Opinion

Blood-Based Biomarkers for Metabolic Syndrome

Sadhbh O'Neill,¹ Mette Bohl,² Soren Gregersen,²
Kjeld Hermansen,² and Lorraine O'Driscoll^{1,*}

Metabolic syndrome (MetS) is a constellation of factors increasing the risk of type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD), and cancer. MetS diagnosis is cumbersome and the precise diagnosis differs throughout the world. Efforts are underway to find MetS biomarkers that could all be analysed in a single blood sample. Here we review recent advances, including progress on circulating exosomes and microvesicles and their molecular contents, as well as DNA, RNAs, and proteins taken directly from blood samples. While additional research is now warranted to advance upon these findings, there is reason for optimising that such blood-based entities will be beneficial for MetS diagnosis and will help reduce risk of T2DM, CVD, and cancers, contributing both societal and economic benefit.

Metabolic Syndrome: A Global Problem in Need of Better Biomarkers

Metabolic syndrome (MetS, see [Glossary](#)) is a serious global issue with substantial clinical relevance. While the precise definition of MetS differs in specific details between the World Health Organisation (WHO), the European Group for Insulin Research (EGIR), the National Cholesterol Education Program–Third Adult Treatment Panel (NCEP:ATPIII), the American Association for Clinical Endocrinology (AACE), and the International Diabetes Federation (IDF) ([Box 1](#)) [1], it is generally accepted that MetS is the compounding of several risk factors including **insulin resistance (IR)**, central obesity, high **triglycerides**, **dyslipidaemia**, and **hypertension** [1].

MetS is of substantial clinical relevance and concern because of its high prevalence and association with the development of more serious pathologies. The prevalence of obesity (one of the components of MetS) has more than doubled from 1980 to 2014ⁱ. In 2013, more than 1.9 billion adults throughout the world were identified as overweight. Of these, 600 million were described as clinically obese. This equates to 39% of the adult population being overweight (38% male, 40% female) and 13% being obese (11% male, 15% female). Overweight status and obesity are not restricted to the adult population, they also occur in children. In 2014, 42 million children under the age of 5 years were diagnosed as obeseⁱ. Results from studies performed around the world show that the global prevalence of MetS ranges from 10% to 84% depending on the ethnicity, age, gender, and race of the population [2]. As expected, MetS increases with body mass index (BMI) and the risk of developing MetS also increases with age [1]. Additional to the health issues directly associated with MetS, this condition contributes to a 5-fold increased risk of **type 2 diabetes mellitus (T2DM)**, 3-fold increased risk of **cardiovascular disease (CVD)**, and also an increased risk of developing certain cancers [3]. Thus, MetS contributes to both reduced quality-of-life and reduced life expectancy.

Additional to the human health costs, MetS – and the pathologies to which it contributes – are huge economic drains on society. Although to the best of our knowledge there are no economic figures relating specifically to MetS, in a study conducted by Nichols and Moler [4] a significant

Trends

Metabolic syndrome (MetS) causes substantial morbidity and increases the risk of developing type 2 diabetes mellitus, cardiovascular disease, and cancer.

MetS diagnosis is cumbersome and so MetS often goes unnoticed and untreated. Early diagnosis might enable early intervention, appropriate and timely MetS management, potentially preventing the development of more serious pathologies, and increasing quality-of-life and longevity.

Minimally invasive biomarkers for MetS diagnosis and, ultimately, for screening those at risk of developing MetS that could be analysed from one blood sample would be of substantial benefit to reducing this problem.

Recent advances in this field suggest that extracellular vesicles and their molecular contents, as well as DNA, RNAs, and proteins taken directly from blood samples might be used as biomarkers to diagnose MetS.

¹School of Pharmacy and Pharmaceutical Sciences and Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin 2, Ireland

²Department of Endocrinology and Internal Medicine, Aarhus University Hospital, Aarhus, Denmark

*Correspondence: lodrisc@tcd.ie (L. O'Driscoll).

URL: <http://pharmacy.tcd.ie/staff/odriscoll-cv.php?uname=lodrisc>.

Box 1. The Numerous Definitions of MetS

Numerous definitions for diagnosing MetS exist, very likely contributing to both false-positive and false-negative diagnoses. Organisations including the World Health Organisation (WHO), the European Group for the Study of Insulin Resistance (EGIR), the National Cholesterol Education Program–Third Adult Treatment Panel (NCEP:ATPIII), the American Association for Clinical Endocrinology (AACE), and the International Diabetes Federation (IDF) have put forward definitions for MetS; the NCEP:ATPIII and IDF are most frequently used. For the most part, all organisations are in agreement on the factors contributing to MetS, but differ on the specific combination of factors and specific threshold points for each factor that defines MetS. The criteria used by these organisations for defining and diagnosing MetS are shown in detail in Table I. The pathologies associated with MetS are illustrated in Figure I.

Figure I. Characteristics of Metabolic Syndrome (MetS). Hallmarks of MetS include insulin resistance (IR), central obesity, high triglycerides, dyslipidaemia, and hypertension. Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table I. Criteria for Defining and Diagnosing MetS as Set Out by the Interested Organisations^a

MetS Definitions	WHO	EGIR	NCEP:ATPIII	AACE	IDF
Fundamental	High insulin level	Insulin resistance		Impaired glucose tolerance	WC (ethnic and gender specific)
Component	+2 of:	+2 of:	Any 3 of:	+2 of:	+2 of:
Abdominal obesity	WC >37 inches BMI >30 kg/m ²	WC ≥94 cm (M) ≥80 cm (F)	WC >40 inches (M) >35 inches (F)		
Triglycerides	>150 mg dl ⁻¹ HDL-C: <35 mg dl ⁻¹ (M) <39 mg dl ⁻¹ (F)	>2 mmol l ⁻¹ HDL-C: <1 mg dl ⁻¹	≥150 mg dl ⁻¹ HDL-C: <40 mg dl ⁻¹ (M) <50 mg dl ⁻¹ (F)	≥150 mg dl ⁻¹ HDL-C: <40 mg dl ⁻¹ (M) <50 mg dl ⁻¹ (F)	≥150 mg dl ⁻¹ HDL-C: <40 mg dl ⁻¹ (M) <50 mg dl ⁻¹ (F)
Blood pressure	≥ $\frac{140}{90}$ mm Hg	≥ $\frac{140}{90}$ mm Hg or BP medication	≥ $\frac{130}{85}$ mm Hg	≥ $\frac{130}{85}$ mm Hg	≥ $\frac{130}{85}$ mm Hg
Microalbuminuria	>30 mg g ⁻¹				
Fasting plasma glucose		≥6.1 mmol l ⁻¹	>110 mg dl ⁻¹		≥5.6 mmol l ⁻¹ or T2DM

^aAbbreviations: BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein; WC, waist circumference; M, male; F, female.

