PAUL BLOW

DIAGNOSTICS

A flow of information

Many non-invasive approaches to detecting bladder cancer are showing promise — including smart toilets.

BY CHELSEA WALD

Your alarm goes off. You roll out of bed and pad to the bathroom in your bare feet. In your morning fug, you are not aware of the date, but your toilet knows. It is the first of the month — time for the test to make sure your bladder cancer has not come back. You sit down and release a stream onto a sensor below you.

Within a few minutes, the toilet has analysed your urine and sent the results to your doctor. It also runs a battery of other tests for infections and chronic diseases, just in case. You do not give it a thought. If there is a problem, the doctor will call. You stand up, flush and head off to start your day.

This may be science fiction, but it is closer to reality than you might think. The lab of Sanjiv 'Sam' Gambhir, director of the Canary Center at Stanford for Cancer Early Detection in California, is developing a prototype of a smart toilet that continually monitors users' health. The first model uses an off-the-shelf test strip that measures ten factors related to common problems such as diabetes and kidney stones. The team is hoping to cover a wider range of conditions by working with more advanced tools, including, Gambhir says, "a nanosensor that can look for RNA or proteins in urine or stool" — molecules that may be present only in tiny amounts but that indicate disease.

The Japanese toilet manufacturer Toto has already brought its own urine-testing toilet to medical institutions in Japan. Its Flowsky model measures changes in the rate of flow and the volume of urine, which can help to diagnose certain problems with the prostate, bladder and sphincter, and monitor the hydration of people in hospitals. Elite athletes might soon benefit from smart toilets too: Russian aircraft company Sukhoi has designed a jet aircraft for sports teams that includes a flow-tracking toilet.

Bladder cancer is an ideal target for all this technology. The tumours are soaked in urine, which the body then expels, so the urine will almost certainly be carrying evidence of the tumours, such as sloughed-off cells, proteins, DNA and RNA. These clues might reveal not just the presence of a tumour, but how advanced it is and how aggressively it is growing. If tests could reliably detect these biomarkers, it would be much cheaper, easier and less painful to screen for possible bladder cancer in healthy people, perform diagnostic testing in suspected cases, and monitor the progression of confirmed disease.

First, however, researchers need to know what to look for in these tests. Urine can contain a bewildering array of potential biomarkers, and researchers are finding more each year. The difficult part is pinning down the best ones and creating tests suitable for use by doctors — something that several teams worldwide are working on. "There is almost definitely more than one set of biomarkers that will work," says Douglas Ward, a biomarker researcher at the University of Birmingham, UK. "Which ones will win out, we don't really know."

GOLD STANDARD

The need for urine tests is acute on several counts. There are currently no general screening tests for bladder cancer, so most cases are found only after people report seeing blood in their urine (haematuria) — about 10% of people with this symptom have bladder cancer. Sometimes cancer is detected by the presence of microscopic amounts of blood in a urine

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Urine samples can potentially be analysed to detect signs of bladder cancer.

sample taken routinely or to test for another disorder, such as a urinary-tract infection or diabetes. But this is an even less useful indicator: fewer than 5% of people with a trace of blood in the urine have bladder cancer. Other signs can also alert doctors, including an increased urge to urinate, increased frequency of urination, or painful urination, but these are not definitive either because they can be caused by a range of conditions.

To follow up on these symptoms, urologists use a cystoscopy to look for bladder cancer, says Thorsten Ecke, a urologist at the HELIOS Hospital in Bad Saarow, Germany. A thin tube containing a camera is inserted into the urethra, and anything out of the ordinary is described and sampled, Ecke says. Cystoscopies are the gold standard, but they are uncomfortable and expensive — and they sometimes fail to find types of tumour that are aggressive but have not yet become invasive. These include carcinoma *in situ*, which is considered more likely than many other tumours to lead to invasive bladder cancer (see 'Scope it out').

Unfortunately, missing these non-invasive tumours can cost lives. Most bladder cancers start in the inner lining of the bladder (the urothelium) and then grow outwards through the layers of the bladder wall. If a tumour is caught and treated before it has invaded the muscle that controls the bladder, the five-year survival rates are good (see page S34). Once a tumour has grown into the muscle, however, it becomes harder to treat, and the five-year survival rate drops dramatically. Some types of bladder cancer are slow-growing (low-grade) and may never invade the muscle, but other types are more aggressive (high-grade), and catching them before they invade is crucial.

"The idea is that we can avoid cystoscopies to avoid the painful examination."

But even if tumours have been successfully identified and removed before they become invasive, bladder cancer has a high rate of recurrence and progression. People with the disease who retain their bladders may need a cystoscopy every few months for several years. This is uncomfortable and expensive, and brings a greater risk of complications, including bleeding, infection, and perforation of the bladder wall. Non-invasive bladder cancer is "long-term and recurrent", says molecular biologist Margaret Knowles of the University of Leeds, UK. "It costs more than any other adult cancer to manage because of that."

