

# Chapter 5

## Role of Biomarkers in Health Care

### Introduction

Study of biomarkers of various diseases will help to improve the management in three ways:

1. By providing a better understanding of the disease pathomechanism
2. By improving the diagnosis and determining the prognosis
3. By providing a basis for development of therapeutics and monitoring the effect of therapeutics on the disease

Biomarkers that will be useful for either disease prediction or treatment should have one or more of several properties, including

- Specific and selective association with illness in a population
- Heritability
- Independently indicate the presence of the disease regardless of the presence or absence of the clinical phenotype
- Co-segregation with disease within families
- Presence in relatives of affected individuals at a higher rate than in the general population

A biomarker should fulfill three criteria to be useful clinically:

1. Accurate, repeated measurements must be available to the clinician at a reasonable cost and with short turnaround times.
2. The biomarker must provide information that is not already available from a careful clinical assessment.
3. The measured level should aid in medical decision making.

Although one single biomarker fulfills all these conditions, multiple relevant disease biomarkers that can be examined concurrently increase diagnostic specificity. Important therapeutic areas that are currently the focus of biomarker discovery are

cancer, metabolic disorders, inflammatory disorders, and diseases of nervous system and cardiovascular system. Some diseases overlap within these categories. Many diseases have multiple biomarkers due to involvement of different pathways. Some biomarkers, e.g., those of inflammation, occur in several diseases characterized by inflammation.

## **Biomarkers of Inflammation**

Inflammation is a component of many disorders that are discussed according to the system involved. Major disorders with inflammation involve cardiovascular and nervous systems. Diseases such as diabetes and rheumatoid arthritis are characterized by inflammation. The complement system is activated in virtually all inflammatory diseases and should therefore serve as a fertile source of biomarkers of inflammation. Traditionally, complement activation has been monitored by measurement of serum C3 and C4, the parent molecules and substrates for enzymatic activation. However, these assays are known to have limited utility for monitoring the inflammatory process.

## **Biomarkers of Oxidative Stress**

Free radical reactions involving reactive oxygen species (ROS) and reactive nitrogen species (RNS) contribute to the pathogenesis and progression of several human diseases. Some of the stress biomarkers are described with other diseases in this report. There is a real need to develop biomarkers that reflect free radical reactions *in vivo*.

### ***Oxidative DNA Damage***

Oxidative injury to macromolecules is implicated in a wide range of pathological conditions. Damage is mediated via free radicals that can be created by a range of agents, e.g., xenobiotics, radiation, ischemia–reperfusion injury, and normal metabolic activity. These free radicals damage DNA leading to mutation, carcinogenesis, or cell death. 8-Oxoguanine is formed by free radical damage to DNA and is a sensitive and specific indicator of oxidative DNA damage. Previously, 8-oxoguanine was difficult to detect, requiring the purification of DNA. However, by utilizing a binding protein with high avidity and specificity for 8-oxoguanine, the OxyDNA Test (Biotrin) provides a simple, convenient, and sensitive fluorescence method for the detection of oxidized DNA.

## ***Proteins as Biomarkers of Oxidative Stress in Diseases***

Proteins are important molecular indicators of oxidative/nitrosative damage. It is not certain whether the presence of oxidatively/nitrosatively modified proteins has a causal role or simply reflects a secondary effect. Only direct identification and characterization of the modified proteins in a given disease can decipher the potential roles played by ROS/RNS-induced protein modifications. MS-based technologies have contributed significantly toward a better understanding of disease processes. The study of oxidative/nitrosative modifications, investigated by redox proteomics, is contributing to establish a relationship between pathological hallmarks of disease and protein structural and functional abnormalities. MS-based technologies can be used for discovery of diagnostic biomarkers of oxidative/nitrosative stress, enabling early detection of diseases. Identification and characterization of oxidatively/nitrosatively modified proteins in human diseases has now started.

### ***1,4-Dihydroxynonane Mercapturic Acid***

4-Hydroxy-2-nonenal (HNE) is a major product of the lipid peroxidation process that is a consequence of free radical reactions. An enzyme immunoassay (EIA) of the major urinary metabolite of HNE, i.e., 1,4-dihydroxynonane mercapturic acid (DHN-MA) has been developed and validated (Gueraud et al. 2006). EIA enabled direct measurement of DHN-MA in rat urine with good sensitivity and precision. Cross-reactivity was very low with 1,4-dihydroxynonene and with different mercapturic acids except with one other HNE urinary metabolite. Good correlation was obtained between EIA and liquid LC/MS quantitation when analyzing urine samples of rats with different oxidative status, due to treatment with either BrCCl<sub>3</sub> or trinitrobenzene sulfonic acid, which are known to induce hepatic lipid peroxidation or colon inflammation, respectively.

## ***Biomarkers in Metabolic Disorders***

There is considerable information available about biomarkers of metabolic disorders. The use of some of these biomarkers is well established. Coverage of all metabolic disorders is beyond the scope of this chapter. Example of acute intermittent porphyria, liver X receptors, diabetes, and the metabolic syndrome will be discussed in this section.

### ***Biomarkers of Acute Intermittent Porphyria***

Acute intermittent porphyria (AIP) is a metabolic disease caused by a deficiency of hydroxymethylbilane synthase, which affects hepatic heme biosynthesis. Clinical

manifestations are abdominal pain and neurovisceral symptoms, accompanied by overproduction of heme precursors in the liver, which frequently remain long-lasting in AIP patients. Treatment is based on symptomatic relief together with carbohydrate loading and in more severe attacks heme therapy. During an acute attack the heme precursors porphobilinogen (PBG) and 5-aminolevulinic acid (ALA) are produced in high amounts by the liver and are found in high concentrations in plasma and urine. These metabolites represent the acute-phase reactants confirming an ongoing attack and are used to evaluate therapeutic measures. Biochemical monitoring of an acute attack is more accurately reflected by plasma PBG than plasma ALA or urinary PBG and ALA (Sardh et al. 2009).

AIP may be associated with alterations of hepatic proteins known to be either increased or decreased in serum according to diverse pathological conditions including malnutrition, inflammation, or liver disease. Most of the serum proteins are within normal limits in these patients; however, insulin-like growth factor 1 (IGF-1) is decreased in 53.8% of AIP patients and transthyretin (prealbumin) is found significantly decreased in 38.5% of them (Delaby et al. 2009). The use of both IGF-1 and transthyretin has been proposed as biomarkers of disease morbidity/severity for the clinical follow-up of AIP patients.

### ***Liver X Receptors***

The liver X receptors (LXRs) are nuclear receptors that play central roles in the transcriptional control of lipid metabolism and are useful biomarkers of metabolic disorders. LXRs function as nuclear cholesterol sensors that are activated in response to elevated intracellular cholesterol levels in multiple cell types. Once activated, LXRs induce the expression of an array of genes involved in cholesterol absorption, efflux, transport, and excretion. In addition to their function in lipid metabolism, LXRs have also been found to modulate immune and inflammatory responses in macrophages. Synthetic LXR agonists promote cholesterol efflux and inhibit inflammation *in vivo* and inhibit the development of atherosclerosis in animal models. The ability of LXRs to integrate metabolic and inflammatory signaling makes them particularly attractive targets for intervention in human metabolic disease. LXR agonists prevent the development and progression of atherosclerosis and inhibit neointima formation following angioplasty of the arterial wall.

### ***Biomarkers of Diabetes Mellitus***

Diabetes mellitus results from abnormal function of pancreatic  $\beta$  cells – specialized structures that produce type 1 diabetes (insulin dependent) generally result from autoimmune destruction of pancreatic islet  $\beta$  cells. Type 2 diabetes mellitus (non-insulin dependent) is caused by the failure of the pancreatic  $\beta$  cells to secrete sufficient insulin to compensate a decreased response of peripheral tissues to insulin

**Table 5.1** Biomarkers of diabetes mellitus

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Biomarkers of hyperglycemia
Increased serum-free fatty acids and ketones
Exhaled methyl nitrate
Biomarkers of diabetes-associated oxidative stress
Elevated serum malondialdehyde, lipid hydroperoxides, and lipoperoxides
Elevated levels of plasma thioredoxin
Elevated superoxide dismutase in RBCs
Elevated plasma protein carbonyl levels
Increased urinary 8-hydroxy-2'-deoxyguanosine
Biomarkers of inflammation
C-reactive protein
Plasma-soluble cell adhesion molecules
Monocyte IL-6
Nitrotyrosine
Biomarkers of renal complications in type 2 diabetes mellitus
Elevated triglycerides
Elevated low-density lipoprotein
Elevated apolipoprotein B
Elevated soluble tumor necrosis factor receptor
Biomarkers of endothelial dysfunction in type 2 diabetes mellitus
E-selectin
Intercellular adhesion molecule 1
Vascular cell adhesion molecule 1
Biomarkers of insulin resistance
Serum retinol binding protein-4
Biomarkers of diabetes with cardiovascular complications
Adiponectin
Glycosylated hemoglobin

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action. LC/MS followed by multivariate statistical analysis has been successfully applied to the plasma phospholipids metabolic profiling in type 2 diabetes mellitus. It is a complement or an alternative to NMR for metabolomics applications. Diabetes has a causative role in many diseases and its complications affect several organs. Biomarkers of diabetes mellitus are shown in Table 5.1.

### **Biomarkers of Hyperglycemia**

Metabolic characteristics of hyperglycemia in the diabetic such as low insulin and increased free fatty acids and ketones in serum are well known. Exhaled methyl nitrate has been shown to strongly correlate specifically with the acute, spontaneous hyperglycemia of type 1 diabetes mellitus in children and can be used as a non-invasive biomarker of hyperglycemia (Novak et al. 2007). Gas analysis was performed on breath samples via gas chromatography using electron capture, flame ionization, and mass selective detection. The kinetic profile of exhaled methyl nitrate, commonly present in room air in the range of 5–10 parts per trillion, was most strongly statistically correlated with that of plasma glucose. In healthy

subjects, exhaled methyl nitrate concentrations are slightly greater than room air concentration, indicating a small net output of this gas by the human body. The biochemical production of this gas has not been postulated; however, the results of this study show that oxidative processes play a major role in its production.

### **Biomarkers of Diabetes-Associated Oxidative Stress**

A study of various biomarkers made in patients with diabetes mellitus, both controlled and uncontrolled, shows that erythrocyte glutathione peroxidase activity, glutathione content, and plasma beta-carotene are significantly lower in diabetic patients compared with normal subjects, with no significant difference between patients in whom the disease is controlled and those in whom it is not. Antioxidant enzyme superoxide dismutase (SOD) activity is significantly higher in the erythrocytes of diabetic patients independently of the presence of microvascular complications. However, the plasma alpha-tocopherol/total lipids ratio is significantly diminished in controlled diabetes group compared with uncontrolled diabetes group. Lipid peroxidation indices measured in plasma included malondialdehyde, lipid hydroperoxides, and lipoperoxides, which are significantly elevated in diabetic patients regardless of the presence of complications, indicate oxidative damage to proteins.

### **Biomarkers of Inflammation Associated with Diabetes**

Type 1 diabetes is associated with increased vascular complications, and monocytes are pivotal cells in atherogenesis. In type 1 diabetic subjects without macrovascular disease, biomarkers of inflammation – C-reactive protein, plasma-soluble cell adhesion molecules, monocyte IL-6, and nitrotyrosine – are significantly elevated compared with control subjects (Devaraj et al. 2006). These results have a major implication on our understanding of the role of inflammation in vasculopathies in type 1 diabetes.

### **Biomarkers of Renal Complications in Diabetes Mellitus Type 2**

Dyslipidemia and inflammation may promote renal disease via mechanisms of vascular endothelial cell dysfunction in type II diabetes mellitus. A cross-sectional study of men with diabetes mellitus type 2 showed that those with a glomerular filtration rate (GFR) <60 mL/min/L had elevated triglycerides, low-density lipoprotein, apoprotein B, fibrinogen, soluble tumor necrosis factor receptor, and vascular cell adhesion molecule-1 (Lin et al. 2006a). There was no association between C-reactive protein and GFR. It was concluded that several potentially modifiable lipid and inflammatory biomarkers are elevated in the setting of moderately decreased GFR in men with type 2 diabetes mellitus and may be the link between renal insufficiency and increased risk for cardiovascular events in this population.

## **Biomarkers of Diabesity**

Diabesity is the term used for the syndrome of gradual progression of obesity to type 2 diabetes with overlapping symptoms of insulin resistance, hyperinsulinemia, hyperglycemia, dyslipidemias, ion imbalance, and inflammation. The Busselton study, in progress in Australia, is trying to identify biomarkers in the blood that will predict the onset of diabetes before clinical symptoms manifest. In 2009, the study was being conducted by Proteomics International, which has expertise in mass spectrometry, in collaboration with the Fremantle Hospital Diabetes Research Group in Australia.

