Hypothyroidism is a common endocrine disorder throughout Europe. Patients suffer from various discomforting symptoms, making accurate diagnosis and prompt treatment crucial to ensuring their welfare. By testing their blood using molecular markers, scientists hope to improve current practice, providing clinicians with accurate data needed to confidently dispense timely and targeted treatment

Improving the diagnosis of thyroid diseases through accurate blood testing

Hypothyroidism is an endocrine disorder which affects over 100 million patients in Europe, typically women in their forties. It is caused when the thyroid gland produces insufficient thyroid hormones, causing several uncomfortable symptoms. These include tiredness, weight gain, cognitive impairment and frequent mood swings, which can make daily life extremely difficult. Diagnosis can be confirmed with blood tests measuring thyroid-stimulating hormone (TSH), but the inaccuracy of current techniques currently prevents many patients from receiving optimum care.

"There's been a long-standing debate concerning what can be done to detect the onset of hypothyroidism with high accuracy," explains Professor Catherine Ronin, a biochemist currently based at the University of Aix-Marseille. According to the International Federation of Clinical Chemistry, she says, there may be as much as a 36 per cent discordance between existing tests. "Furthermore" she continues, "there is a need to develop assays – sampling tools – which are calibrated on a molar basis (measuring the concentration of substances in the blood), and are compliant with recent EU directives."

In response to these challenges, the EU launched its own specific TSH project, which aimed to improve patient outcomes by developing reliable thyroid disorder testing and measurement systems "These are the two fundamental markers used to diagnose thyroid disorder," confirms Professor Ronin, who joined the International Federation of Clinical Chemistry's TSH testing group in 2001. Her leading role in TSH research capitalises on considerable expertise in the field, the scientist having been a pioneer in the area of glycotechnology (the development of methods to analyse ubiquitous natural protein-linked carbohydrates, known as glycans) and elected as President of the French Glycoscience Society in 1996.

During a previous four year TSH research project, conducted between 2001-2005, the professor worked with Dutch hospitals in Amsterdam and Leiden, and observed pronounced variations in TSH tests between different labs. "We published four papers on our findings, with a high impact factor," explains Professor Ronin. "These showed that during the onset of the disease, TSH glycosylation changes. It transforms into highly sialylated, hypofucosylated, and long-lived molecular forms, which are more reactive to most monoclonal antibodies than its typical structures." Based on these findings, the CNRS supported the innovative findings of the TSH researchers by applying for a European patent relating to blood testing techniques, which was filed in 2011.

Their unique methodology relies on using a type of protein marker found in the bloodstream called glycoproteins, which cannot be further extracted from the human body but should be genetically engineered. Relevant antibodies are then used as detectors in a sandwich assay that can be further automated and run thousands of times each day. Measured using an assay in a laboratory, the antibodies can act as 'tracers' in blood samples, and when appropriately selected help to improve assessments of TSH. A preliminary clinical study was performed to demonstrate proof of concept and, to continue her contribution to improving blood testing, Professor Ronin recently launched a company, SiaMedXpress, which is dedicated to offering hyperglycosylated recombinant proteins for both therapeutic and diagnostic use.

SiaMedXpress recently received a grant from the French National Agency to develop new TSH tests. The firm has already celebrated some early successes, having been awarded the National Institute of Intellectual Property's Innovation Trophy 2008, and won the French Ministry of Research's National Contest 2010 for the Creation of Innovating Technologies Company. Concluding in late 2014, the company's latest study is now in its final stages, with its results due to be published in 2015. "This project set out to clinically confirm new procedures to measure early raise in TSH, on a molar basis," says Professor Ronin. "It is truly innovative, and sets out to validate this approach in a clinical setting for the first time."

Divided into three work packages, the project's first step was to examine blood taken from patients with hypothyroidism, to ensure that methods of detecting TSH were sufficiently versatile. The samples were volunteered by more than 1650 patients, located at hospitals in Lyon (Hospices Civils de Lyon, Dr Anne Charrié) and Grenoble (Centre Hospitalier de Chambéry, Dr.Anne Sophie Gauchez), which were subsequently screened and evaluated. "This task was realised in partnership with hospitals, so that we can relate the quantifications produced by our testing with the medical backgrounds of the different patients," says Professor Ronin. "This is something we could not do alone."

stage, deliver a clinical validation of the utmost importance, which can define new and existing assays according to the recent European Directives," says Professor Ronin. "Moreover, it will provide manufacturers with the rare opportunity of optimising existing tests or acquiring new ones, which we hope to stimulate. However, it is not an attempt to reach all patients, or impose a universal standard. It is for the patients who are in the early stages of thyroid disorder, who are at the moment being overlooked."

