7. EVIDENCE BASED LABORATORY MEDICINE IN THE DIAGNOSIS OF METABOLIC SYNDROME

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1. Introduction

Evidence based medicine (EBM) is a formalized system helping medical community cope with numerous different medical information, whereby the end result helps doctors identify the best diagnostic tests and treatments. Medical knowledge is accumulating and changing with such a dizzying speed that medical community has found it needs new methods to cope with it all. EBM provides formal protocols that are applied to the latest data to determine what data best support the best outcomes.

The aims of EBM in laboratory medicine (or EBLM) are to advance clinical diagnosis by research and dissemination of new knowledge, and to combine methods from clinical epidemiology, statistics and social science with the traditional pathophysiological and molecular approach. The evaluation of diagnostic investigations as well as the clinical decision-making process can help in translating the results of good quality research into everyday practice.

EBLM has a few elements which have to be satisfied by order: audit practice, identifying the question, search for evidence, critically appraising the evidence, applying it to practice by modifying the practice, and constant practice audit.

2. Definition of metabolic syndrome (MS)

Until 1998, there was no initiative to develop an internationally recognized definition. World Health Organization (WHO) was the first to publish an internationally accepted definition for metabolic syndrome. Subsequently, third report of the USA National Cholesterol Education Program: Adult Treatment Panel (NCEP:ATP III) has also defined clinical identification of MS. The NECP:ATP III guidelines suggest a diagnosis of metabolic syndrome (previously known as syndrome X) when three or more of the following risk factors are present: central obesity, elevated triglycerides, low HDL, raised blood pressure, and raised fasting plasma glucose. More recently, the International Diabetes Federation (IDF) has defined criteria for MS where metabolic syndrome is diagnosed if the patient has a 'large waist' plus any other two risk factors.

Since several definitions of the syndrome are in use, it is difficult to compare its prevalence and impact between countries. Fortunately, there is a chance for a more rational approach. In 2004, IDF convened a group of experts to establish a unified definition for MS. Table 7.1. presents the comparison of different definitions of diagnostic criteria for metabolic syndrome.

The metabolic syndrome is also known as syndrome X, insulin resistance syndrome, and deadly quartet (upper body obesity, glucose intolerance, hypertriglyceridemia and hypertension).

Table 7.1. *Comparison of different definitions of diagnostic criteria for metabolic syndrome (MS)*

Risk factor	Defining level NCEP ATP III Any three of these:	Defining criteria IDF Large waist plus any two:	WHO All of these:
Central (abdominal) obesity waist circumference	men >102 cm women >88 cm	men >94 cm women >80 cm	BMI >30 or waist-to-hip ratio men >0.9, women >0.85
HDL-cholesterol	men <1.0 mmol/L women <1.3 mmol/L)	men <1.0 mmol/L women <1.3 mmol/L	men <0.9 mmol/L women <1.0 mmol/L)
Triglycerides	≥1.7 mmol/L	≥1.7 mmol/L	≥1.7 mmol/L
Blood pressure	≥135/85 mm Hg	≥130/85 mm Hg	>140/90 mm Hg
Fasting plasma glucose	≥6.1 mmol/L	≥5.6 mmol/l	Diabetes or impaired fasting glycemia or impaired glucose tolerance or insulin resistance
Other			Microalbuminuria: albumin excretion >20 μg/min

3.   Laboratory indicators for the diagnosis of metabolic syndrome

Based on these data, general laboratory tests used in the diagnosis of MS include determination of lipid profile (concentrations of total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides) and glucose metabolism (total glucose concentration, oGTT, and glycated hemoglobin HbA_{1c}).

There are other laboratory tests that are not recommended for diagnosing MS but may be ordered by some doctors to provide additional information. Tests that can also be useful include measuring of lipoprotein particle size (measurement of small dense low-density lipoprotein particles in particular) and determination of high sensitive C-reactive protein (CRP) concentration.

