LEONARDO MONTECILLO/AGENCIA PRESS SOUTH/GETTY

A health worker administers the Oxford–AstraZeneca COVID-19 vaccine in Mexico City. Some countries have restricted its use.

COVID VACCINES AND BLOOD CLOTS: WHAT RESEARCHERS KNOW SO FAR

Scientists are trying to understand why a small number of people develop a mysterious clotting disorder after receiving a COVID jab. **By Heidi Ledford**

It was when the second person with unusual clots came in that Phillip Nicolson knew something was wrong. Blood clots are uncommon in young people, and it's even rarer to see a combination of blood clots and alarmingly low levels of platelets — cell fragments that help to form clots.

Yet in the space of one week in March, two young people with this pairing of symptoms had arrived at the Queen Elizabeth Hospital in Birmingham, UK, where Nicolson works as a haematology specialist. And both had recently been given the Oxford–AstraZeneca COVID-19 vaccine.

Nicolson had been on call at the hospital all weekend, and had been looking forward to a rest on Monday. Instead, he found himself rushing around to get consent to collect samples to study in the laboratory. By the time he arrived at the second patient's bedside, a third had been admitted.

That week, Nicolson was among the first to witness what researchers now call vaccine-induced immune thrombotic thrombocytopenia (VITT), a life-threatening and mysterious condition that affects a very small number of people who have received the Oxford–AstraZeneca or Johnson & Johnson (J&J) COVID-19 vaccines. It is now estimated that VITT occurred in about 1 in 50,000 people aged under 50 who received the Oxford–AstraZeneca vaccine[1]. This and similar observations in other countries have led some officials to delay and then scale back the deployment of these vaccines.

Despite fervent work by researchers such as Nicolson, the mechanism that links the vaccines and VITT is still uncertain. Establishing a mechanism could reveal ways to prevent and treat the condition, and improve the design of future vaccines. Over the past few months, researchers have gathered clues and developed a host of hypotheses.

Working through these possibilities is a daunting task. "You can have your hypothesis, but how do you find which is the one that caused an event in maybe 1 in 100,000 people?" asks John Kelton, a haematologist at McMaster University in Hamilton, Canada. "It's really, really hard."

Clotting concepts

The unusual constellation of symptoms was immediately familiar to some haematologists, particularly those with experience of treating people with a rare reaction to the anti-clotting drug heparin. That syndrome, called HIT, is also characterized by low platelet counts and sometimes the presence of clots.

HIT is caused by heparin, a negatively charged molecule, binding to a positively charged protein called platelet factor 4 (PF4) that is produced by platelets to promote clotting. In some people, the immune system views this complex as foreign, and develops antibodies against it.

These antibodies can also bind to and activate platelets, priming them to clump together and trigger clotting. The clots can clog up important blood vessels, and the condition can be fatal, although some treatments improve the chances of survival.

Only a handful of labs around the world study HIT, and those that do scrambled to get samples from the few people who had been diagnosed with VITT. When researchers analysed the samples, it was clear that vaccine recipients who had this mysterious clotting reaction were also producing antibodies against their own PF4 (refs 2–4). But it was anyone's guess as to what had triggered these antibodies. Kelton, who has been studying HIT for decades, had to wait to get precious specimens from people with VITT, and then his team had to wade through samples of varying quality. Some were contaminated by treatments the people with VITT had received. "Many, many samples were not what I would call pristine," he says. "These people are as sick as can be, and the physicians throw the book at them. They have every kind of chemical in them." And about two-thirds of the samples his team received lacked the PF4 antibodies altogether, suggesting that the patients did not have VITT, but instead had developed a clotting disorder that was probably unrelated to their vaccination, Kelton says.

Eventually, his team was able to get five samples taken from people before treatment for VITT. When researchers characterized antibodies in the samples, they found that some were binding to PF4 at the same site as the one used by heparin, and that they were also capable of activating platelets[5]. The results suggested that the mechanism behind the vaccine-linked syndrome was similar to that of HIT — but the trigger seemed to be the vaccine rather than heparin.

Something in the vaccine or the body's response to it must be binding to PF4 — but what? VITT has been linked to two COVID-19 vaccines, both of which use disabled adenoviruses as a 'vector' to shuttle a gene encoding a coronavirus protein, called spike, into human cells. Once there, the gene is expressed and the protein is made. The immune system detects spike and generates antibodies against it that are crucial for protection against coronavirus infection.

"These people are as sick as can be, and the physicians throw the book at them."

Some researchers have proposed that impurities in the vaccines left over from the manufacturing process — such as snippets of DNA floating around in the solution, or proteins in the broth used to grow the virus — are interacting with PF4 to generate the clumps that are then targeted by antibodies[6].

Others think the culprit could be the adenovirus itself. Previous work has shown that adenoviruses can bind to platelets and trigger their depletion in mice[7]. It's conceivable that those mice might also have developed clots if they had been followed for longer, says Maha Othman, who studies blood clotting at Queen's University in Kingston, Canada, and was lead author of the study.