## **Glycosylated Hemoglobin in Diabetes Mellitus**

In persons with diabetes, chronic hyperglycemia, as assessed by glycosylated hemoglobin level, is associated with an increased risk for cardiovascular disease. Combining fasting plasma glucose and glycosylated hemoglobin (HbA1c) improves the accuracy for detecting patients with diabetes mellitus type 1. Combination of HbA1c level and OGTT enables more precise prediction of progression to diabetes mellitus type 2 in subjects with glucose intolerance. The glycosylated hemoglobin value is the primary target for glycemic control. The American Diabetes Association recommends that the blood test – which measures the average level of glycemia over the preceding 2–3 months – be performed at least twice a year in patients whose treatment goals are being met (and who have stable glycemic control) and quarterly in patients whose treatment has changed or whose goals are not being met.

## **Lack of C-Peptide as Biomarker of Complications of Diabetes Type 1**

Pancreatic  $\beta$  cells produce proinsulin, which splits into C-peptide and insulin, and both are released into circulation. C-peptide binds to a receptor at the cell surface and activates signal transduction pathways that result in stimulation of  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase, and endothelial nitric oxide synthase (eNOS), both of which are enzymes with reduced activities in diabetes. In type 1 diabetes patients no proinsulin is produced and neither insulin nor C-peptide is formed. Lack of C-protein is thus a biomarker of early peripheral neuropathy in diabetes before painful neuropathy develops with structural damage to the nerve. Replacement of C-peptide in clinical trials by Creative Peptides (Stockholm, Sweden) has been shown to improve the early signs of peripheral neuropathy such as sensory impairment. There is concomitant improvement of renal dysfunction (normalized glomerular filtration, decreased albumin excretion) and reduces diabetes-induced structural changes (decreases mesangial expansion) after C-peptide replacement.

C-peptide studies by using surface plasmon resonance (SPR) and electrospray mass spectrometry (ESI-MS) showed how proinsulin was found to influence insulin–insulin interactions (Shafgat et al. 2006). These technologies can be used for measurements of C-protein as a biomarker.

### **Serum Retinol Binding Protein-4 as Biomarker of Insulin Resistance**

Insulin resistance has a causal role in type 2 diabetes. Serum levels of retinol-binding protein-4 (RBP4), a protein secreted by adipocytes, are increased in insulin-resistant states. Levels of serum retinol binding protein-4 (RBP4), an adipocyte-derived “signal,” are elevated in insulin-resistant mice and humans with obesity and type 2 diabetes and are normalized by rosiglitazone, an insulin-sensitizing drug. Transgenic overexpression of human RBP4 or injection of recombinant RBP4 in normal mice causes insulin resistance. RBP4 is elevated in the serum before the development of frank diabetes and is a biomarker to identify insulin resistance and associated cardiovascular risk factors in subjects with varied clinical presentations (Graham et al. 2006). Exercise training is associated with a reduction in serum RBP4 levels only in subjects in whom insulin resistance improves. Adipocyte GLUT4 protein and serum RBP4 levels are inversely correlated. These findings provide a rationale for antidiabetic therapies aimed at lowering serum RBP4 levels.

### ***Biomarkers of Metabolic Syndrome***

The metabolic syndrome, which is very common in the general population, is defined by the clustering of several classic cardiovascular risk factors, such as type 2 diabetes, hypertension, high triglycerides, and high-density lipoprotein cholesterol (HDL). Central obesity and insulin resistance, which are the two underlying disorders of the syndrome, are further risk factors for cardiovascular disease. Novel risk factors of the metabolic syndrome include biomarkers of chronic mild inflammation, increased oxidant stress, thrombophilia, and endothelial dysfunction. Therefore, subjects with the metabolic syndrome are potentially at high risk of developing atherosclerosis and clinical cardiovascular events. In recent years several longitudinal studies have confirmed that subjects with the metabolic syndrome present with atherosclerosis and suffer from myocardial infarction and stroke at rates higher than subjects without the syndrome. The risk of cardiovascular disease is particularly high in women with the syndrome and in subjects with pre-existing diabetes, cardiovascular disease, and/or high CRP. However, an increased risk is already present in subjects with a cluster of multiple mild abnormalities. The risk related to the metabolic syndrome is definitely higher when subjects affected are compared to subjects free of any metabolic abnormality.

### **Adiponectin**

Adiponectin, secreted by adipocytes, accounts for approximately 0.01% of total plasma protein. Unlike other adipocyte products, adiponectin correlates with decreased free fatty acid blood concentrations and reduced body mass index or body weight. It has been well established to be an important biomarker for metabolic syndrome and its complications. Animal and cell culture experiments also support most claims from human observations of its roles in the metabolic syndrome.



Reproducible results of human genetic studies of diverse ethnic origin and by different investigators may provide the evidence for its causative roles in the pathogenesis of the metabolic syndrome and further insight into the genetic constitutions of the metabolic syndrome. Some of the common polymorphisms in the promoter region, exon and intron 2, and the rare non-synonymous mutations in exon 3 of the human adiponectin gene were repeatedly shown in many studies from many different ethnic populations to associate with the phenotypes related to body weight, glucose metabolism, insulin sensitivity, and risk of type 2 diabetes mellitus and coronary artery disease. The common polymorphisms and rare mutations of the human adiponectin gene itself were demonstrated to associate with differential expression of adiponectin at the plasma protein level and mRNA level in adipose tissue (Yang and Chuang 2006). The PPARgamma2 Pro12Ala variants were also shown to influence insulin sensitivity in interaction with adiponectin genotype or to influence plasma adiponectin levels. However, the results were not consistent. Three genome-wide scans for the loci that regulate plasma adiponectin concentration suggest that further exploration on chromosomes 5, 9, 14, 15, and 18 is required. These human genetic studies on adiponectin and the metabolic syndrome strongly suggest that adiponectin is one of the causative factors in its pathogenesis and provide significant insights into the genetic make-up of the metabolic syndrome. Extension from these studies may accelerate the discovery of new molecular targets for future therapeutic interventions.

Clinical studies have confirmed that treatment with thiazolidinediones may increase adiponectin concentrations in patients with type 2 diabetes independent from improvements in blood glucose control or parallel treatment with insulinotropic drugs. These findings suggest that adiponectin may have a diagnostic value and can be used especially for monitoring treatment success.

## **Biomarkers in Immune Disorders**

There are a large number of immune disorders that include rheumatoid arthritis, multiple sclerosis (see Chapter 7), type 1 diabetes (see under metabolic disorders in this chapter), systemic lupus erythematosus, and psoriasis. Some of these have inflammation as well. Many of these conditions are characterized by the expression of cell surface biomarkers and cytokines produced by T and B lymphocytes, which can be used to as an indicator of the disease and to monitor patient's response to therapy (O'Hara et al. 2006). Rejection of allografts also involves immune mechanisms and there is an interest in finding predictive biomarkers of organ rejection after transplantation.

### ***Biomarkers of Failure of Transplanted Organs***

The human leukocyte antigens (HLA) encoded by genes within the major histocompatibility complex display an impressive degree of polymorphism. HLA markers are

proteins found on the surface of certain cells in the body. They are used by the body's immune system to identify material that is foreign, such as viruses or bacteria. HLA antibody identification is important for organ transplant donor–recipient matching because, in the case of organ donation, a patient's immune system may fight cells from the donor, causing organ failure or rejection. DynaChip HLA Antibody Analysis System (Life Technologies) is the only approved automated chip-based system for HLA antibody detection and identification.

### **Immunological Biomarkers of Graft Versus Host Disease**

Following transplantation of major organs such as heart, kidney, and liver, rejection of grafted organs is an important problem. There is a need for non-invasive tests to monitor these patients for adjusting their immunosuppressive drug treatment and early detection of rejection. There is a need for discovery of predictive biomarkers for these patients.

Gene expression signatures have been studied in peripheral blood mononuclear cells isolated from patients with graft versus host disease (GVHD) as well as immunosuppressed transplant recipients. TcLandscape<sup>®</sup> technology (TcLand SA) provides a both global and precise picture of T-cell mobilization by combining a quantitative and qualitative assessment of TCR gene usage. Originally developed for analysis and monitoring of T-cell immune responses, the company now develops specific diagnostic biomarkers as well as a proprietary portfolio of therapeutic molecules for grafted patients. TcLand is currently developing a therapeutic antibody that will selectively inhibit T-cell responses directed against the graft. This molecule is a new antagonist of the human CD28 receptor, an important co-stimulatory protein expressed on T cells. Promising preliminary results show that this molecule will be active in the treatment of graft rejection.

According to the NIH consensus development project on criteria for clinical trials in chronic GVHD, the following applications of biomarkers could be useful (Schultz et al. 2006):

- Predicting response to therapy
- Measuring disease activity and distinguishing irreversible damage from continued disease activity
- Predicting the risk of developing chronic GVHD
- Diagnosing chronic GVHD
- Predicting the prognosis of chronic GVHD
- Evaluating the balance between GVHD and graft-versus-leukemia effects (graft-versus-leukemia or GVT)
- Serving as a surrogate end point for therapeutic response

To date, no validated biomarkers have been established for chronic GVHD, although several candidate biomarkers have been identified. With the advancement of many high-throughput “omic techniques” such as genomics, proteomics,

and metabolomics, efforts have been made to understand potential mechanisms of specific graft injuries and develop novel biomarkers for acute as well as chronic rejection (Sarwal 2009). Microarrays are being increasingly used to identify specific patterns of gene expression that predict and characterize acute and chronic rejection and to improve the understanding of the mechanisms underlying organ allograft dysfunction. It is feasible to develop minimally invasive, rapid tests for prognosis and diagnosis in personalized management of transplantation patients.

### **Biomarkers of Renal Allograft Failure**

Survival of renal allografts is limited by chronic allograft deterioration resulting from processes that are difficult to detect in their early stages, when therapeutic interventions would be most effective. Long-term graft loss is still a major problem in renal transplantation. The occurrence of acute rejection episodes and impaired response of the patient to anti-rejection therapy can lead to adverse graft outcome in both the short term and the long term. Currently, clinical features and morphologic assessment of the renal biopsy serve as the basis for assessment of risk for graft failure. Molecular biomarkers have been tested for the following purposes: monitoring for acute rejection; identifying steroid-resistant rejections; and monitoring for clinical transplant tolerance. Predictive biomarkers from easily accessible specimens, such as blood or urine, might improve early diagnosis of graft-damaging processes and help with the identification of patients at particularly high risk of sustained injury, thereby helping to tailor therapy and appropriate follow-up screening.

Despite a large number of studies for the prediction of renal allograft failure, none of the investigated candidate biomarkers is robustly established for widespread clinical use or able to replace biopsies for graft assessment (Boenisch and Chandraker 2008). The combination of molecular analyses and cellular immunological assays is essential for undisputed detection of rejection, reversibility of the rejection by therapy, absence of rejection, and long-term graft outcome (Eikmans et al. 2008).

PlexMark™ 3 Renal Biomarker Panel Assay (Life Technologies Corporation) is a non-invasive and cost-effective research tool for performing kidney function post-transplantation studies rapidly and easily. It uses Luminex® xMAP®, multiplexing technology for bioassay analysis, in a standard immunoassay format to offer ease-of-use, sensitivity, and rapid, reproducible results. It measures levels of cytokines, chemokines, and receptor levels in urine, which enable researchers to better understand immune function and response. This assay provides researchers with an alternative to invasive and expensive procedures, such as biopsies for obtaining kidney tissue samples to enable research into post-transplantation kidney function.

Quantification of miRNAs in primary cultures of human renal epithelial cells (HRECs) has shown that miR-30a-3p, -10b, and let-7c are highly expressed in HRECs, and that stimulation results in a decreased expression of miR-30a-3p (Anglicheau et al. 2009). These studies, in addition to suggesting a cellular basis for the altered intragraft expression of miRNAs, propose that miRNA expression patterns may serve as biomarkers of human renal allograft status.

## **Biomarkers of Lung Transplant Rejection**

Lung transplant patients face a higher death rate than other organ recipients, with 45% dying within 5 years. Until recently, it was not possible to predict transplant failure, and once the signs of chronic rejection appear it is usually too late to reverse it. The aim is to identify patients at risk of chronic rejection before they have the clinical manifestations. Protein biomarkers are an early sign of lung transplant rejection and give an insight into the physiologic mechanisms of rejection. They may provide a guide to the management of lung transplant patients. The dose of anti-rejection drugs should be increased when the biomarkers appear and reduced in patients without these early signs of rejection.