Glossary

Biological markers (biomarkers):

in 1989 biomarkers were described as measurable and quantifiable biological parameters that serve as indices for physiology- and pathology-related assessments, such as disease risk. In 2001, the National Institute of Health standardised the definition as a 'characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention'. Broadly they are markers of a biological process or state, with many applications including diagnostic tools for disease, and for monitoring, predicting, and understanding mechanism(s) of clinical response to therapy. Biomarkers may also offer a mechanistic understanding of a clinical response to treatment.

Cardiovascular disease (CVD):

the grouping of disorders resulting from narrowing or blocking of blood vessels. These disorders include coronary heart disease, cerebrovascular disease, rheumatic heart disease, deep vein thrombosis, congenital heart disease, and peripheral arterial disease. This can lead to heart attack and stroke.

Dyslipidaemia:

elevated levels of total and low-density lipoprotein (LDL)-cholesterol or low levels of high-density lipoprotein (HDL)-cholesterol.

Extracellular vesicles (EVs):

membrane-enclosed vesicles released by cells into the extracellular milieu (i.e., biofluids), which include exosomes, ectosomes/microvesicles/membrane vesicles/microparticles, and other EV subsets. EVs contain proteins, DNA, and RNA nucleic acids and some have been implicated in cell-to-cell communication.

High-density lipoprotein (HDL):

cholesterol lipoproteins that transport cholesterol from body tissues to the liver for excretion. HDL-cholesterol is considered the 'good' cholesterol.

Hypertension:

abnormally high blood pressure.

Insulin resistance:

is a condition in which the tissues of the body have a reduced ability to respond to insulin, a hormone that is secreted by the pancreas which helps to regulate the level of glucose in the circulation. As a result, larger quantities of insulin are

increase in healthcare costs was found for each additional component of MetS presented. Each component of MetS [i.e., elevated fasting glucose, elevated blood pressure, reduced **high-density lipoprotein (HDL)**-cholesterol, and increased waist circumference (using BMI $>28.8 \text{ kg/m}^2$ rather than waist circumference as, in practice, waist circumference is very rarely recorded in healthcare records)] was analysed for a cohort of 57 420 individuals from the Kaiser Permanente Northwest Medical Centre in Oregon. Individuals were categorised as having zero to five components of MetS present. Each person was subsequently followed for 5 years and the cost of healthcare was estimated. The increase in healthcare costs were found to be significant when advancing from zero components to one, two, and three components ($P<0.001$), and from four components to five components ($P<0.001$); while the increase from three components to four was tending towards significance ($P=0.058$). As would be expected, this study also reported that the further development of T2DM or CVD increased the cost of healthcare. In the USA in 2012 alone, the estimated cost associated with T2DM was \$245 billion [5], with CVD costing \$108.9 billion annually [6]. Cancer diagnosis and treatment are also extraordinarily large draws on the economy. However, the current process of diagnosing MetS is multifaceted, cumbersome, and not ideally for purpose. Thus, substantial efforts are being invested into identifying more and better **biomarkers (biological markers)** for this condition.

Blood-based biomarkers for MetS [7–9] could offer an improved minimally invasive diagnosis (Figure 1, Key Figure), a method for prognosing patients' outcome, a means for predicting response to therapeutic intervention, and, importantly, also an opportunity for earlier intervention

Key Figure

Potential Biomarkers for Metabolic Syndrome (MetS)

Trends in Endocrinology & Metabolism

Figure 1. Blood-based biomarker entities such as miRNAs, mRNAs, DNA, and proteins [existing as extracellular vesicle (EV)-contained cargo and/or freely circulating] hold potential as biomarkers for MetS either singly or in combination. Studies to date have included analysis of the quantities and/or molecular content of EVs isolated from serum or plasma, while others have analysed nucleic acids and proteins directly from these blood products. Abbreviations: WHO, World Health Organisation; EGIR, European Group for Insulin Research; NCEP:ATPIII, National Cholesterol Education Program–Third Adult Treatment Panel; AACE, American Association for Clinical Endocrinology; IDF, International Diabetes Federation.

produced to establish glucose homeostasis.

Low-density lipoprotein (LDL): transfers cholesterol, phospholipids, and triglycerides around the body in the extracellular fluid, it is often referred to as the 'bad cholesterol'.

Metabolic syndrome (MetS): constellation of components resulting in a three-times increased risk of CVD and five-times increased risk of T2DM. The five components of this constellation that result in MetS include obesity, insulin resistance, high triglycerides, low high-density lipoprotein, and hypertension.

microRNA (miRNA): small endogenous ~20–23 nucleotide long noncoding RNA that functions in post-transcriptional RNA silencing by binding to complementary mRNA sequences; typically in the 3'-untranslated region. Owing to their small size and stability, miRNAs are being investigated as biomarkers for many conditions and diseases.

Triglycerides: consist of a combination of three molecules of fatty acids and one molecule of the alcohol glycerol. They are the major fat forms stored by the body and are both produced by the body and come from food.