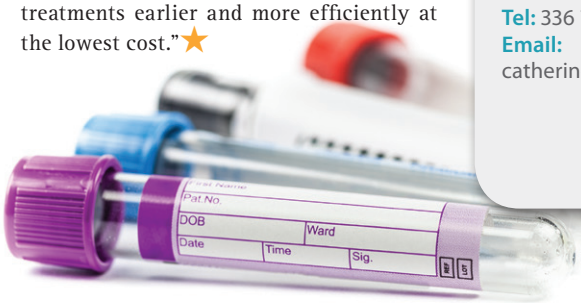
SiaMedXpress's new technology is thus most relevant for patients in the 'basal state' – a period in which TSH does not markedly raise, and can typically be identified only via testing. "If assays are not accurate enough, this means a clinician could miss initial development of the disease," Professor Ronin points out. Earlier interventions could prove critical, since patients typically see clinicians for around two years, but might receive no treatment, if their practitioner is uncertain of the diseases' status. It's a potentially deadly impasse that SiaMedXpress aspires to resolve.

"As we are dealing with hormones, if the thyroid hormone balance is not correctly adapted to a patient in therapy, then there is a risk of metabolic and neurological impact, because the thyroid is acting on all the body's tissues," Professor Ronin cautions. "Yet, conversely, if the hormone level is too high because of inadequate treatment, there is also a risk of heart disease. Enhancing early diagnosis is therefore vital to improving the options available to clinicians and patients. If we can realise better blood testing of the TSH marker, medical specialists can be confident in safely prescribing appropriate treatments earlier and more efficiently at the lowest cost."★

"Because we are developing new technology in the field of blood glycosylated markers, we can engineer proteins which have a carbohydrate structure very similar to that which actually exists in the bloodstream"

After considering the results, SiaMedXpress then set about developing a new test calibrator, based on a genetically modified form of TSH. "The experiments we've conducted on this subject were more accurate, and more evenly distributed than comparative tests," says Professor Ronin. "Because we are developing new technology in the field of blood glycosylated markers, we can engineer proteins which have a carbohydrate structure very similar to that which actually exists in the bloodstream. I think that this is a reason for our test's superiority." The bespoke recombinant product may be sent to manufacturers for in-house comparison in their own analysers, and antibodies anti-TSH screened and further developed by SiaMedXpress, to serve as the basis for new assays.

Industrial producers are also integral to the final component of the work package, which will involve numerous companies in a working group. Collectively, these participants will test the new TSH variant in their assays, using common blood collection, which will help to suggest relevant antibodies to detect them. In order to disseminate project findings worldwide, the IFCC Scientific Division and the IFCC-working group dedicated to the Standardisation of Thyroid Function are also participating. "The project will, at this



AT A GLANCE

Project Information

Project Title:
Improving the diagnosis of thyroid diseases through accurate blood testing

Project Objective:
Our past EU project showed that during the onset of the disease, TSH is turning to highly sialylated, hypofucosylated, long-lived forms which are more reactive to most monoclonal antibodies than normal forms. These findings have been patented and a start up company, SiaMed'Xpress founded to develop the preoof of concept and related products and blood tests. The company now aim at establishing a more accurate measurement of the early raise in TSH during the onset of hypothyroidism to deliver early diagnostic. Measuring such glycoforms will provide a more accurate testing and as a result, will also contribute to define a threshold that inclines the clinician to treat or not treat the patient with thyroid hormone.

Project Duration and Timing:
The work has bee supported by a grant of the French National Agency 2011-2014

Project Funding:
Public Funds and VCs

Project Partners:
French hospitals from the Rhône Alps Region: Lyon and Grenoble/Chambéry

MAIN CONTACT

Catherine Ronin
Professor Catherine Ronin received her PhD degree in Biotechnology and has been a Professor for the University of Provence and a member of the Polytech Bioengineering School for 25 years. Catherine has also been a guest scientist at NIH and Vice Chair and Chair of Innovative Training Networks at the European Research Agency for the past 10 years, before founding SiaMed'Xpress in 2010.

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SiaMed'Xpress
GLYCOTECHNOLOGY AT WORK