Microarrays were used to assess gene expression in bronchoalveolar lavage cell samples from lung transplant recipients with and without acute rejection on simultaneous lung biopsies (Lande et al. 2007). These studies showed increased expression during acute rejection of genes involved in inflammation, apoptosis, and T-cell activation and proliferation. Studies in a murine heterotopic tracheal transplant model of chronic rejection demonstrated specific patterns of gene expression at defined time points after transplantation in allografts, whereas gene expression in isografts reverted back to that of native tracheas within 2 weeks after transplantation. These studies demonstrate the potential power of microarrays to identify biomarkers of acute and chronic lung rejection. The application of new genetic, genomic, and proteomic technologies is in its infancy, and the microarray-based studies are at an early stage of their application to lung transplantation. The massive amount of data generated per tissue or cell sample has spawned an outpouring of invention in the bioinformatics field, which is developing methods to turn data into meaningful and reproducible clinical inferences.

## ***Systemic Lupus Erythematosus***

Systemic lupus erythematosus (SLE) affects more than a million persons in the United States and Western Europe. It is a chronic B-cell-mediated disease manifested by arthralgias, fever, skin rash, and end-stage renal disease. Although considered a prototypic autoimmune disease, the hallmark of SLE is its heterogeneity. Accordingly, manifestations can vary widely from person to person, with the potential involvement of virtually any bodily organ. Genetic abnormalities underlying this condition are complicated, with diverse genetic polymorphisms described in different ethnic groups, strongly suggesting that the actual pathology underlying the immunologic disarray might not be the same for each patient. There is no cure for this disease.

## **Current Management and Need for Biomarkers**

Only three categories of drugs are currently approved for SLE: corticosteroids, antimalarials, and low-dose aspirin. These are used for symptomatic relief or

non-specific immunosuppression. Until recently, antibodies to dsDNA or nuclear antigens such as Sm antigen and phospholipids or measurement of complement activation were used together with clinical scores as indicators of drug efficacy in clinical trials. Clinical scores are not satisfactory as there is a considerable lag period between initiation of treatment and clinical effects. There are two potential biologic drugs, rituximab (anti-CD20) and anti-CD22, but drug-approval agencies are unable to assess their real efficacy because reliable biomarkers are not available.

The lack of reliable, specific biomarkers not only hampers clinical management of systemic lupus erythematosus (SLE) but also impedes development of new therapeutic agents. Based on available data, several laboratory markers have shown promise as biomarkers for susceptibility, diagnosis, and disease activity. Despite the complexities of the many immunologic pathways that are involved in SLE, biomarkers are emerging to characterize patient subgroups, predict prognosis, indicate the exacerbations and remissions of SLE flares, and serve as end points in the determination of the dosing and timing of immune-modulating treatments. Recently several clinical studies have tested new therapies directly targeting B lymphocytes. Flow cytometry of circulating peripheral B lymphocytes have been used to define pathogenic subsets of the disease and assess therapeutic efficacy. Biomarkers for SLE include Fc receptor genes (disease susceptibility), complement C4d-bound erythrocytes (diagnosis or disease activity), CD27 plasma cells (disease activity), “interferon signature” (disease activity), and anti-C1q antibodies (disease activity and organ involvement). These promising candidate biomarkers need to be validated through rigorous, large-scale multicenter studies. There is still an urgent need for better biomarkers with which to monitor disease activity in patients with SLE.

### **Role of Collaborative Efforts and Databases of SLE Biomarkers**

Large databases and multicenter clinical collaborations are needed to identify and validate biomarkers of SLE. Recognizing the urgent need for lupus biomarkers, a Lupus Biomarker Working Group has been initiated in the United States to facilitate collaborative efforts aimed at identifying and validating biomarkers for SLE. In the EU, BIOLUPUS group aims to apply genomics and proteomics, construct translational databases to the identification of biomarkers of clinical utility, and develop a centralized EU database for SLE. Thirty-five participants from 13 European countries are involved in this enterprise involving clinicians, immunologists, and geneticists.

### **C4d-Bearing Reticulocytes**

Abnormal levels of C4d, an activation-derived fragment of complement component C4, are deposited on the surface of erythrocytes from patients with SLE; thus C4d-bearing reticulocytes may serve as biomarkers of disease activity in patients with SLE (Liu et al. 2005f). CD40L has also been reported to be upregulated in patients with SLE and might be a candidate for SLE biomarker. 2'5'-Oligoadenylate synthetase (OAS) was known previously to be related to SLE and was rediscovered

to be involved in type I interferon pathway in SLE by several microarray gene expression studies recently, which show that pattern of OAS isoform expressions, particularly of OASL, may provide useful information in differentiating disease flares from certain infections in SLE (Ye et al. 2006).

### **Adiponectin**

Adiponectin is an adipocyte-derived cytokine that has antiinflammatory properties. Plasma adiponectin levels are increased in patients with renal SLE compared to healthy controls and patients with non-renal SLE. The urine of patients with SLE and kidney involvement, which was shown previously to contain immunoreactive adiponectin, was reexamined for the presence of specific adiponectin isoforms by non-denaturing gel electrophoresis (Song et al. 2009). High molecular weight adiponectin was found in the urine of patients with active lupus nephritis and may contribute to the renal inflammation of SLE.

### **CB-CAPS**

A new proprietary technology platform for SLE developed at the University of Pittsburgh Arthritis Institute (Pittsburgh, PA) is based on cell-bound complement activation products (CB-CAPS), which is superior to measurement of serum C3 and C4 (Calano et al. 2006). More than 10,000 assays of CB-CAPs bound to erythrocytes, platelets, and white blood cells have been performed. The data suggest that CB-CAPs may serve as universal biomarkers for diagnosis, disease activity monitoring, identification of disease subsets in SLE, and identification of those at risk for catastrophic events such as heart attack and stroke. Initial studies were conducted with a cohort of greater than 300 SLE patients compared with healthy controls and patients with other diseases. The data demonstrate that CB-CAPS have diagnostic as well as disease activity monitoring properties that could enhance or even replace current unsatisfactory modalities. Pilot data have also been generated from patients with HCV infection, cardiovascular disease, transplantation, and complications of pregnancy to support the hypothesis that CB-CAPs may serve as universal biomarkers of inflammation.

### **Genetic Loci of SLE**

The risk of SLE is influenced by complex genetic and environmental contributions. Alleles of HLA-DRB1, IRF5, and STAT4 are established susceptibility genes; there is strong evidence for the existence of additional risk loci. Two new genetic loci for SLE – a promoter region allele associated with reduced expression of BLK and increased expression of C8orf13 and variants in the ITGAM–ITGAX region – have been identified and then confirmed through replication (Hom et al. 2008).

### **Epigenetic Biomarkers of SLE**

Epigenetic aberrations play key roles in the etiology of SLE. Interactions between DNA methylation, histone modifications, and miRNAs are factors in pathogenesis of SLE (Zhao et al. 2009). Advances in our knowledge of epigenetics in SLE will lead to discovery of new diagnostic and prognostic biomarkers as well as novel therapeutic targets and strategies.

Scientists at the University of Michigan (Ann Arbor, MI) have discovered that the levels of methylation of certain genes are closely associated with the onset of SLE. Sensigen licensed these epigenetic biomarkers to develop an assay, EpiSense<sup>TM</sup> Lupus, based on its AttoSense technology, which is combined PCR-MS. This is a quantitative methylation-specific assay for the determination of epigenetic changes in lupus-associated biomarkers.

### **Biomarkers of Musculoskeletal Disorders**

Scientists at Duke University (Durham, NC) have devised a method of determining, in a serum sample, the proportion of the total amount of a biomarker molecule that is derived from catabolism due to the presence of age-related molecular alterations in the molecule. An increased amount of D-aspartate and/or advanced glycation end product in the sample of the subject as compared to the amount of D-aspartate and/or advanced glycation end product in the sample of the control subject is diagnostic of a musculoskeletal, arthritic, or joint disorder in the subject and/or identifies a subject at risk of developing such a disorder. A patent has been filed for this invention.

### ***Biomarkers of Rheumatoid Arthritis***

Rheumatoid arthritis (RA) is a complex inflammatory disease with numerous clinical symptoms and inadequately defined biomarkers. Specific disease, tissue, and prognostic biomarker signatures can be identified and characterized without prior selection of clusters of candidate genes directly in the clinic. These strategies not only redefine biomarkers in complex diseases but also reassess our unappreciated disease mechanisms. RA is the most common inflammatory joint disorder.

Tools are now available to evaluate the target tissues in RA proof-of-concept clinical trials. Synovial biomarkers are not yet qualified as surrogate end points for regulatory purposes. However, evaluation of intermediate synovial biomarkers provides unique opportunities for guided development and decision making. Targeted tissue measurements are particularly useful in diseases like RA where patient stratification and target identification are integrated into the early clinical trial process.

Circulating cytokines have been demonstrated to correlate with RA disease activity and participate directly in disease pathogenesis. Presence of tumor necrosis factor (TNF)- $\alpha$  in serum or synovial fluid is a strong indicator of disease activity

in RA. TNF- $\alpha$  serves as both a biomarker and a target for therapy. Neutralization of TNF- $\alpha$  with an antibody relieves pain and improves mobility in RA patients. Regulatory T cells are functionally compromised in RA and indicate that modulation of regulatory T cells by anti-TNF- $\alpha$  therapy may be a further mechanism by which this disease is ameliorated. Etanercept, a TNF- $\alpha$  inhibitor, reduces the oxidative stress biomarker levels (DNA damage, lipid peroxidation, and protein glycosylation) in patients with RA (Kageyama et al. 2007).

Elevated levels of serum VEGF have been reported in patients with RA. Successful disease therapy results in lowering the levels of VEGF. Serum levels of VEGF have been reduced by administration of an IL-6 antibody with improvement in patients' condition.

The OMERACT 9 Soluble Biomarker Group has successfully formulated a levels of evidence scheme and a study design template that will provide guidance to conduct validation studies in the setting of soluble biomarkers proposed to replace the measurement of damage end points in RA, psoriatic arthritis, and ankylosing spondylitis (Maksymowych et al. 2009).

### ***Biomarkers of Spondylarthritis***

Spondylarthritis (SpA) refers to a group of chronic autoimmune arthritic conditions, including ankylosing spondylitis (AS) and the arthritis associated with psoriasis and inflammatory bowel diseases, primarily affecting peripheral joints. For an international alliance of researchers, synovial tissue analysis presented an ideal vehicle for identifying biomarkers of SpA and hold the promise to facilitate the progress of clinical trials dedicated to improving the treatment of SpA (Kim and Inman 2006). The study included patients who met established criteria for SpA and were randomly divided into three groups. Two groups were treated with a tumor necrosis factor (TNF- $\alpha$ ) blocker – infliximab or etanercept, and the remaining patients served as controls. At the study's inception and again at its culmination, each patient participated in a thorough assessment of disease symptoms and pain. At intervals, synovial biopsies were performed on each patient, through needle arthroscopy of the knee. The synovial tissue samples were then subjected to extensive evaluation. The laboratory tests included measures of abnormal cell growth in the lining layer, vascular growth, markers of cellular infiltration, and metalloproteinases (MMPs) in the lining and sub-lining layers.

In both the anti-TNF- $\alpha$  treatment groups, patients experienced significant improvements in disease activity, pain, and swollen and tender joint counts. In contrast, there was no improvement in the control group. Among the synovial features linked to anti-TNF- $\alpha$  effectiveness, the researchers found changes in subsets of synovial macrophages, in the levels of polymorphonuclear cells, and in MMP-3 expression. Taken together, these findings indicate that, for SpA patients, changes in disease activity are accompanied by series of distinct and measurable events in the peripheral joint. The same synovial features showed a highly different standardized response means between SpA patients receiving effective treatment and control



patients. In validation analyses with independent synovial tissue samples, effective treatment was correctly predicted in 80% SpA patients. Further studies are needed to confirm the value of changes observed as biomarkers at early time points across different therapeutic regimens and to combine synovial assessment with predictors of response to treatment in SpA.

Immunopathologic studies have suggested that the features of spondylarthropathy are distinctive, supporting a prominent role for innate immune cells, and can be consistently differentiated from rheumatoid arthritis. Successful treatment of spondylarthropathy synovitis resulted in rapid and sustained decrease in infiltration by macrophage populations and neutrophils and decreased expression of many proinflammatory mediators. Consistent with studies in rheumatoid arthritis, significant correlations between the effects of both methotrexate and infliximab on disease activity and sub-lining macrophage populations have been reported (Bresnihan 2006). These observations highlight the possibility that macrophage populations may be a synovial tissue biomarker of therapeutic intervention in spondylarthropathy. Preliminary studies have evaluated advanced genomic and proteomic methodologies in spondylarthropathy. A surrogate marker of arthritis activity in spondylarthropathy could profoundly enhance screening for efficacy and optimization of dose ranges in early-phase randomized clinical trials.

