Spartan RX™ point-of-care DNA test for identifying the CYP2C19*2 mutation

**SUMMARY**

The Spartan RX™ CYP2C19*2 test is a point-of-care test that aims to detect a mutation in the CYP2C19 gene. This mutation may be associated with reduced response to clopidogrel. If patients with this mutation could be treated with alternative treatments, it is possible that this may help prevent rare but often fatal stent thrombosis after percutaneous coronary intervention. It is not always practical to use existing laboratory tests to detect this mutation due to the urgent setting in which the results may be required. Further research is needed to understand which alternative drug treatment would be most effective in patients with a mutation in this gene. Many more studies are therefore needed to work out whether this test can help prevent stent thrombosis in patients. This is the first point-of-care DNA test to be available in the UK.

**BACKGROUND**

Coronary Heart Disease (CHD) remains the most common cause of death in the UK with around 94,000 deaths each year\(^1\). It results from the build-up of fatty material (plaque) on the lining of coronary arteries (blood vessels that supply the heart). This narrows the vessels, gradually reducing the blood flow and therefore the oxygen supply to the heart muscle\(^2\). There may be associated pain from oxygen starved muscle which occurs on exertion (stable angina)\(^2\). When the disease is more severe and causes blood clotting, narrowing of the blood vessels may cause pain at rest (unstable angina). Further narrowing or blockage of these vessels may result in a heart attack where some of the heart muscle dies (myocardial infarction, MI)\(^3,4\). MI may be classified as either an “ST segment elevation myocardial infarction” (STEMI) or a “non-ST segment elevation myocardial infarction” (NSTEMI)\(^3,4\).
The STEMI is the more serious form of MI which occurs when a complete blockage occurs in the coronary arteries, while the NSTEMI is caused by an incomplete blockage. Unstable angina and MIs are emergencies that require urgent admission to hospital.

Percutaneous coronary interventions (PCIs) are used to treat CHD by widening the coronary arteries in order to restore blood supply to the heart. A small balloon is threaded over a wire through a tube (catheter) and inflated at the site of the plaque, compacting the material against the vessel wall. A stent (an expandable device that resembles a tubular wire mesh) is usually deployed to hold vessels open. Most PCIs are performed for acute, or emergency conditions (unstable angina or MI). It is recommended that PCI in patients with STEMI is performed within 150 minutes of a patient’s call for help and 90 minutes from admission to hospital (and within 60 minutes for some high risk STEMI patients).

Rarely, PCIs with stent placement will result in stent thrombosis (blood clotting around the stent), which will block the flow of blood through the coronary artery once again, causing angina or myocardial infarction. Stent thrombosis may be acute (within 24 hours after PCI), early (between 24 hours and 30 days after PCI), late (between 31 days and 1 year after PCI) or very late (more than 1 year after PCI).

In 2011, the number of PCIs undertaken in the UK was 1,405 per million population. In the UK in 2011, 25% of PCIs were for STEMIs (which should be performed within 90 mins of admission to hospital). This is an average of approximately 35 emergency PCIs per 100,000 population. The number of PCIs performed is increasing every year, although the rate of increase is slowing down. It is thought that 1-2% of patients undergoing PCI will develop early stent thrombosis with fewer patients (0.4-0.6%) developing very late stent thrombosis. The risk of death from stent thrombosis is lower the later it occurs. One estimate is that approximately 7.9% of those with early stent thrombosis will die in hospital.

There is no validated risk assessment for stent thrombosis but risk factors include diabetes, renal failure, previous heart failure and an under-sized stent.

CURRENT PRACTICE

The National Institute for Health and Clinical Excellence (NICE) and the European Society of Cardiology (ESC) recommend the use of aspirin and another anti-platelet agent (such as clopidogrel) for the prevention of stent thrombosis in patients undergoing PCI. Anti-platelet agents help to prevent the clumping together of particles in blood called platelets. This clumping together of platelets is normally needed to form blood clots.

Until recently, clopidogrel with aspirin were the standard anti-platelet agents for patients undergoing PCI in the UK. These drugs are given prior to PCI and for 12 months afterwards. NICE recommendations have also been published for newer agents and there are reports suggesting that there is increasing usage of these (with one of these being used as the default anti-platelet agent for approximately 50% of UK PCI centres). The ESC recommends the use of newer agents before consideration of clopidogrel unless there is a high risk of bleeding complications (thought to be an increased risk with newer drugs).

