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Does Skin Cholesterol Testing Provide Benefit?

Summary

- ✓ **Cholesterol 1,2,3™ is being promoted as a non-invasive way to measure cholesterol that has accumulated in a person's skin.**
- ✓ **The test received a medical device licence from Health Canada in January 2001. It was approved by the U.S. Food and Drug Administration (FDA) in June 2002.**
- ✓ **This test is not intended to be used as a screening tool for coronary artery disease in the general population. Evidence from non-randomized, non-blinded clinical trials suggests a correlation between higher skin cholesterol levels and the presence of severe coronary arterial lesions.**
- ✓ **At this point, technical improvements and more robust evidence are required to determine the significance of this technology in clinical practice.**

The Technology

The role of cholesterol in the pathogenesis of atherosclerotic heart disease has been explored by numerous pathological, epidemiological, genetic, and interventional studies.¹ Cholesterol levels in the skin have been reported to be elevated in patients with atherosclerotic disease.²⁻⁴ The current test is being promoted as a non-invasive way to measure the amount of cholesterol which has accumulated in a person's skin, as measured in the palm of the hand.⁵

The test uses a detector solution, consisting of a cholesterol-binding agent (digitonin) linked to a marker enzyme (horseradish peroxidase), which binds to the skin layer rich in cholesterol. A drop of an indicator solution (horseradish peroxidase

substrate) is incubated with the detector solution, and a colour change is measured, using a hand-held spectrophotometer interfaced with a computer. All the steps of analysis occur while the applicator pad is affixed to the palm of the hand. The result is reported numerically as the hue, or degrees, an outcome that correlates with the saturation of the coloured product. There is no reported difference in the background hue of the palmar skin of racially diverse individuals. Assay validity is assessed by visual interpretation of control wells.

Regulatory Status

Cholesterol 1,2,3™ was developed at McMaster University and is manufactured by Toronto-based International Medical Innovations Inc. (IMI). A medical device licence for Cholesterol 1,2,3 was issued by the Therapeutic Products Directorate at Health Canada in January 2001. The FDA approved the test in June 2002.

Patient Group

This test could be used to provide additional risk factor information for people who may be predisposed to significant coronary artery disease. It is not intended to be used for the general population.

Current Practice

Enzyme assays are used to measure cholesterol directly in serum or plasma.⁶ Skin cholesterol has been previously measured by skin biopsies after extraction from tissue with organic solvents but is not routinely measured in practice.⁷

Administration and Cost

A foam pad is applied to the palmar surface of the patient's hand. Solutions are added to wells in the pad for a total incubation time of three minutes. A hand-held colour reader, applied to the centre well of the pad, measures the amount of colour and gives a skin cholesterol number. The entire process requires about three to four minutes.

Individual tests will cost approximately \$6 to \$7 when purchased directly from the distributor by doctors' offices, pharmacies or laboratories. An additional expense is the one-time capital cost of the spectrophotometer, which is between \$1,500 and \$2,000.

Rate of Technology Diffusion

The concept of "cardiovascular risk factors" did not exist until the 1960s, when the initial findings of the Framingham study were reported. This large, longitudinal cohort sought to identify individuals at risk for coronary arterial lesions and heart disease. In addition to serum cholesterol, over 240 other risk factors were identified. Educational campaigns by the National Cholesterol Education Program (NCEP) in the US continue to promote cholesterol screening while the Heart & Stroke Foundation of Canada continues to promote identification of high-risk patients and their treatment as the standard of care.

This "medicalization" of high blood cholesterol has led many to believe that it is a disease itself, and not a risk factor for disease. If this enthusiasm carries over to skin cholesterol, testing could receive a disproportionate amount of attention before being well established as a reliable predictor of disease. Skin cholesterol testing is also likely to be co-promoted with drugs that modify arterial plaque formation, if proven to be as reliable as standard diagnostic testing.

Concurrent Developments

There is no information on technologies directly competitive with Cholesterol 1,2,3. A variety of office devices for measuring total cholesterol

from pinprick methods have been used for comparison. In addition to the measurement of cholesterol, several other biochemical markers of atherothrombotic risks including homocysteine, fibrinogen and lipoprotein(a), are commonly measured in serum in clinical laboratories. Imaging techniques including carotid and intravascular ultrasonography, electron beam computed tomography and magnetic resonance imaging are also under investigation as methods to identify "silent" plaque. Genetic risk factors have recently attracted increasing attention.