### ***Biomarkers of Osteoarthritis***

Osteoarthritis (OA) is a painful disorder of joints characterized by destruction of articular cartilage and remodeling of bone. At the molecular level, loss of the cartilage proteoglycan is clearly evident at a relatively early stage followed by erosion and degradation of other cartilage components (collagen) until the underlying bone is denuded of cartilage. There are some features in common with RA. The breakdown of the cartilage is thought to be mediated by the release of proteolytic enzymes such as matrix metalloproteinases (MMPs). Nitric oxide (NO) plays a role in the pathogenesis of OA. The activation of synovial cells and chondrocytes in OA involves different phases – an early, altered biomechanical, and chronic cytokine-driven auto-destruction, e.g., IL-1. There is a very modest synovial inflammation in OA as compared to RA, with increased MMP (metalloprotease)-1, and MMP-3, but no cytokine overproduction. In the OA cartilage, however, alterations are much more obvious – increased IL-1, TNF (tumor necrosis factor)- $\alpha$ , and iNOS (particularly in the superficial zone). The multiple effects of NO on chondrocytes include increased IL-1, MMPs, apoptosis, proteoglycan, and type II collagen. The production of NO and PGE2 in OA cartilage appears to be responsible for altered biomechanics accompanied by the local increase in inflammatory cytokines (particularly IL-1, but also IL-6 and IL-8) and the differential expression of extracellular matrix elements. IL-1 induces a catabolic phenotype in chondrocytes and increased apoptosis. Antagonizing IL-1-beta, but not TNF- $\alpha$ , decreases NO and PGE2 production by OA cartilage explants. NO dependence has been demonstrated in the increased susceptibility to oxidant injury and in the oxidant stress-induced

apoptosis in cytokine-stimulated chondrocytes. Furthermore, IL-1 induces peroxynitrite and superoxide anion in chondrocytes. The exposure of chondrocytes to peroxynitrite results in Jun N-terminal kinase activation and translocation of NF- $\kappa$ B to the nucleus. MMP-1 and MMP-9 are regulated in OA cartilage through the fibronectin receptor  $\alpha$ 3b1 integrin. One of the functions of intraarticular osteopontin (a major non-collagenous proteins of bone matrix), which is overexpressed in OA cartilage, is to act as an innate inhibitor of IL-1, NO, and PGE2 production. This function is regulated by the interaction of chondrocytes with differentially expressed proteins within the extracellular matrix.

Diagnosis is usually made by radiological studies, imaging of the involved joints and arthroscopy. Efforts are being made to develop a blood-based approach to identify OA of the knee. Genes differentially expressed between mild knee OA and control samples have been identified through microarray analysis of blood samples. Subsequent real-time RT-PCR verification identified six genes that are significantly downregulated in mild OA: heat-shock 90 kDa protein 1,  $\alpha$ ; inhibitor of kappa light polypeptide gene enhancer in B cells, kinase complex-associated protein; IL-13 receptor,  $\alpha$  1; laminin,  $\gamma$  1; platelet factor 4 (also known as chemokine (C-X-C motif) ligand 4); and tumor necrosis factor,  $\alpha$ -induced protein 6. Logistic regression analysis identified linear combinations of nine genes as discriminatory between subjects with mild OA and controls: the above six genes, early growth response 1;  $\alpha$  glucosidase II  $\alpha$  subunit; and v-maf musculoaponeurotic fibrosarcoma oncogene homolog B (avian). Linear combinations of blood RNA biomarkers thus offer a substantial improvement over currently available diagnostic tools for mild OA. Blood-derived RNA biomarkers may be of significant clinical value for the diagnosis of early, asymptomatic OA of the knee.

In 2004, the National Institute of Arthritis and Musculoskeletal and Skin Diseases started recruitment for the Osteoarthritis Initiative, a public-private partnership between the NIH and industry that funds a multisite contract to create a resource to hasten discovery of biological markers for OA. Men and women aged 45 and older at risk for developing OA and those with early disease were eligible to participate. After an initial screening, four centers around the United States each enrolled and followed 1,250 adults for 5 years (total enrollment of 5,000). Biological specimens (blood, urine, DNA), images (X-rays and MRI scans), and clinical data were collected annually. Regional analysis of femorotibial (FT) cartilage loss in a subsample from the Osteoarthritis Initiative progression subcohort showed that the rate of cartilage loss is greater in central subregions than in entire FT cartilage plates (Wirth et al. 2009). Final results may be available in 2010.

## **Biomarkers of Osteoporosis**

Osteoporosis is a progressive systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture. Osteoporosis is an important public

health concern in older women. More than one-third of women will suffer one or more osteoporotic fractures in their life time. Postmenopausal women are at greater risk of osteoporosis; however, not all women will have the same risk of developing osteoporosis. Life time risk among men is less but still substantial.

Bone mineral density (BMD) and molecular biomarkers of bone turnover are useful end points in phase II dose-ranging trials and have the advantage in that they enable shorter trials with fewer subjects. There is no linear relation between changes in BMD and reduction in fracture risk with antiresorptive agents. Interpretation of BMD changes at the individual level requires calculating the smallest significant change at each measurement center. BMD measurement is essential before administration of antiresorptive or anabolic agents for prevention or treatment of postmenopausal osteoporosis. Biochemical markers of bone turnover can be monitored after 6 months of treatment but their interpretation requires careful assessment of their intraindividual variability (Lespessailles 2006). The validity of bone mineral density and biomolecular markers as surrogates for fracture in clinical trials is not yet established. Thus therapeutic confirmatory studies require fracture as an end point. Various fracture end points have been employed including morphometric vertebral fracture, hip fracture, and all clinical fractures.

### ***Dual X-Ray Absorptiometry***

Dual X-ray absorptiometry (DXA) scanners are widely available and offer an inexpensive and precise method to assess bone mineral density at many skeletal sites including the lumbar spine, proximal femur, forearm, and total body. However, the quality of DXA measurements varies widely from clinic to clinic and among geographical regions. In addition, the performance of the scanner itself impacts the quality of the measurement. When adjusting for differences in calibration between manufacturers, better results may be achieved by using standardization equations developed from scans of human beings. The DXA manufacturers have adopted formal, generalized standardization equations developed through the activities of the International Committee for Standards in Bone Measurement. DXA combined with molecular biomarkers of bone degradation is more predictive of fracture risk than either DXA or biomarkers alone.

### ***Bone Imaging with Quantitative CT and MRI***

Quantitative CT (QCT) and MRI enable independent measurements of trabecular and cortical BMD as well as bone structure and derived measures of bone strength. QCT and MRI produce tomographic images of the bone which provide 3D images of bone geometry which can be used to measure cortical bone dimensions.

### ***Assays for Detection of Biomarkers of Osteoporosis***

Type I collagen molecules in bone matrix are linked together by cross-linking molecules including pyridinoline (PYD) and deoxypyridinoline (DPD) in the region of N and C-telopeptides. DPD, which differs from PYD by the absence of a hydroxyl residue, is specific for bone tissue. During bone resorption, pyridinoline is released into the circulation and excreted in the urine in its free form or linked to C-(CTX) or N-(NTX) telopeptides. The different cross-link forms can be measured in serum and urine using specific immunoassays. A targeted use of biomarkers could optimize identification of high-risk patients, the process of drug discovery, and monitoring of the efficacy of osteoporosis treatment in clinical settings.

Roche scientists have evaluated an automated multiplex assay for biomarkers of bone turnover in osteoporosis in only 20  $\mu$ L of serum (Claudon et al. 2008). It showed performance characteristics similar to those of the corresponding individual measurements with increased analytical sensitivity in the low range for CIX-1, PINP, and OC. This assay would be particularly useful for assessing bone turnover profile in clinical studies involving large number of patients and when sample volume is limited.

### ***Biomarkers of Infectious Diseases***

The first clinical sign of sepsis, fever, is usually not typical or specific. Similarly leukocytosis is non-specific. The more typical signs or laboratory parameters such as arterial hypotension or lactate accumulation are often late symptoms associated with organ dysfunction and a rising mortality rate. Traditional laboratory methods for confirming diagnosis of infectious diseases involve microbial identification that relies on morphological features, growth characteristics, and biochemical substrates. Microbiologists have searched for more rapid and efficient means of microbial identification. Nucleic acid amplification technology, PCR, has opened up new frontiers for microbial identification. Advent of molecular diagnostics has provided a tool for faster diagnosis of infections. Even PCR-based methods have time limitation as they cannot be performed within half an hour required for the POC diagnosis of infections. Non-PCR methods have been developed for this purpose and the role of biomarkers that can be detected more rapidly is being explored. The diagnosis of infections will, however, continue to require a critical clinical awareness, careful patient history, dedicated physical examination, and appropriate cultures. Several biomarkers of sepsis are currently available as listed in Table 5.2.

The diagnostic spectrum of the various biomarkers, however, is different. Many biomarkers have been implicated as playing a harmful mediator role in sepsis. Some primarily indicate severity of inflammation (e.g., IL-6), others respond to infection, but do not indicate the host response well (endotoxin, lipoprotein binding protein, triggering receptor on myeloid cells). There are new markers with limited clinical experience, for example triggering receptor on myeloid cells or mid-pro-atrial

**Table 5.2** Biomarkers of sepsis

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Carbamoyl phosphate synthase-1 (CPS-1)
Chemokines
Coagulation system markers
C-reactive protein
Endotoxin
Lactate
Leukocytosis
Lipoprotein-binding protein
Pro-atrial natriuretic peptide
Procalcitonin
Proinflammatory cytokines
Triggering receptor on myeloid cells

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natriuretic peptide (Seristra, Brahms AG, Hennigsdorf, Germany). Recent data and cumulative analyses indicate that biomarkers of sepsis improve diagnosis of sepsis, but only a few biomarkers have impact on therapy and fulfill the clinical requirements. Characteristics of an ideal biomarker of infection are

- High levels in sepsis
- Positive correlation with severity of infection
- Prolonged persistence in the blood
- Should enable an early diagnosis by rapid and accurate bedside measurement
- Should indicate the course and prognosis of the disease
- Should facilitate therapeutic decisions

Several studies indicate that the prohormone procalcitonin, a marker-mediator of sepsis, possesses great potential for meeting all of the above criteria, and that its therapeutic immunoneutralization in humans merits evaluation.

### **Procalcitonin**

Procalcitonin (ProCT), a precursor peptide from the hormone calcitonin (CT), better fulfills the requirements of a desirable biomarker as compared to others and has a solid scientific basis. After translation from CT-mRNA, ProCT is cleaved enzymatically into smaller peptides, finally to yield the 32 amino acid mature CT. Most CT precursor peptides, including ProCT, are found in the serum of normal persons. In microbial infections and in various forms of inflammation, circulating levels of several calcitonin precursors, including ProCT but not mature CT, increase up to several thousand-fold. This increase and especially the course correlate with the severity of the condition and with mortality. A microbial infection induces an increase of CALC-I gene expression and release of ProCT from all parenchymal tissues and differentiated cell types throughout the body.

A commercially available assay is based on time-resolved amplified cryptate emission (TRACE) technology (Kryptor<sup>®</sup> PCT, Brahms, Hennigsdorf, Germany). It used a sheep polyclonal anti-calcitonin antibody and a monoclonal anti-katacalcin antibody, which bind to the calcitonin and katacalcin sequence of calcitonin precursor molecules. Diagnostic accuracy of ProCT has been shown for a variety of infections, e.g., respiratory tract infections, meningitis, acute infectious endocarditis, and pancreatitis. A colorimetric, “quick” bedside version of the test (PCT<sup>®</sup>-Q) has the advantage of rapid determination of circulating CTpr levels in 30 min but the assay is only semi-quantitative and is not sensitive enough to detect moderately elevated ProCT levels.

A ProCT-based therapeutic strategy can safely and markedly reduce antibiotic usage in lower respiratory tract infections, the major cause of sepsis. Being a hormokine mediator, immunoneutralization of ProCT might offer new hope for more effective treatment options in sepsis. It is now evidence that ProCT provides more information and, thereby, questions the currently used “gold standards” for the diagnosis of clinically relevant bacterial infections. Yet, ProCT is less than a perfect marker. ProCT can be increased in non-infectious conditions and may remain low in infections.

A randomized intervention trial, conducted on patients with suspected community-acquired pneumonia at the University Hospital (Basel, Switzerland), assessed ProCT guidance for the initiation and duration of antibiotic therapy (Christ-Crain et al. 2006). The primary end point was antibiotic use; secondary end points were measures of clinical, laboratory, and radiographic outcome. ProCT guidance reduced total antibiotic exposure, antibiotic prescriptions on admission, and antibiotic treatment duration compared with patients treated according to guidelines. Measurements of ProCT, reduced the length of antibiotic treatment by an average of 7 days. ProCT appears to be a more reliable measure for individual tailoring and early discontinuance of antibiotic therapy as compared with the routinely used clinical and other parameters. It is also time- and cost-effective. It took less than 20 min to detect levels of serum procalcitonin in the laboratory and results were routinely available within an hour.