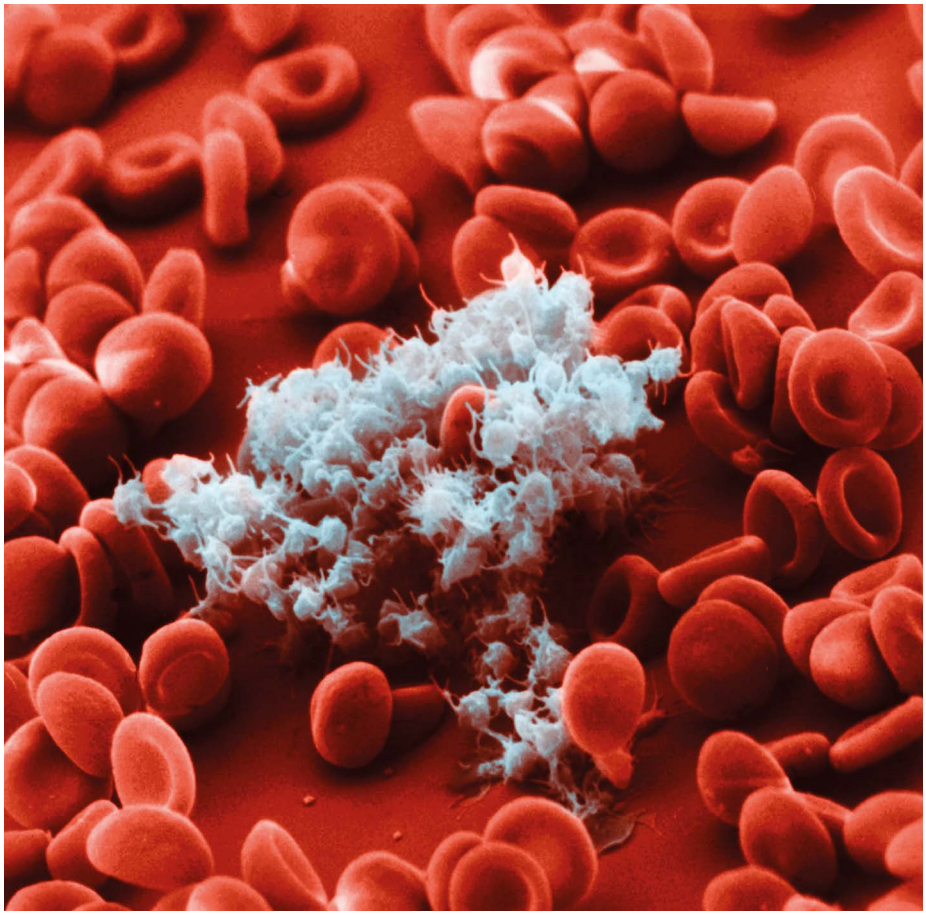
Before the COVID-19 pandemic, adenovirus-based vaccines were being developed against infections such as HIV and Ebola, but had not yet been used in large populations. There have been no reports that these vaccines produced a VITT-like condition; however, they were not tested in nearly as many people as have received the Oxford–AstraZeneca COVID-19 vaccine.

Haematologist Mitesh Borad at the Mayo Clinic in Phoenix, Arizona, and his colleagues have analysed the structure of the chimpanzee adenovirus used in the Oxford–AstraZeneca vaccine and determined that it has a strong negative charge. Molecular simulations suggest that this charge, combined with aspects of the virus's shape, could allow it to bind to the positively charged PF4 protein[8]. If so, it could then set off a cascade much like the rare reaction to heparin, says Borad, although it remains to be seen whether this happens.

Even if the adenovirus is to blame, Borad says he would not advocate that vaccine developers stop using adenoviruses in vaccines. Some adenoviruses could be engineered to reduce their negative charge, he says, and some are less negatively charged than others; the Ad26 adenovirus used in the J&J COVID-19 vaccine does not have as much of a charge as the chimpanzee virus, which might explain why VITT seems to be less common in recipients of the J&J vaccine. And so far, no link to VITT has been reported for the Sputnik V COVID-19 vaccine, which uses both Ad26 and another adenovirus called Ad5 that has still less negative charge, he adds.

Then there's the spike protein itself. One team of researchers wondered whether the antibodies that bind to PF4 in people with VITT are an unintended by-product of the body's immune response to spike. But they found that the PF4 antibodies can't bind to it, suggesting that they are not part of the immune response to the viral protein[9].

But cancer researcher Rolf Marschalek at Goethe University Frankfurt in Germany and his colleagues have shown that the snippets of RNA that encode spike can be cut apart and stitched back together in different ways in human cells; some of these forms, called splice variants, can generate spike proteins that get into the blood and then bind to the surface of cells that line blood vessels[10]. There, they cause an inflammatory response that is



LENNART NILSSON, BOEHRINGER INGELHEIM INTERNATIONAL

Platelets (white) are fragments of cells that encourage clots to form.

also seen in some SARS-CoV-2 infections, which in severely affected people can lead to the formation of clots.