Type 2 diabetes mellitus (T2DM): a chronic condition that adversely affects the way the body metabolises glucose. The body is resistant to insulin and/or does not produce enough insulin to maintain normal blood glucose levels.

to avoid the development of more life-threatening pathologies, such as T2DM, CVD, and cancer. An ideal biomarker for MetS would be an entity that is consistently dysregulated in MetS compared with healthy controls and would be quantifiable, quick to assess, specific, and sensitive. Of course, many of the tests currently contributing to a MetS diagnosis, regardless of the specific definition used, are blood-based. However, additional tests are also required (glucose tolerance, waist circumference). In reality, the more complex a test is and the more self-conscious a patient feels about going for such a test (e.g., having his/her waist circumference measured), the less likely it is that there will be compliance. As outlined in the following section, there is increasing evidence to suggest that exosomes, nucleic acids, and proteins derived from blood all have potential to form useful biomarker panels. Thus, here we review studies of circulating exosomes and microvesicles, nucleic acids, and proteins that may form such a panel of biomarkers, and when further optimised may help diagnose MetS earlier and proactively address MetS-related complications.

Extracellular Vesicles/Exosomes

Although it has long been known that eukaryotic cells release complex vesicular structures into their environment, only in recent years has it been established that these entities, namely exosomes, microvesicles/ectosomes/membrane vesicles/microparticles (collectively termed **extracellular vesicles, EVs**), are not merely junk or debris, but tailor-made specialised mini-maps of their cell of origin [10]. EVs were described by two independent studies, in 1983 [11] and 1984 [12], when it was observed that reticulocytes could discharge the transferrin receptor by its incorporation into EVs that were then released by cells. These vesicles were termed exosomes as they originated from the endosome/multivesicular bodies [13].

The terminology EVs includes exosomes and microvesicles/ectosomes that are often defined and subgrouped first and foremost on size and proposed origin (exosomes are ~30–120 nm and are of endosomal origin; microvesicles/ectosomes are >120–1000 nm and originate from pinching of the cell membrane) (Figure 2). However, there is no reason to believe that pinched vesicles of smaller sizes cannot also exist, thus the relevance of size *per se* is questionable. Furthermore, as unique markers for the different vesicle types have not yet been fully defined, the origin of vesicles released from cells cannot be identified. For this reason, here we use the collective term EVs [10–14].

Studies of circulating EVs as biomarkers for MetS are still in their infancy and it is not possible to directly compare or perform a meta-analysis of emerging data due to different methods being used for their isolation, some of which typically produce relatively pure populations of EVs and others methods that precipitate EVs but are not selective for EVs [15] (Table 1). However, trends are pointing towards a relationship between the quantities of blood-based EVs and MetS. When plasma specimens from individuals (Table 2, row A) with MetS were compared to those from healthy controls, a significant ($P<0.001$) increase in quantities of EVs (as determined by flow cytometry following labelling of EVs with antibodies against surface antigens) was observed with MetS [16]. A follow-up study (Table 2, row B) confirmed these results and found circulating quantities of plasma EVs (as determined by flow cytometry) to be significantly ($P<0.05$) higher in MetS compared with healthy individuals [17]. Thus, these studies support the hypothesis that basic quantification of circulating EVs may offer potential as diagnostic biomarkers for MetS. Although further appropriately designed studies are required, ultimately EV quantities may even prove to have benefit for screening those at increased risk of developing MetS.

Freely circulating **microRNAs (miRNAs)** and those carried in EVs are also under investigation as biomarkers for MetS [18–20]. A particular interest in miRNAs has emerged from the fact that they appear to be causally and mechanistically involved in events that contribute to MetS. Regulation of miRNAs is tightly controlled and each miRNA may, in turn, target multiple mRNAs. This is

Trends in Endocrinology & Metabolism

Figure 2. Biogenesis of Extracellular Vesicles and Their Classification. (A) Exosome formation starts at the cell membrane when the membrane is pinched inwards, this creates an endosomal-like structure. Intraluminal vesicles (ILVs) develop capturing bioactive molecules, such as proteins, lipids, and nucleic acids. The ILV matures to form multivesicular bodies (MVBs). The MVB then takes one of two paths, that is, they are transported to the (i) lysosome for degradation or (ii) cell membrane, fuse to this, and, subsequently, release the ILVs into the extracellular space. Released ILVs are termed exosomes. Alternatively, budding at the cell membrane, capturing cytosolic fragments, results in the formation of microvesicles (MVs), also known as ectosomes/membrane vesicles/microparticles. These vesicles have been reported to contain DNA, RNAs, and proteins, although it is as yet unknown the mechanism by which DNA can become incorporated into exosomes. (B) Some characteristics of EVs as exosomes and microvesicles/ectosomes based on size and origin.

Table 1. Comparison of Exosomes/EV Isolation Methods^a

Isolation Method	Advantages	Disadvantages
Ultracentrifugation (UC)	<ul style="list-style-type: none"> ✓ High EV yield ✓ High RNA yield 	<ul style="list-style-type: none"> ✓ Moderate EV purity ✓ Moderate protein yield ✓ Moderately laborious
Gradient ultracentrifugation (OptiPrep/optical density gradient)	<ul style="list-style-type: none"> ✓ Very high EV yield ✓ Very high purity 	<ul style="list-style-type: none"> ✓ Laborious, time-consuming ✓ Low protein yield ✓ Low RNA yield
Commercial Kits: <ul style="list-style-type: none"> ▪ ExoQuick ▪ Total Exosomes Isolation Solution 	<ul style="list-style-type: none"> ✓ High protein yield ✓ High RNA yield ✓ Easy to use ✓ Very high protein yield ✓ High RNA yield ✓ Easy to use 	<ul style="list-style-type: none"> ✓ Low EV yield ✓ Low EV purity ✓ Low EV yield ✓ Low purity

^aThis published comparison was based on studies of conditioned medium rather than blood-derived serum or plasma. However, our in-house data support this also holding true for serum and plasma. Adapted from [15].