## **Endotoxin**

Endotoxin has been a candidate as a diagnostic tool for infection for several years. However, inconsistently increased levels, variations in sensitivity and specificity in different patients groups and lack of correlation with severity of inflammation and the host response did not support clinical use. It has been reevaluated now by a highly sensitive biological assay, endotoxin activity assay (EAA<sup>TM</sup>, Spectral Diagnostics Inc.), which has been approved by the FDA for use in the United States. This ex vivo whole-blood kinetic luminometric assay for zymosan- and anti-endotoxin-antibody elicited respiratory burst. The antibody is directed against lipopolysaccharides of various Gram-negative bacteria. In clinical studies the assay had a high negative predictive value for diagnosis of Gram-negative infections and was shown to be useful in excluding serious bacterial infections in patients admitted

to ICU. Because of the low response to severity of infection, it may have a limited value as guide to therapy. Also the low specificity may restrict clinical use. Future studies will indicate, whether other biomarkers have a similar sensitivity at a given low specificity.

### **Chemokines as Biomarkers of Infection**

Chemokines are a superfamily of small peptides involved in leukocyte chemotaxis and in the induction of cytokines in a wide range of infectious diseases. These peptides are secreted by tissue cells, leukocytes, and activated epithelial cells. Four different subfamilies can be identified based on the highly conserved presence of the first two cysteine residues, which are either separated or not by other amino acids: the CC chemokines, the CXC chemokines, the CX3C chemokines, and the C chemokines. Chemokines act through a family of chemokine receptors, which are present on cell types such as leukocytes, dendritic cells, and endothelial cells. Chemokines and their receptors play an important role in the innate immunity against infectious diseases such as HIV/AIDS and malaria. Measurement of the serum levels of CXC and CC chemokines during the initial phase of meningococcal sepsis in children can predict mortality and can correlate strongly with disease severity (Vermont et al. 2006). Chemokines may play a key role in the pathophysiology of meningococcal disease and are potentially new targets for therapeutic approaches.

### **Circulating CPS-1 as Biomarkers of Organ Damage in Sepsis**

Mitochondrial damage and dysfunction are considered to play an important role in the pathogenesis of sepsis-induced organ failures. Unfortunately, there is paucity of specific biomarkers of mitochondrial damage in vital organs. Recently, carbonyl phosphate synthase (CPS)-1, a protein primarily localized to liver mitochondria, was found to be present in high concentrations in the plasma of patients with sepsis. A prospective, randomized, controlled animal study has verified that circulating CPS-1 is a marker of mitochondrial damage and depletion in the liver during the subacute phase of sepsis (Crouser et al. 2006). From a mechanistic standpoint, mitochondrial depletion is not due to cell death but is apparently related to the removal of damaged mitochondria by lysosomes, followed by repletion of mitochondrial populations. Restoration of mitochondrial populations in the liver and reduced levels of CPS-1 appears to signal recovery from sepsis. CPS-1 may be superior to conventional markers of liver damage during sepsis. Further studies are needed to determine the clinical utility of CPS-1 as a marker of severity of sepsis.

### ***Application of Proteomics for Discovering Biomarkers of Infections***

Proteomic technologies have been used for detection of protein biomarkers of infection. For example, a novel mass spectral fingerprinting and proteomics approach using MALDI-TOF MS was applied to detect and identify protein biomarkers of

group A *Streptococcus* (GAS) strains (Moura et al. 2008). Specific biomarkers were found for each strain, and invasive GAS isolates could be differentiated. GAS isolates from cases of necrotizing fasciitis were clustered together and were distinct from isolates associated with non-invasive infections.

In 2008, the National Institute of Allergy and Infectious Diseases of NIH granted up to \$10.9 million to the University of Texas Medical Branch (UTMB) at Galveston to seek biomarkers for infectious diseases. UTMB will use the 5-year contract to establish the Clinical Proteomics Center for Infectious Disease and Biodefense. UTMB researchers will search for proteins that are created by pathogens or made by the human body in response to infection. These proteins could be used as indicators of disease to develop diagnostics, therapies, or vaccines. The first two diseases the centers will study include dengue fever and brucellosis. Any candidate biomarkers will be made publicly available for free to the research community for further development. These centers also will assess samples submitted from outside institutions for protein biomarkers.

### ***Systemic Inflammatory Response Syndrome***

Sepsis is now defined as a systemic inflammatory response syndrome (SIRS) in which there is an identifiable focus of infection. SIRS can be also precipitated by non-infective events such as trauma, pancreatitis, and surgery. As a consequence of an overactive SIRS response, the function of various organ systems may be compromised, resulting in multiple organ dysfunction syndrome and death. Efforts are being made to identify biomarkers for prognosis in SIRS.

Chromogranin A (CGA) is a biomarker of stress released with catecholamines by the adrenal medulla and has been previously associated with cardiovascular disease and cancer. Serum CGA concentrations are significantly increased in SIRS patients when compared to healthy controls. Highest increase in CGA is seen in patients where infection is associated with SIRS. CGA concentrations positively correlate with biomarkers of inflammation (procalcitonin, CRP), as well as with Simplified Acute Physiological Score (SAPS). Patients with CGA concentration above 71  $\mu\text{g/L}$  have a significantly shorter survival (Zhang et al. 2009).

### ***Tuberculosis***

*Mycobacterium tuberculosis* is the most common bacterial infection in the world, affecting approximately 2 billion people. However, because of the unique nature of this organism, only 10–15% of those infected will ultimately develop the disease. Tuberculosis (TB) is the world's most neglected health problem, killing 3 million people each year – more than all the other infectious diseases combined. Unless diagnosed, active TB is an often fatal condition, and the patient with active TB will spread the disease to an average of 10–15 others per year. Tuberculosis incidence is



increasing in both developed and developing countries. One reason for the sharp increase in TB infections is the development of antibiotic resistant TB strains, including some that are resistant to multiple drugs. World Health Organization (WHO) estimates that this disease will infect 1 billion persons and claim more than 35 million lives in the next 20 years. In the United States alone, approximately 15 million residents are infected. Worldwide, one in three persons harbors the causative organism, *M. tuberculosis*. According to WHO, over 1 billion TB tests are performed yearly 2,000 and this number is projected to increase.

Tuberculosis is reemerging as an important cause of human disease, particularly in HIV-infected patients who experience severe immunosuppression. Approximately 14% of all cases of TB are associated with HIV, and most tuberculosis infections predominantly involve the lung. Halting the spread of tuberculosis requires a multifaceted approach incorporating early diagnosis, appropriate antimicrobial therapy, proper patient isolation, screening of high-risk populations, and enhanced laboratory biosafety. In the effort to prevent a late-twentieth-century epidemic from becoming a major scourge of the twenty-first century, laboratory personnel and methods will play a key role. Early and prompt diagnosis, particularly in HIV-infected individuals, can reduce the morbidity and mortality of tuberculosis.

### **Conventional Diagnosis of Tuberculosis**

Tuberculosis is generally diagnosed by a combination of generalized and specific symptoms along with findings of various laboratory tests. Two widely used tests are the tuberculin skin test and acid-fast microscopic smear. Although both provide rapid results, neither is especially reliable. Skin testing does not distinguish latent infection from active tuberculosis. In addition, distinguishing *M. tuberculosis* from an atypical mycobacterium can prove difficult under the microscope. For reliable detection, a large number of organisms must be present. Sputum smears are positive in only one-half to three-quarters of cases. Culture to distinguish mycobacteria from atypical forms and to determine antibiotic sensitivity takes as long as 3–6 weeks. This distinction is important because atypical forms of mycobacteria do not respond to conventional antibiotics. This time lag in diagnosis, however, delays both the isolation of this contagious disease and the initiation of treatment. The emergence of multidrug-resistant (MDR) tuberculosis has further aggravated attempts to eradicate this infection. MDR organisms are not only resistant to conventional and antimicrobial therapy but also associated with a high mortality and rapid occurrence of death.

### **Molecular Diagnostics for Tuberculosis**

Molecular technology is now available to provide detection, identification, and antimicrobial sensitivity testing of mycobacteria. Ideally, a molecular probe would provide these functions directly in a clinical sample, with the sensitivity of a culture,

but in a matter of hours rather than weeks. Such rapid results will be essential to provide optimal care for patients infected with *M. tuberculosis* or other mycobacterium species and to limit the spread of tuberculosis.

The FDA approved the Amplified Mycobacterium Tuberculosis Direct (AMTD) test (Gen-Probe, San Diego, California) in 1996. In various studies where the AFB (acid-fast bacilli) smears were cultured, the sensitivity of AMTD was 85.5% and its specificity was 100%. This test, which combines Gen-Probe's transcription-mediated amplification and HPA technologies, yields results in 4–5 h. It can be used on patients who do not have cultivable *M. tuberculosis* but continue to shed these microorganisms. In addition, AMTD can aid in monitoring patients who have been treated with antitubercular drugs.

### **Biomarkers for Tuberculosis**

Large-scale studies have been initiated aiming to identify biomarkers of *M. tuberculosis* infection and disease. Key finding from recent times is that no one factor seems able to explain the complex course of *M. tuberculosis* infection. Multifactorial analyses have identified a variety of genes and proteins, mostly involved in bacterial persistence or host responses, that offer promise as biomarkers for different disease stages. Candidate biomarkers should differentiate people with active tuberculosis from healthy individuals, normalize with therapy, and reproducibly predict clinical outcomes in diverse patient populations (Wallis et al. 2009). Although a large number of promising candidate biomarkers have been examined to date, few patients in these studies have reached clinically meaningful outcomes, and few of the studies have been conducted to international research standards. The challenge now is to validate the suggested biomarkers being described and then reduce them to clinical practice (Doherty et al. 2009). If this can be done, it offers the possibility of greatly improved clinical management of tuberculosis, allowing segregation of patients and contacts into appropriate treatment regimens.

Diagnosis of tuberculous meningitis (TBM) is difficult. Rapid confirmatory diagnosis is essential to initiate required therapy. The presence of 65 kD heat-shock protein (hsp) antigen in the CSF of confirmed and suspected cases of TBM would indicate that the selected protein is specific to *M. tuberculosis* and could be considered as a diagnostic biomarker for TBM (Mudaliar et al. 2006).

### **Biomarkers of Pulmonary Tuberculosis in the Breath**

Pulmonary tuberculosis may alter volatile organic compounds (VOCs) in breath because mycobacteria and oxidative stress resulting from mycobacterial infection both generate distinctive VOCs. A study was conducted to determine if breath VOCs contain biomarkers of active pulmonary tuberculosis (Phillips et al. 2007). Head space VOCs from cultured *M. tuberculosis* were captured on sorbent traps and assayed by gas chromatography/mass spectroscopy (GC/MS). Breath VOCs were assayed by GC/MS in patients hospitalized for suspicion of pulmonary tuberculosis and in healthy controls. Sputum cultures were positive for mycobacteria

in 23/42 and negative in 19/42 patients. Pattern recognition analysis and fuzzy logic analysis of breath VOCs independently distinguished healthy controls from hospitalized patients with 100% sensitivity and 100% specificity. The study concluded that volatile biomarkers in breath were sensitive and specific for pulmonary tuberculosis: the breath test distinguished between “sick versus well,” i.e., between normal controls and patients hospitalized for suspicion of pulmonary tuberculosis, and between infected versus non-infected patients, i.e., between those whose sputum cultures were positive or negative for mycobacteria. However, since these findings were derived from a comparatively small pilot study, confirmation will require additional studies in larger numbers of patients.

### ***Biomarkers of Viral Infections***

Whereas most viral infections can be tested by either immunoassays or DNA, the latter provides the benefit of an earlier, more specifically accurate test. This is because an immunoassay detects only the presence of an antibody to the virus, which cannot be measured until the immune system has actually produced an antibody in the blood. In diseases such as HIV and hepatitis, antibody generation can lag behind infection by as long as 6 months. DNA tests, on the other hand, look for the antigen or the virus itself; it is not necessary to wait for the body to produce antibodies, thus providing earlier detection. Biochemical tests for infections are more prone to human error and require extensive quality control with each new lot. Biochemical-based identification can take up to several days, compared to just several hours for DNA-based tests.

#### **Viral Hepatitis**

Approximately 85% of all acute viral hepatitis cases are due to the familiar hepatitis A–E viruses. For the diagnosis of hepatitis A, D, and E, serologic markers are usually adequate. In contrast, molecular diagnosis is important in hepatitis B and C. The cause of hepatitis infection in nearly 15% of the cases continues to baffle physicians.