The Evidence

An open-label trial demonstrated that skin cholesterol levels measured by Cholesterol 1,2,3 were significantly correlated with changes in serum low-density lipoprotein (LDL) cholesterol in 10 patients (Pearson correlation 0.608, $P < 0.001$).⁸ In this study, patients on cholesterol-lowering therapy had skin cholesterol and serum LDL measured three times at three-month intervals. The results showed a parallel change in skin cholesterol and LDL levels. This direct relationship is consistent with the data from a study by Bjornheden et al. in which there was a positive correlation between serum and skin cholesterol.⁹

A second manufacturer-sponsored trial involved 111 healthy patients, ages 34-77 years who received a treadmill test due to chest discomfort.¹⁰ The objectives of this trial were to determine the relationship between the Cholesterol 1,2,3 results and exercise stress testing and serum lipids. Skin cholesterol levels were significantly correlated with a positive stress test (Pearson correlation 0.262, $P < 0.023$) in this trial. Contrary to the first trial, no evidence of a correlation between skin and serum cholesterol levels or skin cholesterol and serum triglycerides levels was found.

In another study carried out by the same group, skin cholesterol was tested using Cholesterol 1,2,3 in 241 patients who subsequently underwent diagnostic coronary catheterisation. The results of this trial showed skin cholesterol levels were associated with the presence and extent of CAD based on angiographic lesions, after adjusting for age and other risk factors¹¹ (Pearson correlation statistic not reported).

Data from a trial of similar design involving 649 patients were reported. Skin cholesterol measurement by Cholesterol 1,2,3 was shown to correlate with angiographic severe multi-vessel CAD (defined as 50 percent closure of two or more arteries) (Pearson correlation 0.11, $p=0.005$).¹² The highest angiographic burden and history of myocardial infarction are seen in subjects with elevated skin cholesterol and low serum high-density lipoprotein (HDL) cholesterol ($p<0.001$).¹³ In a more recently-conducted trial, significant and positive correlations were also found between skin cholesterol measurement using Cholesterol 1,2,3 and Framingham risk calculation, systolic blood pressure, total cholesterol, LDL cholesterol and inflammatory marker intercellular adhesion molecule-1.¹⁴

Implementation Issues

Although skin cholesterol has been suggested as an independent risk factor for CAD, this assertion will require further exploration and more robust evidence. Conventional risk factors, such as serum cholesterol, have been established by a large succession of appropriately blinded prospective studies in broad populations. Establishing "skin cholesterol" as a risk factor, will, at the very least, require similar methodological rigour.

Although the digitonin conjugate in this test binds to free cholesterol with the desired affinity, it is not absolutely specific to cholesterol. It can also bind to other sterols (especially β -sitosterol), as well as stigmastanol, cholesterol sulfate, ceramides and other molecules. The composition of the three main lipid classes found in human skin (free fatty acids, cholesterol, and ceramides) exhibits a large variation.¹⁵ Individual differences in the relative amounts of these lipid classes can be greater than 100%. It may be misleading therefore, to state that Cholesterol 1,2,3 strictly measures "skin cholesterol". A conversion of chromogenic hue into skin cholesterol mass has not been performed in any of these trials. Exactly what is being measured and the impact of what is being measured requires further characterization using larger patient groups.

Variations in skin thickness could also affect test reliability. Free fatty acid analysis in relation to human skin depth has been examined, with data revealing a change in the amount and composition of lipid in the stratum corneum,¹⁶ a factor that could interfere with the accuracy of the test.

Data showed correlation between skin cholesterol measured by Cholesterol 1,2,3 and severe CAD, but the test was not shown to be useful in identifying people with less severe CAD. This test is therefore not intended to be used as a screening tool to determine risk of CAD in the general population. Even if good correlations are found, the significance of the test in clinical practice is unknown because the comparators such as LDL, HDL, blood pressure and angiography are surrogate outcomes for death due to CAD.

The ultimate value of a diagnostic or screening test lies in its ability (a) to improve outcomes for patients following changes in management resulting from use of the test, or (b) to provide equivalent outcomes at reduced cost, or with less pain or less invasiveness. Investigations of this test using properly randomized trials are needed to determine the effect of testing on the management of cardiovascular patients. Prospective cohort studies are also required to examine the effectiveness of this technology in predicting cardiovascular morbidity and mortality.

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