There also are some tests that can be used in research setting, such as plasminogen activator inhibitor-1 (PAI-1), fibrinogen, proinsulin and homocysteine.

4.   General laboratory tests

1.   Glucose

Insulin as a hormone enables glucose to move into the tissue. The liver produces glycogen and/or fatty acids from glucose. Insulin resistance causes an additional release of insulin by the pancreas. This can lead to increased glucose and insulin concentrations in the bloodstream.

Blood glucose may be measured on the fasting basis (8- to 10-hour fasting), randomly, or as part of oral glucose tolerance test (oGTT), a series of blood glucose testing.

### 2.	Triglycerides

In MS, high triglycerides are the consequence of increased glucose and insulin concentrations. Diminishing glucose entering the cells increases the production of fatty acids (supported by high insulin concentration) and triglyceride production.

### 3.	Total cholesterol

Measuring of total cholesterol concentration in the diagnosis of MS is included in the lipid profile determination and is used to assess the risk of heart disease. As high blood cholesterol is associated with arteriosclerosis, heart disease and an increased risk of death from heart attack, cholesterol testing is considered a routine part of preventive health care as well as a tool to assess complications of some diseases such as MS.

### 4.	High-density lipoprotein cholesterol

The concentration of HDL-cholesterol is decreased in MS, probably due to the increased triglyceride concentration. HDL particles become enriched with triglycerides and are more rapidly removed from the circulation. Triglyceride-enriched HDL particles are smaller and become better substrates for hepatic lipase. The test for HDL-cholesterol is used along with other lipid tests to determine the risk of heart disease.

### 5.	Low-density lipoprotein cholesterol

Insulin resistance has an unfavorable effect on lipid production. It decreases HDL-cholesterol, increases triglycerides, and also VLDL and LDL. So, determination of LDL-cholesterol is also important in the diagnosis of MS as well as a prognostic factor for some complications such as cardiovascular diseases.

## 5.	Alternative laboratory tests

### 1.	Insulin

Fasting insulin values may be too variable to be clinically useful in the diagnosis of MS but if measured, they will usually be elevated in these individuals. The methods of determination are different immunoassays.

### 2.	High-sensitivity C-reactive protein (hs-CRP)

Although determination of CRP is not useful in the diagnosis of MS, it may be tested as part of the cardiac risk assessment. People who have hs-CRP results at the upper normal limit have a 1.5- to 4-fold risk of sustaining heart attack found in those with CRP values at the lower normal limit. It may come from cells in the fatty deposits in arterial walls that reflect the process of atherosclerosis; however, it may also come from other tissues. So, CRP determination is not used for large-scale screening of the general adult population but is useful as an independent marker of the risk of cardiovascular disease to help determine the course of treatment. Methods of determination are different immunoassays.

3. Microalbumin

Increased urinary albumin excretion precedes and is highly predictive of diabetic nephropathy. Microalbuminuria is between normal and overt proteinuria. Microalbumin is an early indicator of kidney disease. This test is used to help monitor diabetics and is recommended under the WHO criteria. Detection of microalbuminuria can be accomplished by using semiquantitative rapid tests or by performing quantitative immunochemical determination of albumin. Test strips used for screening purposes fail to detect most cases of microalbuminuria.

4. Small dense LDL

LDL varies in size and the smaller denser particles, which tend to form when elevated triglycerides and VLDL are present in the blood, are thought to be more aggressive in causing atherosclerosis. Determination of small dense LDL particle can be useful because LDL cholesterol values can be misleading. Optimal levels of LDL cholesterol can mask an increased number of LDL particles.

5. Homocysteine

Elevated plasma homocysteine may cause or result from insulin resistance and could be actively involved in atherogenesis or is just an indicator of vascular risk. Numerous clinical studies have shown that total homocysteine is a risk factor for cardiovascular disease and stroke in humans and predicts mortality independently of traditional risk factors in patients with CAD. The possible cellular mechanisms by which homocysteine may contribute to cardiovascular disease include unfolded protein response, oxidative stress, and the induction of proinflammatory factors. Interpretastion of some laboratory test results in the diagnosis of metabolic syndrome are presented in Table 7.2.