*Hepatitis A virus (HAV).* This is the most common cause of viral hepatitis worldwide. This small, single-stranded RNA virus belongs to the enterovirus genus of the picornavirus family. HAV infection usually produces a brief illness and does not lead to a chronic carrier state or chronic hepatitis. A PCR-based assay can be used to detect HAV antibodies as well as to differentiate between genotypes I and II. In addition, a modified PCR test can detect intact virus particles while ignoring fragments of genetic material from any virus destroyed during the sterilization process. In this method, the blood product is incubated with an HAV-specific monoclonal antibody before the viral RNA is transcribed, amplified, and identified as DNA. The antibody captures any intact virus and the PCR test then identifies the viral nucleic acid, making a false-positive result less likely.

*Hepatitis B virus (HBV)*. There are approximately 300 million chronic carriers of HBV worldwide, representing a global health-care challenge. Exposure to contaminated blood is the major source of infection, but other modes of transmission are possible (e.g., inoculation of the ocular surface during corneal transplants). Chronic HBV infection is responsible for much of the world's liver cirrhosis and is implicated in a high percentage of cases of liver cancer. Situations in which screening for this virus is warranted include the following:

- Clinical suspicion of hepatitis B
- Blood donors and blood products
- Monitoring of responses to vaccination

Because HBV is difficult to culture, its presence is typically demonstrated by electron microscopy. Although the existence of HBV surface antigen (HbsAg) in serum or plasma indicates HBV infection, detection of HbsAg does not provide information on the replicative activity of the virus. Hepanostika HBsAg Ultra assay (bioMerieux), a CE-approved test launched in Europe/Middle East, offers state-of-the-art sensitivity and excellent specificity for the detection of HBV surface antigen in human plasma or serum. The level of HBV DNA in serum or plasma probably reflects the replicative activity of HBV. Various techniques for detection of HBV DNA have been developed, including hybridization assays (which generate quantitative results but lack sensitivity) and PCR (which offers superior sensitivity but predicts only qualitative results, though these findings are important as treatment guides). Methods for quantitative assessment of HBV DNA include Bayer's HBV DNA assay and Abbott's HBV DNA assay. Monitoring the level of HBV may help identify those individuals who are most likely to respond to antiviral therapy, evaluate the efficacy of therapy, and track the infection and viral burden after therapy. Such monitoring provides several benefits:

- It facilitates the tracking of viral load reduction and enables early identification of relapses.
- HBV RNA level at any given time point is predictive of response to therapy.
- The standardization and reproducibility of the assay, as demonstrated on specimens taken at different times and in different places in clinical trials, are helpful in evaluating test results.

Viral hepatic diseases, especially those induced by the HBV, can progress into more serious pathological outcomes and eventually to hepatocellular carcinoma. A growing body of evidence indicates that many trace elements play important roles in a number of carcinogenic processes that proceed through various mechanisms. Markedly elevated Cu:Zn ratios are found in patients having hepatic cirrhosis or hepatocellular carcinoma. These findings imply that the levels of some trace elements, such as selenium, iron, copper, and zinc, and Cu: Zn ratios, might serve as biomarkers for the increased severity of viral hepatic damage (Lin et al. 2006).

*Hepatitis C virus (HCV)*. HCV affects roughly 170 million people around the world. Approximately 5 million persons have chronic HCV infection in the United States, 30,000 new infections are diagnosed each year, and 8,000 infected patients die. In Europe, the number of patients with chronic HCV infection is estimated to be 5–10 million. The number of HCV-antibody-positive individuals is as high as 10–20 million. Acute HCV infection develops into chronic disease in 85% of all cases, setting the stage for development of liver cirrhosis and hepatocellular carcinoma.

The introduction of the approved immunoassay EIA has reduced the incidence of HCV transmission via blood transfusion. It is the first test administered to patients with clinical liver disease. Another test available for HCV is an immunoblot assay (RIBA-2). Both methods are of limited use, however, because a period of several weeks separates infection and seroconversion. In addition, loss of antibody in some persistently infected individuals has been reported. Another monitoring method, which measures the levels of the liver enzyme alanine aminotransferase (ALT), can also give misleading results because fluctuations do not correlate with the levels of HCV. For example, ALT levels may normalize during therapy despite persistent, detectable levels of HCV RNA. If hepatic damage is minimal or has not developed yet, ALT levels may remain normal during active HCV infection. Illnesses other than HCV (e.g., alcoholism) may also produce abnormal ALT values, complicating diagnosis. Direct detection of HCV is valuable in the following situations:

- Diagnosis of neonates born to a seropositive mother
- Diagnosis of HCV infection in seronegative individuals
- Diagnosis in organ transplant recipients
- Assessment of antiviral therapy with interferon-alpha (IFN- $\alpha$ )

Although the recommended treatment for chronic HCV infection involves a 48-week course of PegIFN- $\alpha$ -2b or PegIFN- $\alpha$ -2a combined with ribavirin (RBV), the therapy cures ~40% of those with HCV, and the response is even lower in African-American populations. In addition to limited efficacy, treatment is often poorly tolerated because of side effects that prevent some patients from completing therapy. For these reasons, identification of a biomarker of response to treatment is a high priority. A genetic polymorphism near the IL28B gene, encoding IFN-lambda-3, has been reported to be associated with an approximately twofold change in response to treatment, both among patients of European ancestry and African-Americans (Ge et al. 2009). Almost 80% of those with the favorable response genotype eradicated the virus, while only about 30% with the less favorable response genotype did so. Because the genotype leading to better response is in substantially greater frequency in European than African populations, this genetic polymorphism also explains approximately half of the difference in response rates between African-Americans and patients of European ancestry. On the other hand, among African-Americans who did carry the CC genotype, treatment response was 53.3% – higher than the 33.3% treatment response observed among individuals of European descent who had the TT genotype. The favorable C allele also tended to

be found less frequently in those with chronic HCV infections, suggesting a role in overall viral clearance. Unexpectedly though, the authors reported that the C alleles actually appeared to be linked to higher rather than lower baseline viral loads. More research is needed to determine whether the newly identified SNP is a biomarker for other important genetic changes or whether the change itself directly influences treatment outcomes.

### **Biomarkers of SARS**

SARS (severe acute respiratory syndrome)-associated coronavirus (SARS-CoV) has been confirmed as the pathogen for SARS. Several companies are working to develop diagnostics for SARS virus, which are based on detection of the virus.

Cytokines, growth factors, and other biomarkers are important indicators of the inflammatory response to infection. Cytokine profiles can also provide a useful tool for the diagnosis and management of the SARS. Acute infections can cause a rapid increase in cytokine levels, an exaggerated response to a high viral load which can be detrimental to the individual. Huge elevations in a variety of cytokine biomarkers may alert clinicians to the severity of the infection and ensure that priority patients are managed immediately to prevent further spread. Biomarkers that have been elevated in SARS are IFN-g, IL-1b, IL-6, IL-12, and MCP-1. Cytokine response has been shown to affect the mortality and morbidity of the patient with communicable diseases. Biochip array technology (Randox Laboratories) offers a blood testing system that can offer rapid cytokine profiling of patient samples. The system uses a panel approach to diagnostic profiling enabling simultaneous measurement of numerous markers in minutes. Ease-of-use and minimal operator intervention are key benefits of a system that can measure and quantify many clinical markers. Risk of infection to laboratory personnel is limited as sample handling is fully automated and onboard disposal compartments ensure isolation of contaminated waste. The system boasts a test throughput of over 800 cytokine test results per hour, enabling rapid profiling of numerous patients within the time constraints of the WHO regulations.

### **Biomarkers of HIV**

The major cause of AIDS in the world is the retrovirus HIV type 1 (HIV-1). Worldwide, an estimated 18 million persons are infected with HIV, including more than a million individuals in the United States. HIV infection is predominantly a sexually transmitted disorder, although other modes of transmission (e.g., infected blood transfusion and intravenous drug abuse via infected needles) are well recognized.

Direct detection of HIV-1 is difficult because only a small number of cells harbor the virus, a small number of proviral copies exist in each infected cell, and the viral genome has a tendency toward transcriptional dormancy. Nevertheless, a number of assays have been developed to detect the presence of HIV-1 infection and quantify the level of virus in the blood of infected individuals:

- EIA tests for the detection and quantification of HIV-1 p24 antigen
- Western blot
- Latex agglutination
- Radioimmunoprecipitation
- Immunofluorescence for the detection of antibodies to HIV-1
- Viral cultures for the isolation and semi-quantification of HIV-1

HIV-associated neuropsychological impairment is frequent among HIV-1-infected patients but the incidence of HIV dementia has declined since the introduction of HAART therapy (zidovudine, lamivudine, and ritonavir boosted indinavir). Improvement of neurocognitive function parallels by normalization of CSF neural markers (NFL, Tau, and GFAP) levels and a decline in CSF and serum neopterin and CSF and plasma HIV-1 RNA levels (Andersson et al. 2006).

APOBEC3G (apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like 3G; also known as CEM15 or hA3G) is a novel cellular factor of innate immunity that inhibits HIV replication in vitro by causing G to A hypermutations, and consequently reduced relative infectivity of each virus produced by infected cells. Quantification of CEM15 mRNA levels in patient samples as a prognostic indicator of innate HIV/AIDS disease resistance and predicting whether a viral-infected patient will be categorized as a long-term non-progressor (LTNP), which has a much slower disease progression rate (Jin et al. 2007). This also provides a method of predicting the level of CD4 cells in a patient, as well as a method of optimizing antiviral therapy in a viral-infected patient, and has significant implications on new development of diagnostic tools and therapeutic targets to treat viral infections.

### ***Biomarkers in Parasitic Infections***

Parasitic infections are still endemic in developing countries. Role of biomarkers in two common parasitic infections, malaria and schistosomiasis, will be discussed here. Biomarker studies in parasitic infections are complicated by the simultaneous infection with multiple parasites in the same individual.

#### **Role of Biomarkers in Malaria**

The incidence and severity of malaria infection continue to be on the rise in many parts of the world. The situation is exacerbated by the emergence of multidrug resistance to *Plasmodium falciparum* and *Plasmodium vivax*, the two most important human malaria parasites. Malaria is a complex infectious disease in which the host response to infection is dependent on the parasite stage, parasite virulence factors, and host genetic background. Diagnosis can be established by identification of the parasite in blood smears. There is still a need to understand the molecular processes

that regulate transcriptional activity and gene networks involved in the pathogenesis of or protection from disease as they may provide insights into protective mechanisms of immunity that aid in the design of more effective vaccines.

An analysis of the gene expression profiles has identified a set of host biomarkers, which distinguish between lethal and non-lethal blood stage murine malaria infections with *Plasmodium yoelii* (Schaecher et al. 2005). Multiple biological replicates sampled during the course of infection were used to establish statistically valid sets of differentially expressed genes. Genes that correlated with the intensity of infection were used to identify pathways of cellular processes related to metabolic perturbations, erythropoiesis, and B-cell immune responses and other innate and cellular immune responses and provide insights into transcriptional regulatory mechanisms that influence both the pathogenesis of disease and the host's recovery from infection. While immune responses in human *P. falciparum* and *P. vivax* malaria may share many similar features of the global gene expression program observed in murine malaria, important differences in expression profiles in humans infected clinically or experimentally with malaria will depend on the type of tissue (peripheral blood, bone marrow, spleen, or brain) and the stage of infection (early asymptomatic versus clinical malaria) that is studied.

Because acquisition and maintenance of antimalarial antibodies depend on exposure to malaria infection, such antibodies might be used as biomarkers of transmission intensity. Measurement of these antibodies by serological tests may detect variations in malaria transmission over time and will be invaluable for monitoring trends in malaria endemicity and the effectiveness of malaria control programs. Molecular biomarkers have been investigated for assessing resistance to antimalarial drugs but no conclusive information is available as yet. Efforts to use plasma levels of sTNF-R75 and circulating parasite DNA to estimate sequestered loads of *P. falciparum* have not been successful so far.

It is important to identify individuals infected with *P. falciparum* who are at risk of developing serious complications such as cerebral malaria. Serum angiopoietin-1 and the angiopoietin-2/1 ratio are promising clinically informative biomarkers for cerebral malaria (Lovegrove et al. 2009). Further studies should address their usefulness as prognostic biomarkers and potential therapeutic targets in severe malaria.

### **Identification of Biomarkers in Schistosomiasis Infections**

Schistosomiasis is the second most prevalent human parasitic disease after malaria and affects more than 200 million people worldwide. The eggs produced when infected by *Schistosoma mansoni* produce complex and unique protein- and lipid-linked glycans, which are important activators and modulators of the host's immune response.

Current diagnosis of schistosomiasis is not ideal. The egg detection by microscopy is specific, but lacks sensitivity and suffers from highly fluctuating egg output. Antibody-based diagnosis is sensitive but fails to reliably identify active



infections. Antigen detection-based assays have a number of advantages but fail to detect minimal infections. This has prompted search for biomarkers of the disease.