Clopidogrel may not work in some patients. Factors that may affect this are age, diabetes, how well the kidneys work and genetics. Mutations in genes called ABCB1 and CYP2C19 may be associated with reduced response to clopidogrel and an increased risk of adverse events. It is thought that 2-14% of the population are “poor metabolisers” of clopidogrel (have significantly reduced response due to having two copies of a mutation in the CYP2C19 gene). These are most likely to be mutations called CYP2C19*2 or CYP2C19*3. People of African-Caribbean and East Asian descent are at most likely to have these mutations.
The United States Food and Drug Administration (FDA) considered the evidence on CYP2C19 gene mutation association with poor response to clopidogrel to be robust enough to issue a safety announcement in 2010. This states that patients who are “poor metabolisers” of clopidogrel will not respond to this drug, placing them at an increased risk of adverse events. Alternative drugs are suggested for these patients although there is no explicit recommendation to routinely test for mutations and no particular tests are recommended. The ESC states that reports on this subject are contradictory but that DNA testing (or other methods of predicting poor response to clopidogrel) may be considered in selected patients with NSTEMI or unstable angina.

It is possible to test for CYP2C19 mutations using laboratory testing but this is not practical for testing patients prior to undergoing emergency PCIs or in the first 24-48 hours afterwards. During this time, the patient may be at an increased risk of thrombosis if they are not responding to clopidogrel. It is also possible to measure the extent to which platelets are clumping together in a patient (platelet function test). This may provide information to doctors about whether clopidogrel is likely to work or whether clopidogrel is working in that patient. These tests are still being evaluated and are not currently recommended by NICE or the ESC for routine use. However, the ESC suggests that where clopidogrel is used, DNA testing or platelet function testing may be considered in selected patients with NSTEMI or unstable angina.

NEW TECHNOLOGY

The Spartan RX™ CYP2C19*2 test developed by Spartan Bioscience Inc is the first point-of-care DNA test to be made available in the UK. The test identifies patients with a mutation called CYP2C19*2 in at least one of the two copies of the CYP2C19 gene. One way this test may be used is to rapidly identify patients who will not respond to clopidogrel in order to select patients (undergoing PCI) in whom alternative anti-platelet agents would be needed to help prevent thrombosis. The company estimate that approximately 30% of patients will have at least one copy of the mutation.

The CYP2C19 gene codes for a protein that is needed to break down the drug clopidogrel into its active form. If the drug is not broken down effectively, patients may be less likely to benefit from the anti-platelet effects of the drug which are intended to prevent stent thrombosis from occurring.

The test is the first to be developed to run on the Spartan RX™ platform which rapidly and automatically extracts and analyses DNA. The device dimensions are 17cm x 36cm x 12cm and it weighs 9.3lbs. The company state that the device may be suitable for use in a catheterisation laboratory at the time of PCI. This may allow personalisation of anti-platelet treatment using this test to begin prior to emergency PCI. The test is designed to be easy to use by those who are not laboratory-trained. The company estimate that obtaining a sample and activating the machine will take approximately eight minutes and the automated process provides results within one hour. The sample required for the test is a buccal (cheek) swab which is inserted into a cartridge. This is then inserted into the Spartan RX machine. DNA analysis is triggered by a button on the device. The test will identify whether a patient has two functioning copies of the CYP2C19 gene, one CYP2C19*2 mutation or two of these mutations. If a patient has a different mutation in the CYP2C19 gene, the test will not identify this. Therefore the test result will not be distinguishable from that of a patient with no mutation in the CYP2C19 gene.

The test was CE marked in December 2010 for the detection of the CYP2C19*2 mutation, but not specifically for the prediction of response to clopidogrel. It is available in the UK.
Currently it is being used in private practice, but not in the NHS. A new version of this test is also currently being developed to include other mutations in the CYP2C19 gene and in the ABCB1 gene\textsuperscript{16,17,18}. No cost information was available at the time of writing for the Spartan RX™ platform on which the test will be run. The cost of the test is currently approximately $200 per test (equivalent to approximately £125) but discounts would apply based on the volume of tests purchased.