Table 2. Details of Studies Investigating Blood-Based Biomarkers for MetS^a

	Individuals (n)	Age (years)	MetS Definition	Controls (n)	Age (years)	Specimen Analysed	Refs
A	MetS, n=43 M – 74% F – 26%	52±1.7	NCEP:ATPIII	n=37 M – 57% F – 43%	52.7±1.7	Plasma	[16]
B	MetS, n=21 M – 81% F – 19%	56±2	NCEP:ATPIII	n=17 M – 71% F – 29%	54±2	Plasma	[17]
C	Diabetic, n=100 MetS, n=88 M – 50% F – 50%	M – 48±12 F – 48±10	NCEP:ATPIII (modified for Asians)	Nondiabetic, n=100 MetS, n=18 M – 50% F – 50%	M – 47±11 F – 45±12	Serum	[29]
D	n=661 M – 479, MetS 22.8% F – 182, MetS 3.6%	M – 53±10 F – 54±10	NCEP:ATPIII (modified for Asians)	–	–	Plasma	[30]
E	MetS – 1215 M – 561 F – 654	M – 47.19±10.9 F – 48.23±10.2	IDF	n=1830 M – 907 F – 923	M – 41.5±12.0 F – 40.2±11.4	Serum	[32]
F	n=367 MetS IDF: M – 127, F – 52 MetS NCEP:ATPIII: M – 121, F – 41	11–16	IDF+NCEP: ATPIII	–	–	Plasma	[33]
G	MetS, n=936 M – 53.5% F – 46.7%	11–16	IDF	–	–	Serum	[35]
H	n=383 M – 70% F – 30%	63±0.4	Specific definition not included	n=368 M – 65% F – 35%	62.3±0.6	Plasma	[36]
I	n=31 M – 16 F – 15	M – 55±8 F – 58±9	NCEP:ATPIII	n=71 M – 36 F – 35	M – 56±9 F – 55±6	Serum	[37]
J	CAD, n=155 M – 105 F – 100 Non-CAD, n=100 M – 57 F – 43	CAD – 65.3±11 Non-CAD – 65.2±10.2	–	n=100 M – 35 F – 65	63±10.7	Plasma	[40]
K	Obese F – 13	37.8±2.8	–	n=30	46.3±0.4	Serum	[42]

^aAbbreviations: F, female; M, male; CAD, coronary artery disease.

necessary for regulating and fine-tuning different aspects of metabolism. However, when miRNAs become disrupted, pathology may follow, as can be seen in the case of metabolic diseases [19]. Changes in miRNA expression have now been directly associated with many components of MetS. For example, cholesterol is regulated at the cellular level by both classical transcription factors and also by miRNAs. With regard to their relevance as blood-based biomarkers for MetS, Karolina *et al.* [21] investigated both total miRNAs isolated from whole blood and EV-contained miRNAs isolated from plasma of individuals with MetS, T2DM,

Table 3. Details of Individuals Recruited to the Karolina *et al.* [21] Study and Detailed Results of that Study

MetS (n)	MetS – Age (years)	Results	MetS (vs controls)	T2DM (vs controls)	HCL (vs controls)	HPT (vs controls)
Cohort #1: 18 Cohort #2: 29	37±8.1 45.7±11.3	miR-17	Increased (1.93±0.19-fold)	Decreased (1.55±0.07-fold)	Increased (1.72±0.08-fold)	Decreased (1.63±0.23-fold)
Healthy Controls (n)	Healthy Controls – Age (years)	miR-197	Increased (1.77±0.28-fold)	Decreased (1.49±0.09-fold)	Increased (1.62±0.15-fold)	Increased (1.14±0.23-fold)
Cohort #1: 17 Cohort #2: 29	39±8.1 45.7±11.3	miR-509-5p	Increased (1.96±0.23-fold)	Decreased (1.39±0.25-fold)	Increased (1.66±0.06-fold)	Decreased (1.85±0.21-fold)
T2DM (n)	T2DM – Age (years)	miR-92a	Increased (1.52±0.13-fold)	Decreased (1.47±0.11-fold)	Decreased (1.63±0.04-fold)	Increased (1.77±0.15-fold)
Cohort #1: 21 Cohort #2: 29	39±15.1 44.2±8.4	miR-320a	Increased (1.56±0.23-fold)	Increased (1.41±0.11-fold)	Decreased (1.16±0.13-fold)	Decreased (1.35±0.16-fold)
HCL (n)	HCL – Age (years)					
Cohort #1: 41 Cohort #2: 48	39±6 40.7±11.7					
HPT (n)	HPT – Age (years)					
Cohort #1: 14 Cohort #2: 16	14±11 43±8.1					

hypercholesterolaemia (HCL), hypertension (HPT), and healthy controls (Table 3). Five miRNAs were identified as present in EVs from all groups. From those, four, namely miR-17, miR-197, miR-509-5p, miR-92a, were found to be at higher quantities in the MetS and hypercholesterolaemic groups and lower in the T2DM and hypertensive groups. miR-320a was increased in the MetS and T2DM groups and decreased in the hypercholesterolaemic and hypertensive groups. In this study, the detection patterns of these miRNAs were reported to be similar in EVs and in whole blood.

Whether or not extracellular miRNAs are predominant in EVs or not is still under debate and is very likely to differ with different miRNAs and different cellular/clinical conditions. For example, it has been reported that in serum and saliva the majority of miRNAs are concentrated in the exosomes rather than freely circulating [22], while a study of plasma miRNAs found them to be mainly concentrated in microvesicles [23]. Conversely, an analysis of three miRNAs in plasma found them to be predominantly (>97%) exosome-free [24]. Furthermore, Arroyo *et al.* [25] claimed that 90% of miRNAs in the circulation are not present in a membrane-bound form. However, regardless of whether or not miRNAs are contained within EVs, it is apparent that blood-based miRNAs have promise as biomarkers for full-blown MetS and for many of its contributing components (see section on Circulating miRNA Biomarkers for MetS).

Circulating Protein Biomarkers for MetS

Adiponectin is an antiatherogenic, antidiabetic [26], and antihyperlipidaemic adipokine and its low circulating levels (<4 µg/ml) are associated with MetS. Plasma adiponectin levels were found to be significantly ($P<0.0001$) decreased in obese (3.7 ± 3.2 mg/ml) compared with nonobese (8.9 ± 5.4 mg/ml) individuals [27]. A strong inverse correlation was also found between plasma adiponectin protein concentration and BMI for both males and females [27]. Plasma adiponectin protein concentration was also reported to be lower in individuals with hypertension compared with normotensive individuals [28]. Thus, the association of low adiponectin protein levels with MetS was subsequently investigated in a cohort of diabetics compared with nondiabetics, with and without MetS (Table 2, row C). In this study, serum adiponectin protein was significantly lower in the diabetic cohort. Of the individuals with MetS, adiponectin protein was decreased in both males and females, when compared with individuals without MetS, inversely correlating with MetS. Interestingly, adiponectin protein was found to decrease progressively with

increasing number of MetS components present. Furthermore, for those individuals with diabetes and MetS, compared with individuals with only MetS, the plasma concentration of adiponectin was again much lower [29].