Scientists have discovered that in addition to the glycoprotein and glycolipid antigens, *Schistosoma* eggs also excrete unique unconjugated oligosaccharides, which can be identified by using an affinity purification method based on a specific antigenic MAb. These oligosaccharides appear as biomarkers of infection in the urine of infected individuals and can be detected by mass spectrometry. The identification of new small-molecule biomarkers may lead to a new egg-load-related assay for light infections in schistosomiasis but may also be used as a measure of infection and morbidity.

## **Biomarkers of Liver Disease**

Over the past decade, there has been a renewed enthusiasm to develop non-invasive serum markers or tests to assess the presence and severity of fibrosis in chronic liver disease. Although a single marker or test has lacked the necessary accuracy to predict fibrosis, different combinations of these markers or tests have shown encouraging results. However, inter-laboratory variability and inconsistent results with liver diseases of varying etiologies have made it difficult to assess the reliability of these biomarkers in clinical practice. Current toxicity testing methods often fail to identify human liver toxicity issues. Therefore, liver toxicity is often detected for the first time when drugs are in phase II of clinical testing after a considerable amount of money has been spent on a drug. Efforts are being made to develop biomarkers for detecting hepatotoxicity at preclinical stage of drug development.

### ***Breath Biomarkers of Liver Disease***

Breath biomarkers have the potential to offer information that is similar to conventional clinical tests or they are entirely unique. Preliminary data support the use of breath biomarkers in the study of liver disease, in particular non-alcoholic fatty liver disease (Solga et al. 2006). It was evaluated whether breath ethanol, ethane, sulfur compounds, and acetone would be associated with hepatic histopathology among morbidly obese patients presenting for bariatric surgery. Breath samples were collected during a preoperative visit and compared with liver biopsies obtained during the surgery. A Student's two-tailed *t*-test was used to compare differences between the two groups. Linear regression was used to analyze associations between the concentrations of breath molecules and independent predictor variables. It was found that breath ethanol, ethane, and acetone can be useful biomarkers in patients with non-alcoholic fatty liver disease. In particular, breath ethanol can be associated with hepatic steatosis, and breath acetone can be associated with non-alcoholic steatohepatitis.

## ***Biomarkers of Viral Hepatitis B and C***

Histologic examination of a liver biopsy specimen is regarded as the reference standard for detecting liver fibrosis. Biopsy can be painful and hazardous, and assessment is subjective and prone to sampling error.

Among the non-invasive alternatives to liver biopsy, several studies have demonstrated the predictive value and superior benefit/risk ratio to biopsy of two combinations of simple serum biochemical markers in patients infected with hepatitis B virus (HBV) and hepatitis C virus (HCV). These include FibroTest (BioPredictive) for the quantitative assessment of fibrosis and ActiTest (BioPredictive) for the quantitative assessment of necroinflammatory activity (HCV-FibroSURE, LabCorp). These tests, which are now available in several countries, can facilitate the screening and management of the most frequent liver diseases. Other biomarkers are being investigated.

A panel of sensitive automated immunoassays was developed to detect matrix constituents and mediators of matrix remodeling in serum to evaluate their performance as biomarkers in the detection of liver fibrosis in comparison with biopsy specimens obtained from subjects with chronic liver disease at fibrosis stage (Rosenberg et al. 2004). Discriminant analysis of a test set of samples was used to identify an algorithm combining age, hyaluronic acid, amino-terminal propeptide of type III collagen (PIIINP), and tissue inhibitor of matrix metalloproteinase 1 (TIMP-1) that was subsequently evaluated using a validation set of biopsy specimens and serum samples. The algorithm detected fibrosis (sensitivity, 90%) and accurately detected the absence of fibrosis (negative predictive value for significant fibrosis 92%). Performance was excellent for alcoholic liver disease and non-alcoholic fatty liver disease. The algorithm performed equally well in comparison with each of the pathologists. In contrast, pathologists' agreement over histologic scores ranged from very good to moderate. It was concluded that assessment of liver fibrosis with multiple serum markers used in combination is sensitive, specific, and reproducible, suggesting they may be used in conjunction with liver biopsy to assess a range of chronic liver diseases. This study is the basis of ELF<sup>TM</sup> test (iQur Ltd) for serum biomarkers of liver fibrosis: hyaluronic acid, PIIINP, and TIMP-1.

A study has documented response to lamivudine in patients with chronic HBV over a 24-month period using surrogate serum biomarkers (Fibrotest-Actitest, FT-AT) without corroborating histological data (Poynard et al. 2005). Investigators found improvement in fibrosis and inflammation in 85 and 91%, respectively, despite the emergence of YMDD mutation in 41.5% of patients. The higher improvement rates reported in this study should be interpreted with caution for a number of reasons including the absence of data on virological response rates, corresponding histological data, and data on the validity of FT to evaluate fibrosis in a longitudinal manner. Although FT has been studied extensively by the authors of the current study, to date there are only few independent studies. In addition to significant inter-laboratory variations, these studies have shown that significant fibrosis could be missed or conversely significant fibrosis diagnosed in the absence of minimal or no fibrosis in about 15–20% of patients.

Collagen IV is a component of basement membranes and it is released into the blood during basement membrane turnover. Increased deposition of collagen is associated with increased serum levels of collagen IV and serum IV may be a very early and specific biomarker for active fibrosis, particularly in alcoholic liver disease and hepatitis C. Elevated serum collagen IV levels are also associated with resistance to interferon therapy.

In another study, HCV-infected hemophilia patients, FT correctly identified clinically advanced or minimal liver disease (Maor et al. 2006). Discordance among the various biomarkers of fibrosis was considerable; nevertheless, practical combination of FT, AST-to-platelet ratio index, and Forns may predict stage of fibrosis with accuracy, potentially avoiding liver biopsy in the majority of the patients.

### ***Biomarkers of Liver Injury***

Alpha glutathione *S*-transferase (alpha GST) is a uniquely sensitive and specific biomarker of hepatocyte injury. Unlike the aminotransferases, which are predominantly found in the periportal hepatocytes, alpha GST is found in hepatocytes, alpha GST is found in hepatocytes throughout the periportal and centrilobular regions of the liver. This uniform hepatic distribution, together with high intracellular levels and a short half-life (~90 min) means that alpha GST is a more sensitive and specific indicator of hepatocyte injury in situations such as hepatotoxicity, transplantation, ischemia–reperfusion injury, and hepatitis.

### ***Biomarkers of Liver Cirrhosis***

The current “gold standard” for liver cirrhosis detection is an invasive, costly, often painful liver biopsy. Therefore, there is a need for biomarkers that could obviate biopsy in cirrhosis patients. High serum levels of tropomyosin and MFAP-4 were demonstrated in patients with hepatic cirrhosis due to different causes by using a proteomic approach (Möller et al. 2009). A quantitative analysis of MFAP-4 serum levels in a large number of patients showed MFAP-4 as a novel candidate biomarker with high diagnostic accuracy for prediction of non-diseased liver versus cirrhosis.

### ***FibroMax***

FibroMax™ (Lab21 Limited) is a combination of five algorithm tests:

- FibroTest™ measures the level of liver fibrosis.
- ActiTest™ measures active liver disease.
- SteatoTest™ measures hepatic steatosis or “fatty liver.”

- NashTest™ measures the level of non-alcoholic steatohepatitis.
- AshTest™ is used to monitor liver damage in cases of severe alcoholic steatohepatitis.

FibroMax™ uses a unique combination of serum biomarkers plus age, gender, weight, and height data which, when entered into patented algorithms, accurately determines the level of liver disease without the need to undertake an invasive liver biopsy. Globally, over 120,000 HCV patients are now being clinically managed using FibroTest™–ActiTest™ as an alternative to liver biopsy. In a comparative study on patients with alcoholic liver disease, other biomarkers FibrometerA and Hepascore, did not improve the diagnostic and prognostic values of FibroTest (Naveau et al. 2009).

## **Biomarkers of Pancreatitis**

Acute pancreatitis is an acute inflammation of the pancreas, the most common causes being gallstones and alcohol abuse. The condition ranges from mild to life threatening and the severity of the condition may not be obvious at presentation. A key event in the development of severe pancreatitis is the activation of proenzymes in the pancreas, the most important of which is trypsinogen. Trypsinogen is converted into active trypsin, which lyses pancreatic tissue releasing further pancreatic enzymes and leading to tissue necrosis and inflammation. During the activation of trypsinogen a small peptide, trypsinogen activation peptide (TAP) is split from trypsinogen and its presence in body fluids is a sensitive and specific indicator of severe acute pancreatitis. TAP is a better discriminator of severe acute and acute pancreatitis than other laboratory tests, e.g., amylase or lipase. TAP is difficult to measure, requiring sensitive immunoassay techniques, but the TAP assay (Biotrin) is valuable in the following situations:

- Monitoring the effects of different therapies for acute pancreatitis
- Investigating the effects of surgery on the pancreas
- Investigating the toxic effects of drugs on the pancreas, e.g., anti-HIV therapy
- Staging subjects in studies of pancreatitis

## **Biomarkers of Renal Disease**

Several biomarkers have been tested in prospective studies in chronic kidney disease and end-stage renal disease patients. C-reactive protein (CRP) has consistently emerged as an early marker of renal dysfunction. Measurement of CRP is recommended for monitoring the risk of atherosclerotic complications in patients with chronic kidney disease and end-stage renal disease, particularly in those cardiovascular complications. The usefulness of this measurement for predicting the evolution

of chronic kidney disease or for monitoring the response to renoprotective treatment, however, still remains unproven. There is growing interest in homocysteine and asymmetric dimethylarginine as biomarkers of cardiovascular and renal risk but the usefulness of these biomarkers in clinical practice remains to be proven. Brain natriuretic peptide and troponin T are strongly related to cardiovascular outcomes in end-stage renal disease patients but their value in this population still requires to be properly tested in specifically designed intervention studies.

### ***Cystatin C as Biomarker of Glomerular Filtration Rate***

Cystatin C is a non-glycosylated protein of low molecular weight (13 kDa) in the cystatin superfamily, which is produced at a constant rate in all nucleated cells. Cystatin C belongs to the cysteine proteinase inhibitor group and is associated with several pathological conditions. Imbalance between Cystatin C and cysteine proteinases is associated with diseases such as inflammation, renal failure, cancer, Alzheimer disease, amyotrophic lateral sclerosis, multiple sclerosis, and hereditary Cystatin C amyloid angiopathy. Cystatin C is removed from blood plasma by glomerular filtration in the kidneys. It is reabsorbed by the proximal tubular cells and degraded. There is a linear relationship between the reciprocal Cystatin C concentration in plasma and the glomerular filtration rate (GFR). Cystatin C is suggested to be a better biomarker for GFR than other markers as its serum concentration is not affected by factors such as age, gender, and body mass. There is association of Cystatin C levels with the incidence of myocardial infarction and coronary death, presenting a risk factor for secondary cardiovascular events. The DetectX™ Cystatin C Immunoassay Kit (Luminos LLC, Ann Arbor, MI) is designed to measure Cystatin C levels. This kit uses a native human Cystatin C molecule as a standard.

### ***Proteomic Biomarkers of Acute Kidney Injury***

Acute kidney injury (AKI), previously referred to as acute renal failure, is an important problem in clinical medicine. Despite significant improvements in therapeutics, the mortality and morbidity associated with AKI remain high. The reasons for this include (a) an incomplete understanding of the underlying pathophysiologic mechanisms and (b) the lack of early biomarkers for AKI, and hence an unacceptable delay in initiating therapy. Application of functional genomics and proteomics to human and animal models of AKI has uncovered several novel genes and proteins that are emerging as biomarkers and novel therapeutic targets. Identification of protein biomarkers in the plasma (NGAL and cystatin C) and urine (NGAL, KIM-1, IL-18, cystatin C, alpha 1-microglobulin, fetuin-A, Gro-alpha, and meprin) holds promise for the investigation of ischemic AKI (Devarajan 2008). It is likely that the AKI panels will be useful for timing the initial insult and assessing the duration of

AKI. Based on the differential expression of the biomarkers, it is also likely that the AKI panels will distinguish between the various causes of AKI and predict clinical outcomes.

### ***Biomarkers of Lupus Nephritis***

Systemic lupus erythematosus can affect kidneys, which may sometimes lead to end-stage renal disease. Lupus nephritis is divided into six classes and scored according to activity and chronicity indices based on histologic findings. Treatment differs based on the pathologic findings. Renal biopsy is currently the only way to accurately predict class and activity and chronicity indices.

Several proteins have been identified in urine samples of patients with lupus nephritis that can be used as biomarkers to indicate the type and severity of renal disease in these patients, as well as the extent of damage to the kidney. An assay based on antibodies against these spots could eliminate the need for renal biopsy, allow frequent evaluation of disease status, and begin specific therapy for patients with lupus nephritis. Further studies are needed to determine whether urine protein analysis could replace the use of biopsies to assess kidney damage in lupus.