Most doctors and people with bladder cancer would love to replace cystoscopies with urine tests, for both the initial diagnosis and long-term surveillance. But it is a tall order: tests would ideally catch all types and stages of tumour, and they all shed their own characteristic biomarkers. The benefit to patients would be enormous, Ecke says. "The idea is that we can avoid cystoscopies to avoid the painful examination."

URINE TROUBLE

Medics have been studying urine for evidence of bladder cancer for decades, and doctors regularly supplement cystoscopy with cytology — a visual inspection of the urine for abnormal cells. But the procedure has its limitations. "Cytology will identify nasty, aggressive tumours or carcinoma *in situ* where the cells look very different" from normal cells, says Knowles. However, she says, "for the low-grade, non-invasive tumours where the individual cells look normal, cytology's been pretty useless really."

But there may be smaller clues. For the past 20 years, researchers have been sifting through the various molecules present in urine. In some cases they have created biomarker tests to assist diagnosis or monitoring that have been approved by the US Food and Drug Administration (FDA). Some of these tests, for example, measure the number of fragments of cytokeratins — proteins that help cells to withstand mechanical stress. Tumours tend to shed more cytokeratins than healthy tissue does, probably because of their increased rate of cell turnover. Other tests seek out chromosomal abnormalities that have been linked to the development of bladder cancer.

However, as with cytology, none of these tests has identified slow-growing, non-invasive tumours accurately enough to make it into the medical guidelines for diagnostics or monitoring, much less to replace cystoscopy for these purposes. A 2015 article[1] declared: "It may be time to abandon urine tests for bladder cancer." The authors wrote: "No advancement in non-invasive testing has occurred in recent years capable of altering the current endoscopic surveillance scheme."

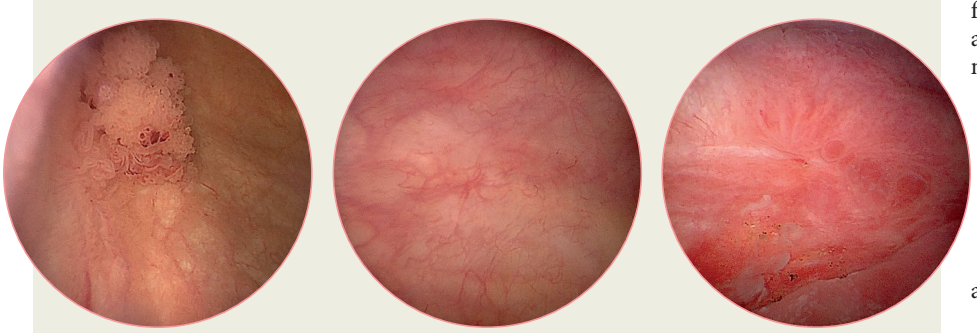
But many researchers are optimistic that better biomarkers can be developed that will be particularly helpful when used together. For example, proteins in the urine can sometimes be used for fast, point-of-care tests, says Ecke. He has been evaluating a commercially available, but not yet FDA-approved, cytokeratin test that could be useful for monitoring patients, perhaps reducing the cystoscopy schedule from every three months to every six. "You put three drops of urine on the cassette, you wait 10 minutes and the computer gives you the results," he says.

Proteomics techniques make it possible to discover multiple potential markers by allowing researchers to compare the proteins present in the urine of people with bladder cancer with those of healthy controls. Scientists also study the proteins secreted by bladder-cancer cell lines. In a 2016 review[2] of the bladder-cancer literature, Ward and his colleagues found that 161 possible protein biomarkers had been investigated. Panels comprising about eight of these proteins perform better as biomarkers than single proteins, but they all suffer from the same weakness, Ward says. Tumour cells may produce large amounts of some of the proteins, but non-tumour cells can produce the same proteins, so there is always the risk of a false-positive result, even when several proteins are combined.

The volatile organic compounds in urine, which rise quickly as vapour, are another potential source of biomarkers. These compounds are products of the cells' metabolic processes and could serve as signatures of cancer. Dogs exposed to human breath, tissues or urine can learn to distinguish whether or not a person has cancer, and they are probably detecting chemicals in these vapours. Researchers have started to develop 'electronic

SCOPE IT OUT

Using a cystoscope, a papillary tumour (left) is clearly different from the healthy bladder lining (centre). However, the rarer but more dangerous carcinoma *in situ* (right) is less easy to detect.



Papillary tumour | Healthy bladder | Carcinoma *in situ*

PIYUSH K. AGARWAL, NATIONAL CANCER INSTITUTE

noses' that can sniff out many kinds of cancer, including bladder cancer, but these are still in the early stages. Nevertheless, if successful, they could be used in the form of hand-held devices, Ward says. "You wouldn't have to send the tests off and get a result a week later."