Table7.2. Interpretation of laboratory test results in the diagnosis of metabolic syndrome (MS)

Triglycerides:	0.5-1.13 mmol/L is optimal, 1.13-1.7 mmol/L is moderate and over 1.7 mmol/L is high.
Cholesterol:	HDL 1.0 mmol/L is good, although higher is even better. In studies, women with HDL of 1.8 mmol /L had low cardiac risk. LDL of less than 2.6 mmol/L is good. Total cholesterol should be less than 5 mmol/L or lower.
Fasting glucose:	Normal is 3.9-5.5 mmol/L. Values of 5.6-6.9 mmol/L are indicative of pre-diabetes. Values greater than or equal to 7.0 mmol/L are indicative of type 2 diabetes.
Oral glucose tolerance (with 75 g glucose load):	Results greater than or equal to 7.8 mmol/L at 2 hours – normal glucose tolerance; 7.8-11.1 mmol /L – impaired glucose tolerance; over 11.1 mmol/L on more than one testing occasion – indicate type 2 diabetes.
Fasting insulin (insulin assay):	10 IU/mL and below is optimal; over 10 IU/mL is high.
High sensitive CRP	Less than 1.0 μU/mL is optimal.
Homocysteine:	Less than 6 μmol/L is optimal; greater than 9 μmol/L is high.
Plasminogen activator inhibitor (PAI -1):	Greater than 31. This test is not yet commonly performed.

Fibrinogen:	This test is a general measure of inflammatory processes in the body and results vary greatly with the patient's age, sex and test method. Results that are both too high and too low are problematic. Refer to your specific laboratory for interpretation of the results.

6.   Laboratory tests used only in research setting

There are a few tests that are primarily used for research purposes. The molecular basis for the link between adipose tissue, metabolic disorders and cardiovascular diseases has not been fully clarified. Research on adipocyte biology has revealed that adipocytes produce and secrete a variety of bioactive substances named 'adipocytokines'; these include growth factors, cytokines and complement factors. Adipose tissue probably acts as an endocrine organ that may affect the function of other organs. The explanation of the molecular mechanism and their impact in the development of MS would in the future be helpful in the diagnosis.

7.   EBM studies of metabolic syndrome and laboratory indicators

EBLM studies have confirmed some of laboratory assays as relatively simple metabolic markers in the diagnosis of MS. Evaluation of the ability of metabolic markers has shown that plasma triglyceride concentration, the ratio of triglyceride/HDL-cholesterol concentrations, and insulin concentration are most useful markers in identifying insulin resistant individuals ATP III criteria for the diagnosis of MS were used to identify insulin-resistant individuals among 258 nondiabetic, normotensive, overweight individuals. The optimal cut-off points were 1.47 mmol/L for triglycerides, 1.8 for triglyceride/HDL-cholesterol, and 109 pmol/L for insulin. The respective sensitivity and specificity for these cut-off points were 67%, 64% and 57%, and 71%, 68% and 85%.

Different EBM studies which include treatment of MS can be useful in the evaluation of laboratory tests in the diagnosis of MS. Sixty nondiabetic adults with NCEP defined MS from local metropolitan Philadelphia area, with HDL-cholesterol lower than 1.0 mmol/L in men and 1.3 mmo/L in women, were treated with pioglitazone-PIO (a synthetic peroxisome proliferator activated receptor γ ligand), which has been approved for the treatment of hyperglycemia in diabetes mellitus type 2. The concentration of HDL-cholesterol increased by 11% as compared with 4% reduction in those who received placebo. Small LDL particles were reduced significantly, and the level of hs-CRP was reduced by 31%. Insulin resistance showed a modest decrease.

