took blood samples from mouse models of heart transplantation and isolated all the exosomes. Then, they used antibody-conjugated bead technology—in which antibodies attached to magnetic beads bind to target molecules—to isolate T cell exosomes. This allowed them to quantify this specific exosome population and study the cargo inside it. They used a technique called quantitative reverse transcription polymerase chain reaction (RT-qPCR) to identify RNAs and the western blot technique for proteins.

The team discovered dramatic differences in the content of T cell exosomes taken from mouse models undergoing ACR.

Intrigued, the researchers repeated their methodology in heart transplant patients. They obtained both blood samples and cardiac biopsies. Once again, in patients whose biopsies indicated ACR, they found that the T cell exosomes obtained from the blood were also significantly altered. "We believe that for the first time we have a platform that is sensitive to

detect T cell-mediated rejection in organ transplantation," says Vallabhajosyula.

The study also shed light on the underlying pathophysiology of ACR. When T cells reject a transplanted organ, they travel to the site of the organ and kill it cell-by-cell through a process called apoptosis. The researchers discovered that the exosomes act similarly.

"We showed that exosomes released by T cells can also mediate injury to the transplanted tissue," says Vallabhajosyula. "This leads us to believe that T cell exosomes are also playing an important part in the rejection process itself."

Future studies could lead to a life-changing diagnostic blood test

The team is now conducting further studies on a larger cohort of heart transplant recipients to better understand how reliable their newly identified biomarker is compared with biopsies. They are also translating these findings to lung transplant patients, where lung biopsy has remained the gold standard for rejection monitoring.

Vallabhajosyula is hopeful that this work will help pave the way to replacing surgical biopsies with a blood test capable of detecting ACR. This would not only improve patients' quality of life but also could potentially save even more lives.

Furthermore, clinicians don't have a way to titrate (calibrate) immunosuppression drugs for their patients. "Everybody gets the same high dose," Vallabhajosyula says. However, this therapy comes with its own risks—patients with suppressed immune systems are more prone to infections and cancer development. A reliable blood test could allow

clinicians to titrate immunosuppression on a patient-by-patient basis to minimize the risks of immunosuppressive therapy.

This vision could become a reality within the next decade, Vallabhajosyula says. "I'm imagining a future in which a heart transplant patient could go once a month to a local [diagnostics] lab," he says. "They don't have to come to a cath lab and get a biopsy of their transplanted heart. They just go to a local lab, give a blood sample, and go home, and clinicians would receive molecular information about the overall immune health of the transplanted heart."

More information: Laxminarayana Korutla et al, Circulating T cell specific extracellular vesicle profiles in cardiac allograft acute cellular rejection, *American Journal of Transplantation* (2023). DOI: 10.1016/j.ajt.2023.10.021

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