A study of Japanese males and females (Table 2, row D) showed plasma levels of adiponectin in both males and females to inversely correlate with BMI, waist circumference, visceral fat, triglycerides, and positively correlated with HDL levels [30]. Studies of each component of MetS independently showed that an adiponectin concentration of $<4 \mu\text{g/ml}$ of plasma correlated with a higher prevalence of each of the MetS components, when compared with adiponectin protein levels for healthy individuals. For example, a concentration of $<4 \mu\text{g/ml}$ was observed in a higher percentage of obese compared with nonobese males and females. Furthermore, MetS had a significantly higher prevalence in both males ($P<0.001$) and females ($P<0.01$) who had adiponectin plasma concentrations $<4 \mu\text{g/ml}$ compared with those who had higher circulating levels [30].

While more extensive double-blinded studies are warranted, data produced to date suggest that low level circulating adiponectin protein (i.e., less than a $4 \mu\text{g/ml}$ threshold) has potential as a biomarker that could contribute to a biomarker panel for MetS diagnosis. In fact, based on its associated activities in the body (antiatherogenic, antidiabetic, and antihyperlipidaemic), it could be cautiously extrapolated that introducing adiponectin may have therapeutic benefit. However, this would have to be thoroughly investigated, initially in preclinical models.

Leptin, a satiety hormone [31], also has potential as a blood-based biomarker for MetS. This is supported by a study from the Iranian Third National Surveillance of Risk Factors of Non-Communicable Diseases (SuRFNCD) involving 3045 participants (Table 2, row E) where MetS was diagnosed according to the IDF definition. Individuals with MetS had higher serum level of leptin compared with non-MetS individuals. Leptin levels were independent of overall obesity or abdominal/central obesity and indicated an independent role in the development of MetS [32]. In parallel, a study including 367 Mexican–American individuals from the Cameron County Hispanic Cohort (CCHC) (Table 2, row F) showed that both decreased adiponectin protein and increased leptin correlated with MetS [33]. Again, these data suggest that more extensive analysis of leptin protein, for example, via a double-blinded study would help to confirm its relevance as a biomarker. It would make sense to study adiponectin and leptin in the same blood (plasma/serum) specimens. Of course to be useful as a biomarker, a robust threshold for leptin assessment would also need to be established.

Circulating DNA Biomarkers for MetS

Genomic (gDNA) polymorphism in the adiponectin (*AdipoQ*) gene is also a potential biomarker for MetS. Genome-wide scan studies have identified a susceptibility locus for obesity, T2DM, and coronary heart disease (CHD) in the same chromosomal region as *AdipoQ* (i.e., 3q27). Forty-two SNPs in the linkage disequilibrium blocks of the adiponectin gene were found to exist and these SNPs confer a risk of obesity, T2DM, and diabetic nephropathy [34]. Therefore, not only do the protein levels of adiponectin show potential as a biomarker but also the gDNA polymorphisms in the *AdipoQ* gene could be of relevance. A study that recruited 936 Chinese adolescents diagnosed with MetS based on the IDF definition (Table 2, row G) extracted gDNA from peripheral blood and found that the frequency of a SNP in the adiponectin gene at position 45 with two G-alleles (i.e., SNP+45 GG) was significantly increased in the MetS cohort compared with the frequency of the same SNP at position 45 but with either two T-alleles (i.e., SNP+45 TT) or with T- and G-alleles (i.e., SNP+45 TG). It was also determined that the risk of MetS was significantly higher in subjects with the SNP+45 GG genotype compared with those with the SNP+45 TT or TT +TG genotypes [35], suggesting that the SNP+45 GG genotype is a biomarker/risk factor for MetS. However, SNP+45 GG genotype had no effect on phenotype, that is, on serum adiponectin protein concentrations [35]. Ohashi *et al.* [36] also genotyped

gDNA isolated from whole blood specimens procured from 383 coronary artery disease (CAD) individuals and 368 controls (Table 2, row H). MetS was diagnosed if total cholesterol >5.6 mmol/l, triglycerides >1.69 mmol/l, HDL-cholesterol <1.03 mmol/l, and blood pressure of systolic ≥ 140 mm Hg and diastolic ≥ 90 mm Hg were present. The presence of SNP-276, SNP-94, and missense mutation I164T in *AdipoQ* was assessed. The frequency of the I164T mutation compared with wild-type was significantly ($P < 0.001$) higher in individuals with three abnormalities (MetS, T2DM, CAD). Furthermore, the prevalence of MetS was significantly higher in CAD individuals with the I164T mutation than in CAD individuals without this mutation. Therefore, this study suggests that the I164T mutation in the *AdipoQ* gene is associated with MetS-related CAD.

These emerging data, although limited so far, suggest that assessing DNA mutations could add further value to forming a panel of biomarkers for MetS diagnosis, rather than assessing proteins alone.

Circulating miRNA Biomarkers for MetS

A number of miRNAs have been identified that regulate components of MetS (Table 4), cholesterol, and fatty acid (FA) metabolism. These include miR-122 and let-7 g that were found

Table 4. miRNAs Associated with Components of MetS, All of which Are Increased in MetS

