

New breath test shows possible biomarker for early-stage liver disease diagnosis

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A natural compound called limonene, which is found in oranges and lemons, could be indicative in early-stage diagnosis of liver disease, according to research published in the journal *EBioMedicine* by researchers in the Molecular Physics Group at the University of Birmingham.

Limonene occurs in the greatest abundance in citrus fruits, but it is also found in a large variety of other fruit and vegetables. It can be ingested or inhaled as it is a common additive in commercial food and drinks, is used to give the fruit flavour to some sweets and is used in cosmetics, perfumes and cleaning products.

In the UK [liver disease](#) has risen sharply over the past few decades and is the third biggest cause of premature mortality, with three quarters of liver deaths due to alcohol. Patients do not often present with symptoms until the disease is advanced. Even then diagnosis is difficult and the symptoms and signs are often general and can be mistaken for other pathologies. For advanced cirrhosis liver transplant is the only treatment.

The Molecular Physics Group's study was carried out in two phases - breath samples from a group of 31 patients suffering from cirrhosis were first compared with a healthy control group. Then pre-transplant samples of the liver disease sufferers were compared with a sub-cohort of 11 patients who went on to have a liver transplant.

When the patients were tested before transplant surgery, the level of

limonene in the breath was found to be very high - higher than in a healthy person. This resulted from patients being unable to fully metabolize limonene.

When the team tested the same patients who had received a new liver, the tests showed that the limonene levels gradually dropped over several days. The researchers deduced that the unmetabolized limonene had been stored in the body fat of people suffering with cirrhosis.

To carry out the test, the patients and control group were asked to provide breath samples, which were collected using a breath sampling protocol developed by Ms. Raquel Fernandez del Rio, a Marie Curie Early Stage Researcher in the Molecular Physics Group. The [breath samples](#) were then put into a highly sensitive analytical instrument that measures the intensities of 'aroma molecules' or molecules that give rise to the experience of smell.

Dr Margaret O'Hara, from the Molecular Physics Group and primary investigator on the project, said: 'Previous studies have found potential biomarkers for liver disease, such as isoprene and acetone, but they are not specific enough because they are possible biomarkers for other diseases or can arise from numerous normal metabolic processes. We wanted to find a biomarker that is unambiguously associated with diseased liver.

'We already knew that people with liver disease have a very distinct smell on the breath and we wanted to find out what caused that smell. Now that we have found a biomarker for the disease in limonene, we can continue to verify how good it is for diagnosing liver disease.

'If our further research is successful, in the future we can envisage a small portable breath analyser that can be used by GPs and other health professionals to screen for [early stage](#) liver disease , leading to earlier

treatment and better survival rates.'

Dr Chris Mayhew, Head of the Molecular Physics Group, said: 'The group's results are astounding because they link limonene to the diseased liver rather than simply the diseased patient. A particularly important advantage of breath tests is that they offer the opportunity to assess the global function of the liver, rather than a localised test such as biopsy.'

'Importantly, our work provides for the first time a potential pathway for non-invasive real-time detection of early-stage cirrhosis. If that is possible, then the disease could be reversed by drugs and lifestyle change which would lead to major socio-economic impacts.'

Together with Dr. Margaret O'Hara and Ms. Raquel Fernandez del Rio, and in collaboration with a UK commercial company, KORE Technology Ltd, Dr Chris Mayhew is now seeking additional funding to continue this research programme to evaluate the diagnostic accuracy of breath volatile analysis for early stage cirrhosis. Dr Fraser Reich of Kore said: 'The possibility of breath analysis as a means of providing rapid and non-invasive medical diagnostics has been proclaimed for years, but in practice the clinical data produced has been extremely complex. In this case the University of Birmingham researchers appear to have found a unique biomarker on the breath that correlates with liver disease, and the early trials are both persuasive and exciting. We have confidence that more research will add to the statistical significance of the results, and together with the Birmingham team we aim to develop a cost-effective commercial means of delivering this diagnostic tool.'

More information: *EBioMedicine*, [DOI: 10.1016/j.ebiom.2015.07.027](https://doi.org/10.1016/j.ebiom.2015.07.027)

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