Sixteen Finnish patients diagnosed with diabetes mellitus type 2 were on a strictly defined low energy diet. The treatment led to body weight reduction by 6±1 kg and BMI reduction by 6%. Laboratory tests showed a 14% reduction of blood glucose concentration and 13%-24% reduction in serum triglycerides. Serum cholesterol concentrations were unchanged.

CRP has also been confirmed as a clinically important prognostic parameter in the diagnosis of MS. Examples of EBLM studies:

1. Inflammatory markers modified by multitarget treatment were investigated in a prospective, randomized study. A series of 300 nondiabetic patients (of Greek descent) with MS (according to NCEP definition), free from CVD were studied over a 12-month period and treated for hypertension, hyperlipidemia, impaired fasting glucose and obesity.

2. Increased CRP concentrations in obese men of Canadian descent with MS were significantly reduced with gemfibrozil, a lipid lowering drug.

3. A series of 179 men and 166 women (of Japanese descent) with MS according to NECP ATP III criteria were included in a study evaluating cut-off points. The optimal cut-off point of CRP for MS might be 0.65 mg/L in Japan and this value could be useful in routine clinical practice and studies of MS.

So, it is concluded that triglyceride concentration, the ratio of triglyceride/HDL-lipoprotein cholesterol concentrations, and insulin concentration as well as CRP and blood glucose concentrations are useful markers in the prognosis and treatment of MS.

As one of the characteristics of MS is insulin resistance, and the risk sequel is development of diabetes mellitus type 2, determination of glycated hemoglobin, HbA1c, plays a role in the diagnosis and prognosis of MS. Hemoglobin A1c (HbA1c) is considered a standard measure of long-term glycemic control, and HbA1c levels are strongly associated with complications of diabetes. Examples of EBLM studies:

1. A Swedish study which included patients with clinically diagnosed type 2 diabetes showed a combination of HbA1c, fasting plasma glucose and BMI to be effective in screening for individuals at risk of future clinical diagnosis of type 2 diabetes. This study also showed that oGTT or familial history of diabetes were not necessary.

2. French subjects with clinically diagnosed diabetes from the Epidemiological Study on the Insulin Resistance Syndrome were investigated. Incident diabetes was defined by fasting plasma glucose ≥ 7.0 mmol/L or treatment by antidiabetic drugs. Results of the study showed that HbA1c predicted diabetes, even though the diagnosis of diabetes was based on blood glucose. It could be used as a test if fasting blood sampling was not available or in association with fasting plasma glucose. In subjects with impaired fasting glucose, HbA1c is better than glucose to evaluate the risk of diabetes, and it could be used to select subjects for intensive early intervention.

There are few EBM studies that indicate the importance of homocysteine in assessing complications in subjects with MS. Examples of EBLM studies:

1. Italian patients with MS and diabetes mellitus type 2 were treated with different antidiabetic drugs. There was a statistically significant decrease in basal homocysteinemia in glimepiride-treated patients (-27.3%) but not in rosiglitazone-treated patients.

2. Weight-reduction diet as well as replacement meal (Slim-FastTM products) in overweight/obese Australians with raised triglycerides showed a significant decrease in homocysteine values.

3. Folate and vitamin B12 treatment improved insulin resistance and endothelial dysfunction, along with decreasing homocysteine levels in Italian patients with MS, suggesting that folic acid has several beneficial effects on cardiovascular disease risk factors.

It is very difficult to compare different EBM studies and to define standards in laboratory diagnosis of MS. At first, difficulties arise from different names and definition of MS (differences in concentration limits for general laboratory indicators of MS such as triglycerides, HDL-cholesterol and glucose. Furthermore, the criteria used for obesity in

Caucasians could be different from those used in Asians and other populations. And finally, significant diversity in the design of EBLM studies of MS laboratory indicators can be documented (e.g., inclusion/exclusion criteria for diabetes mellitus, age, sex, treatment, etc.).

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