And the lower rate of clots in J&J's vaccine compared with Oxford–AstraZeneca's could be because the version of spike generated by the J&J vaccine was engineered to remove the sites that allow the RNA to be processed into splice variants, says Marschalek. He thinks that if this idea is borne out, then the Oxford–AstraZeneca vaccine and other adenovirus-based vaccines could be rendered safer if their versions of spike were similarly engineered.

There are reports that the teams behind the Oxford–AstraZeneca and J&J vaccines are working to develop safer adenoviral vectors, and Marschalek says he would be surprised if companies abandoned adenoviral vectors altogether. Others agree. "I think they are very popular and will remain popular," says Othman, citing the ease with which the vaccines can be produced and manipulated, and the wealth of data suggesting that, for most people, the vaccines are safe. Instead of abandoning them, she says, "we should study more about the immune responses to them."

One possible factor affecting the safety of adenoviral vaccines is how they are administered. The COVID-19 vaccines are given as injections into muscle, but if the needle happens to puncture a vein, the vaccine could enter the bloodstream directly. Leo Nicolai, a cardiologist at Ludwig Maximilian University of Munich, Germany, and his colleagues found in a mouse study that platelets clump together with adenovirus and become activated when the Oxford–AstraZeneca vaccine is injected into blood vessels, but not when it is injected into muscle[11].

It's possible, says Nicolai, that on rare occasions, a vaccine is inadvertently injected into a vein — as was done in the earlier mouse studies that found that adenovirus could bind to platelets. If so, many cases of VITT might be avoided by asking vaccinators to first draw a small amount of fluid from the injection site with the syringe to check for blood before they actually push the plunger to administer the vaccine. This is already standard practice in some countries, and Denmark has added it to its official guidelines for COVID-19 vaccine administration.

Improving treatments

Better treatments are still needed for VITT, which according to a UK study[1] killed 49 of the 220 people who were diagnosed with the condition between March and June 2021. Currently, doctors treat VITT by giving anti-clotting treatments other than heparin, and administering high doses of naturally occurring antibodies from blood-plasma donors. The antibodies compete with the anti-PF4 antibodies for binding sites on platelets, and reduce the latter's ability to promote blood coagulation. "The hope is to try to confuse the body and hide the dangerous antibodies within a huge fog of normal antibodies," says Kelton. "That's a very, very blunt tool."

In Birmingham, Nicolson has been working to develop more-specific approaches. He has tested blood serum from people with VITT to see whether he can repurpose drugs developed for other conditions to treat it. In particular, he is focusing on treatments that interfere with a protein on platelets, to see whether any drugs can prevent platelet activation and the cascade of events that leads to clots in VITT.

But even if he were ready to launch a clinical trial of these therapies, there are few people in whom to test them. Since he saw the first cases in March, the United Kingdom has changed its vaccination policy, and now recommends the Oxford–AstraZeneca vaccine only for people over 40. VITT is more frequent in younger vaccine recipients, possibly because of their more-robust immune responses.

It is unclear whether other countries will have the same luxury of restricting Oxford–AstraZeneca vaccines to older people, given that it is relatively cheap and widely available compared with the mRNA vaccines, for example. Until now, VITT has primarily been reported in Europe and the United States, but researchers don't yet know whether this reflects regional differences in susceptibility to VITT, or differences in reporting systems that gather data on potential vaccine side effects. In Thailand, for instance, researchers reported in July that there had been no cases of VITT after 1.7 million doses of the Oxford–AstraZeneca vaccine were given[12].

Nicolson says the number of people referred to his hospital with VITT has declined drastically: "We're not seeing it any more, it's almost stopped happening."

Heidi Ledford is a senior reporter for *Nature* in London, UK.

1. Pavord, S. *et al. N. Engl. J. Med.* https://doi.org/10.1056/NEJMoa2109908 (2021).
2. Schultz, N. H. *et al. N. Engl. J. Med* **384**, 2124–2130 (2021).
3. Greinacher, A. *et al. New Engl. J. Med.* **384**, 2092–2101 (2021).
4. Scully, M. *et al. N. Engl. J. Med.* **384**, 2202–2211 (2021).
5. Huynh, A., Kelton, J. G., Arnold, D. M., Daka, M. & Nazy, I. *Nature* https://doi.org/10.1038/s41586-021-03744-4 (2021).
6. Greinacher, A. *et al.* Preprint at Research Square https://doi.org/10.21203/rs.3.rs-440461/v1 (2021).
7. Othman, M., Labelle, A., Mazzetti, I., Elbatarny, H. S. & Lillicrap, D. *Blood* **109**, 2832–2839 (2007).
8. Baker, A. T. *et al.* Preprint at bioRxiv https://doi.org/10.1101/2021.05.19.444882 (2021).
9. Greinacher, A. *et al. Blood* https://doi.org/10.1182/blood.2021012938 (2021).
10. Kowarz, E. *et al.* Preprint at Research Square https://doi.org/10.21203/rs.3.rs-558954/v1 (2021).
11. Nicolai, L. *et al.* Preprint at bioRxiv https://doi.org/10.1101/2021.06.29.450356 (2021).
12. Noikongdee, P. *et al.* Preprint at medRxiv https://doi.org/10.1101/2021.07.15.21260622 (2021).