Urine also contains various forms of genetic material, including messenger RNA (mRNA) and microRNA (miRNA), which provide a measure of gene expression in the sample. Researchers have found that some of these molecules are more abundant in bladder cancer than in non-cancerous tissue. One potential complication, however, is that mRNA is more prone to degradation than miRNA or DNA, Knowles says. Another problem, adds Ward, is that the number of tumour and normal cells in the sample can vary significantly. Commercial mRNA tests are becoming available, but Ward says that researchers are still searching for an ideal panel of RNAs that would include genes that are active only in malignant tissue.

"We are exploring several biomarkers for different cancers in the Smart Toilet."

Many researchers think it would be better to examine the DNA directly. Some teams are looking for genetic mutations that have been linked to bladder cancer because, for example, they promote uncontrolled growth. Others are looking for epigenetic alterations associated with the disease — changes in the number or types of chemical tag, such as methyl groups, that attach to specific sites in DNA and influence gene expression. Some of these epigenetic changes can interfere with the function of tumour-suppressor genes.

Bladder cancer is well suited to these kinds of approach, Ward says, partly because high-grade bladder cancer has more mutations than most other forms of cancer. Furthermore, genetic, and sometimes epigenetic, changes are cancer-specific and would not be found in healthy patients, dramatically reducing the problem of false positives that has dogged so many other biomarkers. "They're a yes-or-no answer," says Ward.

Ward and his colleagues have started to use next-generation sequencing to find a panel of genetic mutations that could act as a diagnostic tool. Last year the team published[3] promising results from a panel of seven genes, and an ongoing three-year study includes 20 genes — a combination of established and recently identified mutations (see page S44). The difficult part is finding a combination of genes that will always find cancer if it is present. Such a test may be ten years from entering the clinic, he says, but it could ultimately be practical and affordable. "I'm sure it could be done for less than €50 (US$59) per patient," he says — a fraction of the price of cystoscopies.

Other researchers have been using panels of methylation biomarkers[4], sometimes in combination with mutations[5], and are also getting promising results. "The tumour profiling that's gone on is pretty comprehensive — we know a huge amount," says Knowles. "It's just a question of now coming up with which of the key molecular features to look for in the urine."

REALISTIC TESTS

With so many biomarker tests being developed, the problem is working out which are the best. Some researchers think it would be better if they collaborated on a single large study to compare the most promising urine-based tests with one another, as well as with cystoscopy and cytology. It is also important, Ward says, to ensure that the study population and conditions are as close as possible to those of the doctor's surgery where the test will be used, or the clinic used for further testing or surveillance. In these locations, the people being tested will have inconclusive symptoms, but most will not have bladder cancer — and the test needs to accurately pick out those who do.

Many studies lack such realism because researchers would need to collect a large number of samples from the clinics before they would accumulate the required number from people with bladder cancer. But Ward and his colleagues are trying to do this. In the meantime, he is using 1,000 samples from people with positive cystoscopy results obtained from the West Midlands Bladder Cancer Prognosis Programme. About 10% of the tumours found are not malignant, which means that these patients can serve as controls, but Ward says the approach still fails to fully recapitulate the situation when people are first referred to a specialist clinic.

As well as creating urine tests to diagnose and monitor bladder cancer, researchers would like to screen people who have no symptoms. If the test is effective and cheap enough, Knowles says, it could be deployed in the general population to detect bladder cancer earlier. But it is more likely to be targeted at people with an elevated risk of the disease, such as the elderly, smokers or those exposed at work to chemicals known to promote bladder cancer, such as the aromatic amines that are sometimes used in the dye industry. Detecting bladder cancer earlier could improve the efficacy of treatments — and earlier diagnosis raises the possibility of developing better treatments, she says. "It would make drug companies a little bit more interested in developing local therapies for these non-invasive tumours."

Ultimately, these tests could be deployed not only in clinics, but in people's homes, says Gambhir. "We are exploring several biomarkers for different cancers in the Smart Toilet. These include colorectal, bladder, prostate and renal cancers," he says. Biomarker tests for proteins, volatile compounds, RNA and DNA may all soon be small and cheap enough to put in the average bathroom. The first commercial toilet to include these tests will probably be an all-in-one smart toilet engineered from the ground up. But the ultimate goal is more challenging: a device that could be retrofitted to any of the world's existing toilets, do assays on-site, and then upload them wirelessly for further analysis, he says. "It is still the early days of prototypes, but things are moving relatively quickly."

So you will probably not see these features next time you renovate your bathroom and buy a new toilet. But the time after that? Your doctor might just prescribe one. ■

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