Metabolic Process	miRNAs Regulating the Metabolic Process	Targets of Each miRNA and Area of Inhibition ^a	Refs
Lipid Metabolism + Cholesterol Efflux	miR-122	Targets <i>ALDOA</i> and <i>CS</i> in primary mouse hepatocytes	[45]
	miR-33a, miR-33b	Targets <i>ABCA1</i> in liver cells and macrophages	[45]
	miR-758	Targets <i>ABCA1</i> in human and mouse macrophages	[45]
	miR-106b	Targets <i>ABCA1</i> in human hepatocytes	[45]
Glucose Homeostasis + Insulin Signalling	miR-132	Targets <i>SIRT1</i> in adipocytes	[46]
	miR-29a, miR-29b	Expressed in pancreatic islet cells of obese mice and target <i>MCT1</i>	[19]
	miR-126	Inhibits <i>IRS1</i> to promote insulin resistance. Targets <i>IRS2</i> in liver cells and macrophages	[19]
	miR-33a, miR-33b	Target <i>SIRT6</i> and <i>AMPKα1</i>	[47]
	miR-223	Expressed in skeletal muscle and targets <i>GLUT4</i>	[19]
	miR-103, miR-107	Expressed in liver and adipose tissue and targets <i>CAV1</i> and <i>DICER</i>	[48]
	Let-7	Targets <i>IRS2</i> , <i>IGF1R</i> , <i>INSR</i> in muscle and adipose tissue	[19]
Adipocyte Differentiation	miR-143	Increased during adipocyte differentiation and targets <i>ERK5</i>	[49]
	miR-204	Increased in hADSCs during adipogenesis in concert with <i>C/EBPα</i> , <i>PPARα</i> , <i>FABP4</i> , and targets <i>DVL3</i>	[50]
	miR-200	Expression is up in mouse <i>ST</i> mesenchymal stem cells during adipocyte differentiation and targets Wnt signalling	[51]
	miR-17/92 cluster	Cluster shown to target Rb2 and p130 in 3t3 L1 cells during differentiation. miR-92a was found to be higher in MetS and hypercholesterolaemic individuals compared with controls	[51]
	miR-130	Expressed in human adipose tissue. Downregulated in adipose tissue of obese females, targeting <i>PPARα</i>	[52]

^a*ABCA1*, cholesterol efflux regulatory protein; *ALDOA*, aldolase A; *AMPK α 1*, anti-AMP activated protein kinase alpha 1; *CAV1*, caveolin 1; *C/EBP α* , enhancer binding protein alpha; *CS*, citrate synthase; *DVL3*, dishevelled segment polarity protein 3; *ERK5*, extracellular signal-regulated kinase 5; *FABP4*, fatty acid-binding protein 4; *GLUT4*, glucose transported type 4; hADSCs, human adipose-derived stem cells; *IGF1R*, insulin-like growth factor receptor 1; *INSR*, insulin receptor; *IRS1* and 2, insulin receptor 1 and 2; *MCT1*, monocarboxylate transporter 1; *PPAR α* , peroxisome proliferator-activated receptor alpha; Rb2, retinoblastoma-like protein 2; *SIRT1*, Sirtuin 1; *SIRT6*, Sirtuin 6.

elevated in the serum of an Asian cohort with MetS (Table 2, row I) [37]. Levels of both miRNAs increased in parallel with increasing number of MetS components presented. For individuals presenting with four to five components of MetS, let-7 g and miR-122 were both significantly increased. Moreover, the elevation of serum let-7 g was significantly associated with a low level of HDL-cholesterol and high blood pressure [37]. miR-122 has been shown to regulate FA metabolism, cholesterol synthesis, and cholesterol levels [38]. As outlined earlier, Karolina *et al.* [21] conducted a profiling study including 265 individuals comprising five subgroups [i.e., MetS, T2DM, HCL, HPT, and controls] and found three miRNAs to be increased in MetS (miR-197, miR-23a, miR-509-5p) as potential contributors of dyslipidaemia in MetS, correlating with BMI. From this study, miR-130a and miR-195 were proposed as potential contributors of hypertension in MetS, correlating with blood pressure. A plausible association between miR-27a and miR-320a with MetS and T2DM were proposed, as both miRNAs were altered in these conditions.

miR-370 is another miRNA associated with MetS [39]. To the best of our knowledge, data on circulating miR-370 in individuals with full-blown MetS have not been reported. However, circulating levels of miR-370 and miR-122 have been reported for individuals with hyperlipidaemia, a risk factor for MetS [39]. Gao *et al.* [40] reported that in a cohort of 255 hyperlipidaemia individuals presenting with or without CAD in comparison with 100 healthy controls (Table 2, row J), plasma levels of four lipid metabolism-associated miRNAs (miR-122, miR-370, miR-33a, and miR-33b) were investigated. Here, miR-122 and miR-370 were both found to be significantly increased in the plasma of hyperlipidaemia individuals compared with that of healthy controls, while miR-33a and miR-33b were undetected. Interestingly both miR-122 and miR-370 correlated positively with levels of total cholesterol, triglyceride, and **low-density lipoprotein (LDL)**-cholesterol in the plasma of both patient and control cohorts. Of note, miR-122 and miR-370 were both found to be decreased with statin treatment [40], suggesting these miRNAs as both potential diagnostic biomarkers for hyperlipidaemia and predictive biomarkers for response to statins. As management of MetS in practice involves treatment of each component individually and so many individuals diagnosed with MetS are prescribed statins, this highlights the potential importance of miR-122 as a biomarker for MetS and supports an investigation of miR-370.

Circulating mRNA

mRNAs represent a class of RNAs that could be used as minimally invasive tools for the diagnosis and monitoring of MetS [41]. As previously explained, adiponectin gDNA and protein both have potential as biomarkers for MetS. Although it seems that adiponectin mRNA levels have only been studied in adipose tissue only so far, the results warrant investigation of adiponectin mRNA in the circulation of MetS individuals. Specifically, Lihn *et al.* [42] recruited 13 obese females and 30 healthy control females (Table 2, row K) and collected visceral and subcutaneous abdominal adipose tissue biopsies. mRNA analysis revealed a 6-fold decrease in adiponectin mRNA levels in the obese cohort compared with the healthy controls. Additionally, adiponectin mRNA levels were 33% lower in visceral adipose tissue compared with subcutaneous adipose tissue. This result is of interest as visceral fat is a substantial player in MetS.

In a similar study, neprilysin (NEP), a zinc metalloendopeptidase was investigated. NEP is expressed by adipocytes [43], plays a role in inhibiting peptide signalling events, and is involved in the metabolism of a number of regulatory proteins [44]. This study showed that the circulating levels of NEP mRNA in a mouse model of obesity were higher after 15 weeks on a high-fat diet, with a progressive increase from week 7 to week 15 [43]. This suggests a potential of adipose tissue-derived NEP mRNA in the plasma as a biomarker for MetS. However, analysis of NEP mRNA and, indeed, NEP protein in the plasma of appropriate human cohorts is required.