Another test called Verigene CYP2C19 developed by Nanosphere Inc. was CE marked in April 2011 and FDA approved in November 2012\textsuperscript{19}. The test identifies two mutations in the CYP2C19 gene (CYP2C19*2 and CYP2C19*3). The available information on the company website suggests that this test does not require laboratory trained personnel to operate the device. The test uses a whole-blood sample, takes approximately 5 minutes to set up and provides a result within 2.5 hours. This test would therefore seem not to be suitable for use prior to emergency PCI but could also be used within the 24-48 hour window after PCI to personalise anti-platelet treatment.

**CLINICAL STUDIES AND RESEARCH QUESTIONS**

There are currently no randomised clinical trials of CYP2C19*2 mutation testing in a UK PCI setting comparing clinical outcomes with those resulting from current standard UK practice. There are also no studies looking at the feasibility of using the Spartan RX™ prior to emergency PCI. Four recent systematic reviews of the association of CYP2C19 mutation with clinical outcomes and one proof-of-concept study of the Spartan RX™ are described here\textsuperscript{20,21,22,23,24}.

The earliest of the four systematic reviews identified is a meta-analysis of 9,685 patients from 9 trials (6 of which evaluated stent thrombosis)\textsuperscript{20}. The studies included were published between 2000 and 2010. The majority (91.3%) underwent PCI and 54.5% were for MI or unstable angina. The majority of the studies were concerned with the CYP2C19*2 mutation and 28.5% of patients were found to have at least one copy of this mutation. The analysis suggests that patients with at least one copy of a mutation have an increased risk of death due to a cardiovascular problem, MI or ischaemic stroke (hazard ratio 1.57; 95% CI, 1.13-2.16; p=0.006). The risk of stent thrombosis was also found to be increased (hazard ratio 2.81; 95% CI, 1.81-4.37; p<0.00001).

The majority of the studies in the review described above were also included in a systematic review and meta-analysis of studies published up to October 2011\textsuperscript{21}. This analysis included 42,016 patients from 32 trials including patients with MI or unstable angina (21 studies), stable angina (8 studies) and diagnosis unknown (3 studies). The majority of studies were concerned with identifying the most common CYP2C19*2 mutation. The authors conclude that mutation in the CYP2C19 gene is associated with significant effect on platelets reacting to clopidogrel. They also conclude that overall there is no increased risk of adverse events such as death from any cause, coronary heart disease and stroke. After analysing the data for stent thrombosis, the authors conclude that there was some evidence of an increased risk in patients with a mutation (relative risk 1.75; 95% CI, 1.50-2.03). The authors describe a trend of decreasing effect in larger studies and conclude that the studies available for review were significantly flawed.

A systematic review and meta-analysis of studies published up to the end of 2010 was conducted\textsuperscript{22}. The association between mutations in the CYP2C19 gene and major adverse cardiovascular events or stent thrombosis was investigated using data from 18,529 patients (adverse events) and 9,128 patients (stent thrombosis). The analysis showed no significant increased risk of major adverse cardiovascular events in those with the mutation and an
increased risk of stent thrombosis (odds ratio 1.67 95% CI, 1.34-2.08; p<0.001). The authors state that they found evidence of smaller, earlier studies over-estimating the effect of the mutation. They state that taking these effects into account negated the association between stent thrombosis and mutation in the CYP2C19 gene found during the analysis.

A systematic review and meta-analysis of 13 studies published before October 2010 was conducted23. No significant increase in risk of major adverse cardiovascular events was found in patients with a mutation. Analysis of 7 studies of 8,686 patients showed an increased risk in stent thrombosis (hazard ratio 2.24; 95% CI 1.52-3.30; p<0.001).