Concluding Remarks and Future Perspectives

MetS, if not diagnosed and managed early on, can contribute to the development of more serious pathologies. So far a simple set of blood tests has not been developed that can screen for or diagnose MetS. Early diagnostic tools will aid towards active management to reduce risks of subsequent morbidities.

Recent research including that of circulating exosomes and microvesicles/microparticles (EVs) and their molecular contents, as well as DNA, RNAs, and proteins taken directly from blood specimens suggest that such blood-based entities may facilitate MetS diagnosis and thus prevent the development of further clinical problems. However, because changes in some or all of these entities (EV quantities, miRNAs, mRNAs, etc.) in blood have been associated with other conditions and pathologies, a unique, reliable, sensitive, and specific biomarker or, panel of biomarkers, must be identified for MetS. Because research to date has not found one single entity that is present only, or absent only, in MetS, compared with healthy controls, when using a panel of biomarkers, the difference would most likely be threshold-based, in which case individual thresholds might have to be determined for each entity involved in the panel. Possibly assessing one entity type will suffice or, most likely, the ideal panel will be a combination of these, for example, changes in circulating EV quantities, protein amounts, and DNA mutations. Although the notion of a (minimally invasive) individual blood specimen is ideal, the downstream analysis could be very complex and may require standardised analytical tests performed by central laboratory facilities rather than single clinics. However, efforts to develop platforms for combinatorial analysis of different blood-based entities for other pathologies are on the way, and they could be adapted for MetS.

Ultimately, beyond the relatively small studies by individual research groups on their choice of circulating entity type, large multicentre adequately powered blinded studies inclusive of males and females, different ethnic backgrounds, ranges in age, etc., are necessary (see Outstanding Questions). Controls should include not only healthy controls but also specimens from individuals with other pathologies (e.g., cancer, autoimmune conditions) to help establish a blood-based biomarker panel that is specific for MetS. Biomarker panels may be identified and validated, but one panel may-not-fit-all, rather different panels may be identified as most suitable for those younger than a certain age or of a different ethnic background. In addition, it is possible that when baseline levels of an eventual panel are established, tracking their changes in longitudinal studies may be informative for the future management of the individual, further aiding personalised medicine, saving healthcare funding and – most importantly – lives.

Acknowledgments

This work was supported by grants from the Danish Council for Strategic Research (DSF 0603-004193), the Danish Dairy Research Foundation, Arla Food Ingredients Group P/S, and the Higher Education Authority's Programme for Research in Third Level Institutions Cycle 5 support of Trinity Biomedical Sciences Institute (TBSI).

Resource

¹ <http://www.who.int/mediacentre/factsheets/fs311/en/>

References

1. O'Neill, S. and O'Driscoll, L. (2015) Metabolic syndrome: a closer look at the growing epidemic and its associated pathologies. *Obes. Rev.* 16, 1–12
2. Kaur, J. (2014) A comprehensive review on metabolic syndrome. *Cardiol. Res. Pract.* 2014, 943162
3. Esposito, K. *et al.* (2012) Metabolic syndrome and risk of cancer: a systematic review and meta-analysis. *Diabetes Care* 35, 2402–2411
4. Nichols, G.A. and Moler, E.J. (2011) Metabolic syndrome components are associated with future medical costs independent of cardiovascular hospitalization and incident diabetes. *Metab. Syndr. Relat. Disord.* 9, 127–133
5. American Diabetes Association (2013) Economic costs of diabetes in the U.S. in 2012. *Diabetes Care* 36, 1033–1046
6. Heidenreich, P.A. *et al.* (2011) Forecasting the future of cardiovascular disease in the United States: a policy statement from the American Heart Association. *Circulation* 123, 933–944
7. Vasan, R.S. (2006) Biomarkers of cardiovascular disease: molecular basis and practical considerations. *Circulation* 113, 2335–2362

Outstanding Questions

Are there blood-based molecules and entities that can reliably be used as biomarkers for MetS?

Will one panel of blood-based biomarkers be adequate – in terms of reliability, sensitivity, and specificity – for diagnosis of MetS?

Will the optimal panel of biomarkers include a combination of EV quantities, DNA, mRNA, and miRNA or will one entity/molecule type prove superior to the others in validation studies?

Can such analysis of relatively simply and painlessly procured blood specimens lead to earlier intervention for those with MetS – and possibly identify those at risk of developing MetS – and thus reduce the societal and economic burden of MetS?