The Spartan RX™ CYP2C19*2 test has been evaluated in a proof-of-concept study in 187 patients undergoing PCI for NSTEMI or stable angina24. All patients were treated with 600mg of clopidogrel at least 24 hours before PCI. Patients were either randomised to standard treatment (98 patients) or to be tested by the Spartan RX™ in order to guide treatment (102 patients). Patients with a mutation were prescribed 10mg of Prasugrel daily after PCI. All other patients (including those not tested using the Spartan RX™) were prescribed 75mg clopidogrel daily. Platelet function was compared between patients on standard treatment and those in whom treatment decisions were informed by the Spartan RX™ test. This test was performed immediately after PCI and one week later. When compared with laboratory testing, the Spartan RX™ correctly identified 100% of those with a mutation and 99.3% of those without it. Of those with the mutation, there was a significantly greater proportion of patients with high platelet reactivity in those having standard treatment (30%) than in those who were tested with the Spartan RX™ (0%, p=0.0092). There was no significant difference between the two groups in the rates of adverse events reported.

There are two randomised trials of the Spartan RX™ device ongoing using the device for guiding treatment after PCI25,26. One is a study of 100 patients with STEMI estimated to complete in 201225. Testing for CYP2C19*2 will be combined with testing for other mutations. Patients classified as having a high genetic risk of poor response to clopidogrel will be treated with either clopidogrel or a newer agent (prasugrel). A third group with low genetic risk will be treated with clopidogrel. Platelet reactivity whilst having drug treatment will be compared between the three groups. The second study is of 4,000 patients undergoing PCI and is estimated to complete in 201526. One group will be given standard care (where the clinician may prescribe any of three anti-platelet agents including clopidogrel). The second group will be given anti-platelet agents depending on risk assessment for non-response to clopidogrel. This risk assessment will be based on testing for the CYP2C19*2 mutation as well as other mutations in the CYP2C19 gene, mutation in the ABCC1 gene and platelet reactivity. The time to first occurrence of cardiovascular death, MI, stroke or bleeding events will be compared between the groups.

Randomised controlled trials of the Spartan RX™ test compared with standard UK practice in patients who have undergone PCI are needed to determine whether use of this test can result in benefits for patients and the NHS. Evaluation of the test in combination with other methods of risk-stratifying patients (such as the use of clinical risk factors) for stent thrombosis is awaited. Further research is needed to determine whether it is feasible to use this test prior to emergency PCI and whether there are benefits associated with this. In addition, further study is required to quantify the risks associated with treating patients with rarer mutations in the CYP2C19 gene as though they have no mutation.
POTENTIAL IMPACT

Coronary heart disease is the most common cause of death in the UK and is a priority for the NHS. Stent thrombosis is a rare but life threatening condition which may occur after PCI treatment for STEMI, NSTEMI or angina. The use of clopidogrel as a standard anti-platelet agent plays an important role in the prevention of stent thrombosis in most patients but may not be effective in some people. The time taken from admission to hospital to the PCI being performed is critical and subject to national audit. The ability to predict response to clopidogrel has the potential to help reduce stent thrombosis in those who would not respond to this drug and enable clinicians to use clopidogrel (a cheaper alternative to newer agents with a lower risk of bleeding events) in the majority of patients.

The various possible methods of risk-stratification for clopidogrel resistance and stent thrombosis, including platelet function testing, have not yet been fully standardised and evaluated. Doctors are also beginning to prescribe newer anti-platelet agents to patients undergoing PCI. There is also no consensus yet on the most effective course of action in patients testing positive for at least one copy of CYP2C19*2. Because of these factors, the impact of the Spartan RX™ test in UK practice is uncertain and will depend upon the evaluation of the test alone and in combination with other risk-stratification tools and treatment strategies. However, as the first available point-of-care DNA test in the UK, this test enables the evaluation of genetic testing in patients undergoing emergency PCIs (including testing prior to PCI) for the first time.

Lay summary

The Spartan RX™ CYP2C19*2 test is the first automatic and quick test to be able to find a mutation in a patient’s DNA. This test looks for a change in a gene which is thought to stop certain types of drugs from working in patients. One of these drugs is given when a patient has a treatment for a heart attack or angina called a “PCI”. This is where a small mesh tube is placed into the patient’s blood vessel that supplies the heart because it has become narrower. Very rarely the blood will clot around the tube and this can be very serious and sometimes the patient will die. This new test may help a doctor know quickly if the drug being given to stop this clotting will work. Many more studies are needed to find out how well this test can do this. Because the test is very quick, this would be the first time a DNA test would be able to be used in an emergency like heart attack.

REFERENCES