8. Pletcher, M.J. and Pignone, M. (2011) Evaluating the clinical utility of a biomarker: a review of methods for estimating health impact. *Circulation* 123, 1116–1124
9. Biomarkers Definitions Working Group (2001) Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin. Pharmacol. Ther.* 69, 89–95
10. Raposo, G. and Stoorvogel, W. (2013) Extracellular vesicles: exosomes, microvesicles, and friends. *J. Cell Biol.* 200, 373–383
11. Harding, C. *et al.* (1983) Receptor-mediated endocytosis of transferrin and recycling of the transferrin receptor in rat reticulocytes. *J. Cell Biol.* 97, 329–339
12. Pan, B.T. and Johnstone, R.M. (1983) Fate of the transferrin receptor during maturation of sheep reticulocytes in vitro: selective externalization of the receptor. *Cell* 33, 967–978
13. Lässer, C. (2012) Exosomal RNA as biomarkers and the therapeutic potential of exosome vectors. *Exp. Opin. Biol. Ther.* 12 (Suppl. 1), S189–S197
14. Borges, F.T. *et al.* (2013) Extracellular vesicles: structure, function, and potential clinical uses in renal diseases. *Braz. J. Med. Biol. Res.* 46, 824–830
15. Van Deun, J. *et al.* (2014) The impact of disparate isolation methods for extracellular vesicles on downstream RNA profiling. *J. Extracell. Vesicles* 3, 24858
16. Agouni, A. *et al.* (2008) Endothelial dysfunction caused by circulating microparticles from patients with metabolic syndrome. *Am. J. Pathol.* 173, 1210–1219
17. Agouni, A. *et al.* (2011) Microparticles from patients with metabolic syndrome induce vascular hypo-reactivity via Fas/Fas-ligand pathway in mice. *PLoS ONE* 6, e27809
18. Jensen, S. *et al.* (2011) Evaluation of two commercial global miRNA expression profiling platforms for detection of less abundant miRNAs. *BMC Genomics* 12, 435–447
19. Rottiers, V. and Naar, A.M. (2012) MicroRNAs in metabolism and metabolic disorders. *Nat. Rev. Mol. Cell Biol.* 13, 239–250
20. Bartel, D.P. (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 116, 281–297
21. Karolina, D.S. *et al.* (2012) Circulating miRNA profiles in patients with metabolic syndrome. *J. Clin. Endocrinol. Metab.* 97, E2271–E2276
22. Gallo, A. *et al.* (2012) The majority of microRNAs detectable in serum and saliva is concentrated in exosomes. *PLoS ONE* 7, e30679
23. Li, L. *et al.* (2012) Argonaute 2 complexes selectively protect the circulating microRNAs in cell-secreted microvesicles. *PLoS ONE* 7, e46957
24. Turchinovich, A. *et al.* (2011) Characterization of extracellular circulating microRNA. *Nucleic Acids Res.* 39, 7223–7233
25. Arroyo, J.D. *et al.* (2011) Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. *Proc. Natl. Acad. Sci. U.S.A.* 108, 5003–5008
26. Iwaki, M. *et al.* (2003) Induction of adiponectin, a fat-derived antidiabetic and antiatherogenic factor, by nuclear receptors. *Diabetes* 52, 1655–1663
27. Arita, Y. *et al.* (1999) Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem. Biophys. Res. Commun.* 257, 79–83
28. Iwashima, Y. *et al.* (2004) Hypoadiponectinemia is an independent risk factor for hypertension. *Hypertension* 43, 1318–1323
29. Mohan, V. *et al.* (2005) Association of low adiponectin levels with the metabolic syndrome – the Chennai Urban Rural Epidemiology Study (CURES-4). *Metabolism* 54, 476–481
30. Ryo, M. *et al.* (2004) Adiponectin as a biomarker of the metabolic syndrome. *Circ. J.* 68, 975–981
31. Halaas, J.L. *et al.* (1995) Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* 269, 543–546
32. Esteghamati, A. *et al.* (2011) Contribution of serum leptin to metabolic syndrome in obese and nonobese subjects. *Arch. Med. Res.* 42, 244–251
33. Mirza, S. *et al.* (2011) Adiponectin/leptin ratio and metabolic syndrome in a Mexican American population. *Clin. Invest. Med.* 34, E290
34. Gu, H.F. (2009) Biomarkers of adiponectin: plasma protein variation and genomic DNA polymorphisms. *Biomark. Insights* 4, 123–133
35. Li, P. *et al.* (2015) Correlation of serum adiponectin and adiponectin gene polymorphism with metabolic syndrome in Chinese adolescents. *Eur. J. Clin. Nutr.* 69, 62–67
36. Ohashi, K. *et al.* (2004) Adiponectin I164T mutation is associated with the metabolic syndrome and coronary artery disease. *J. Am. Coll. Cardiol.* 43, 1195–1200
37. Wang, Y.T. *et al.* (2013) Circulating microRNAs have a sex-specific association with metabolic syndrome. *J. Biomed. Sci.* 20, 72
38. Lewis, A.P. and Jopling, C.L. (2010) Regulation and biological function of the liver-specific miR-122. *Biochem. Soc. Trans.* 38, 1553–1557
39. Fernandez-Hernando, C. *et al.* (2013) MicroRNAs in metabolic disease. *Arterioscler. Thromb. Vasc. Biol.* 33, 178–185
40. Gao, W. *et al.* (2012) Plasma levels of lipometabolism-related miR-122 and miR-370 are increased in patients with hyperlipidemia and associated with coronary artery disease. *Lipids Health Dis.* 11, 55
41. Miura, N. *et al.* (2008) Serum messenger RNA as a biomarker and its clinical usefulness in malignancies. *Clin. Med. Insights Oncol.* 2, 511–527
42. Lihn, A.S. *et al.* (2004) Lower expression of adiponectin mRNA in visceral adipose tissue in lean and obese subjects. *Mol. Cell. Endocrinol.* 219, 9–15
43. Standeven, K.F. *et al.* (2011) Neprilysin, obesity and the metabolic syndrome. *Int. J. Obes.* 35, 1031–1040
44. von Lueder, T.G. *et al.* (2014) Current role of neprilysin inhibitors in hypertension and heart failure. *Pharmacol. Ther.* 144, 41–49
45. Rotllan, N. and Fernandez-Hernando, C. (2012) MicroRNA regulation of cholesterol metabolism. *Cholesterol* 2012, 847849
46. Yamakuchi, M. (2012) MicroRNA regulation of SIRT1. *Front. Physiol.* 3, 68
47. Davalos, A. *et al.* (2011) miR-33a/b contribute to the regulation of fatty acid metabolism and insulin signaling. *Proc. Natl. Acad. Sci. U.S.A.* 108, 9232–9237
48. Trajkovski, M. *et al.* (2011) MicroRNAs 103 and 107 regulate insulin sensitivity. *Nature* 474, 649–653
49. Esau, C.C. (2008) Inhibition of microRNA with antisense oligonucleotides. *Methods* 44, 55–60
50. He, H. *et al.* (2015) miR-204-5p promotes the adipogenic differentiation of human adipose-derived mesenchymal stem cells by modulating DVL3 expression and suppressing Wnt/beta-catenin signaling. *Int. J. Mol. Med.* 35, 1587–1595
51. McGregor, R.A. and Choi, M.S. (2011) microRNAs in the regulation of adipogenesis and obesity. *Curr. Mol. Med.* 11, 304–316
52. Lee, E.K. *et al.* (2011) miR-130 suppresses adipogenesis by inhibiting peroxisome proliferator-activated receptor gamma expression. *Mol. Cell. Biol.* 31, 626–638