### ***Biomarkers of Diabetic Nephropathy***

Clinical management and therapeutic intervention at earlier stage of diabetic nephropathy (DN) is of major importance in preventing the progression to end-stage renal disease. Currently, the measurement of albumin in the urine is used as a standard non-invasive test for the diagnosis of early DN. This test, however, does not detect kidney disease in some cases. Therefore, efforts have been made to find better diagnostic biomarkers of DN. Proteomics approaches have isolated potential biomarkers of DN. Further investigations have identified several proteins that can be used as diagnostic biomarkers of DN, including urinary immunoglobulin G, transferrin, ceruloplasmin, and serum cystatin C. A summary of the diagnostic biomarkers developed over the last decade, and comments on their impacts in the diagnosis and management of this disease have been published (Ito et al. 2008).

### ***Biomarkers of Pulmonary Diseases***

Lungs and airways are affected by several pathologies, the most important of which are inflammations, infections, and cancer. Some of the biomarkers of these pathologies are similar to those found in involvement of other organs. This section will briefly discuss general issues of biomarkers of pulmonary disorders listed in Table 5.3. Biomarkers of lung cancer are described in Chapter 6.

**Table 5.3** Biomarkers of pulmonary diseases

Biomarkers	Sample	Applications
Alpha1-antitrypsin/AAT gene polymorphism	Blood: finger prick	Detection of AAT deficiency predisposing to emphysema
Angiogenic growth factor overexpression	Bronchoalveolar lavage fluid	Overexpression of VEGF and PIGF expression is a biomarker of COPD
Brain natriuretic peptide (BNP)	Plasma	Detection of pulmonary hypertension in patients with chronic lung disease
Calprotectin	Sputum and serum	Track changes in lung inflammation during an exacerbation of cystic fibrosis
CF-specific serum proteomic signature	Plasma	Cystic fibrosis (CF)
Chromagranin A (CgA)	Serum	A neuroendocrine activity biomarker that is increased in male smokers with impaired lung function
Copeptin, the precursor of vasopressin	Serum	A prognostic biomarker for poor prognosis in exacerbation of COPD requiring hospitalization
C-reactive protein (CRP)	Serum	Elevated in acute exacerbation of COPD
H <sub>2</sub> O <sub>2</sub>	Exhaled breath condensate	Measurement of oxidative stress in pulmonary diseases
F2-isoprostanes Malondialdehyde 4-hydroxy-2-nonenal antioxidants IgE level	Serum	The dose of omalizumab is that required is to reduce circulating free IgE levels to less than 10 IU/mL
Nitric oxide (NO)	Exhaled breath	Inflammatory lung disorders, e.g., asthma Rhinosinusitis
	Urine	Higher levels of urinary NO are strongly associated with improved survival in acute respiratory distress syndrome
Osteoprotegerin (OPG)	Serum	Increased specifically in COPD
Serum amyloid A (SAA)	Serum	Exacerbation of COPD by respiratory tract infections
Surfactant proteins: A (SP-A) D (SP-D)	Tracheal aspirates	Interstitial lung disease
	Bronchoalveolar lavage	Acute respiratory distress syndrome
	Pleural effusions	Radiation pneumonitis

### ***Biomarkers of Oxidative Stress in Lung Diseases***

Oxidative stress is the hallmark of various chronic inflammatory lung diseases. Increased concentrations of ROS in the lungs of such patients are reflected by elevated concentrations of oxidative stress markers in the breath, airways, lung tissue, and blood. Traditionally, the measurement of these biomarkers has involved invasive procedures to procure the samples or to examine the affected compartments, to the patient's discomfort. Non-invasive approaches to measure oxidative stress have been investigated. The collection of exhaled breath condensate (EBC) is a non-invasive sampling method for real-time analysis and evaluation of oxidative stress biomarkers in the lower respiratory tract airways. The biomarkers of oxidative stress such as H<sub>2</sub>O<sub>2</sub>, F<sub>2</sub>-isoprostanes, malondialdehyde, 4-hydroxy-2-nonenal, antioxidants, glutathione, and nitrosative stress such as nitrate/nitrite and nitrosated species have been successfully measured in EBC. Oxidative stress biomarkers also have been measured for various antioxidants in disease prognosis. EBC is currently used as a research and diagnostic tool in free radical research, yielding information on redox disturbance and the degree and type of inflammation in the lung. It is expected that EBC can be exploited to detect specific levels of biomarkers and monitor disease severity in response to treatment.

### ***Biomarkers of Survival in Acute Respiratory Distress Syndrome***

#### **Urinary NO as Biomarker of ARDS**

Acute respiratory distress syndrome (ARDS) is the rapid onset of respiratory failure – the inability to adequately oxygenate the blood – that often occurs in the critically ill. Acute lung injury (ALI) precedes ARDS as severe respiratory illnesses progress. Both conditions can be life threatening. In a large-scale, multicenter trial of patients with ARDS or ALI, higher levels of nitric oxide (NO) in urine were strongly associated with improved survival, more ventilator-free days, and decreased rates of organ failure (McClintock et al. 2007). The authors speculated that NO has a beneficial effect on ALI since it scavenges oxygen-free radicals that are generated during oxidative stress. Since NO increases microcirculation, it helps to better perfuse tissue beds in the lungs. The investigators offered an alternative hypothesis to explain their findings: NO created inside the body may have a beneficial effect on organs other than the lung during ALI. It might help prevent further tissue damage by improving oxygen and nutrient delivery to the tissues, while helping to decrease the amount of toxic oxygen species. The authors also speculated that NO might have antibacterial effects that could be important in infectious conditions that predispose patients to ALI.

#### **Plasma Biomarkers Related to Inflammation**

Plasma biomarkers related to inflammation – IL-8 and enhanced neutrophil recruitment to the lung (ICAM-1) – are independently associated with increased mortality



in patients with ALI. Higher levels of IL-8 and ICAM-1 independently predicted death (McClintock et al. 2008). In addition, lower levels of the coagulation marker protein C were independently associated with an increased risk of death. The association of lower protein C levels with non-survivors continues to support the role for disordered coagulation in ALI/ARDS. These associations exist despite consistent use of lung protective ventilation and persist even when controlling for clinical factors that also impact upon outcomes. The two biomarkers with an independent association with mortality, IL-8 and ICAM-1, need to be studied further for their potential value in stratifying patients in clinical trials.

### ***Pulmonary Surfactant Proteins as Biomarkers for Lung Diseases***

Pulmonary surfactant, a complex of lipids and proteins, functions to keep alveoli from collapsing at expiration. Surfactant proteins A (SP-A) and D (SP-D) belong to the collectin family and play pivotal roles in the innate immunity of the lung. Pulmonary collectins directly bind with broad specificities to a variety of microorganism and possess antimicrobial effects. These proteins also exhibit both inflammatory and antiinflammatory functions. The collectins enhance phagocytosis of microbes by macrophages through opsonic and/or non-opsonic activities. The proteins stimulate cell surface expression of phagocytic receptors including scavenger receptor A and mannose receptor. Since the expression of SP-A and SP-D is abundant and restricted within the lung, the proteins are now clinically used as biomarkers for lung diseases. The levels of SP-A and SP-D in bronchoalveolar lavage fluids, amniotic fluids, tracheal aspirates, and pleural effusions reflect alterations in alveolar compartments and epithelium, and lung maturity. The determination of SP-A and SP-D in sera is a non-invasive and useful tool for understanding some pathological changes of the lung in the diseases, including pulmonary fibrosis, collagen vascular diseases complicated with interstitial lung disease, pulmonary alveolar proteinosis, acute respiratory distress syndrome, and radiation pneumonitis (Takahashi et al. 2006).

### **Cytokine/Chemokine Biomarkers of SARS**

Pathological changes in severe acute respiratory syndrome (SARS) suggest that SARS sequelae are associated with dysregulation of cytokine and chemokine production. A study from Taiwan showed that cytokine or chemokine profiles in patients with SARS differ markedly from those in patients with community-acquired pneumonia (CAP) and control groups (Chien et al. 2006a). Serum levels of three cytokines were significantly elevated in SARS patients versus the CAP: Interferon- $\gamma$ -inducible protein-10 (IP-10), interleukin (IL)-2, and IL-6. Cytokine levels began to rise before the development of chest involvement and peaked earlier than did lung injury assessed by chest X-ray. Conversely, in CAP patients but not SARS patients or controls, levels of interferon- $\gamma$ , IL-10, and IL-8 were elevated and rose in tandem with radiographic changes. A further difference between groups was the ratio of IL-6 to IL-10, at 4.84 in SARS patients versus 2.95 in CAP

patients. However, in both sets of patients, levels of IL-6 correlated strongly with the severity of lung injury. The early induction of IP-10 and IL-2, as well as the subsequent overproduction of IL-6 and lack of IL-10, probably contributes to the main immunopathological processes involved in SARS lung injury and may be early biomarkers of lung injury. These findings differ from those observed in subjects with CAP.

### ***Biomarkers of Chronic Obstructive Pulmonary Disease***

There has been increasing interest in using pulmonary biomarkers to understand and monitor the inflammation in the respiratory tract of patients with chronic obstructive pulmonary disease (COPD). Bronchial biopsies and bronchoalveolar lavage provide valuable information about inflammatory cells and mediators, but these procedures are invasive, so that repeated measurements are limited. Sputum provides considerable information about the inflammatory process, including mediators and proteinases in COPD, but samples usually represent proximal airways and may not reflect inflammatory processes in distal bronchi. Analysis of exhaled breath is a non-invasive procedure so that repeated measurements are possible, but the variability is high for some assays. There is relatively little information about how any of these biomarkers relate to other clinical outcomes, such as progression of the disease, severity of disease, clinical subtypes, or response to therapy. More information is also needed about the variability in these measurements. In the future pulmonary biomarkers may be useful in predicting disease progression, indicating disease instability, and predicting response to current therapies and novel therapies, many of which are now in development (Barnes et al. 2006).

Measurements of C-reactive protein (CRP), a marker of inflammation, provide incremental prognostic information beyond that achieved by traditional biomarkers in patients with mild-to-moderate COPD and may enable more accurate detection of patients at a high risk of mortality (Man et al. 2006). Lung function decline was significantly related to CRP levels, with an average predicted change in FEV1 of  $-0.93\%$  in the highest and  $0.43\%$  in the lowest quintile. However, respiratory causes of mortality were not significantly related to CRP levels.

### **Increased Expression of PIGF as a Biomarker of COPD**

Decreased expression of vascular endothelial growth factor (VEGF) and its receptor has been implicated in the pathogenesis of COPD. Levels of placenta growth factor (PIGF), another angiogenic factor, are increased in the serum and bronchoalveolar lavage (BAL) fluid of patients with COPD and are inversely correlated with FEV1 (Cheng et al. 2008). Serum levels of PIGF in patients with COPD were more than double those in smokers and non-smokers without COPD. These findings suggest that bronchial epithelial cells can express PIGF, which may contribute to the pathogenesis of COPD. Both PIGF and VEGF expression levels were increased in cultured bronchial epithelial cells exposed to proinflammatory cytokines such

as TNF $\alpha$  and IL-8. Although the mechanisms underlying the observed detrimental effects of PIGF remain to be clarified, persistent PIGF expression might have adverse effects on lung parenchyma by downregulating angiogenesis.

### **Chromagranin A (CgA) as Biomarker of Airway Obstruction in Smokers**

A study has revealed that serum levels of the neuroendocrine activity biomarker chromagranin A (CgA) are increased in male smokers with impaired lung function and are associated with both respiratory symptoms and the degree of airway obstruction (Sorhaug et al. 2006). The subgroup of airway epithelial cells belonging to the diffuse neuroendocrine system, termed pulmonary neuroendocrine cells, may represent a putative regulatory function of CgA as a prohormone. They are considered to control growth and development of the fetal lung and regulation of ventilation and circulation, but may also have a role in the pathogenesis of smoking-induced airway disease. The findings indicate that neuroendocrine activation may be important in smoking-related airway inflammation and remodeling and raise the possibility that CgA could be of predictive value as a biomarker of prognosis in smoking-associated diseases.

### **BNP as a Biomarker of Chronic Pulmonary Disease**

Circulating BNP levels were evaluated as a parameter for the presence and severity of pulmonary hypertension (PH) in patients with chronic lung disease (Leuchte et al. 2006). During a follow-up time of approximately 1 year, significant pulmonary hypertension (mean pulmonary artery pressure >35 mmHg) was diagnosed in more than one-fourth of patients and led to decreased exercise tolerance and life expectancy. Elevated BNP concentrations identified significant pulmonary hypertension with a sensitivity of 0.85 and specificity of 0.88 and predicted mortality. Moreover, BNP served as a risk factor of death independent of lung functional impairment or hypoxemia. It is concluded that plasma BNP facilitates non-invasive detection of significant PH with high accuracy and can be used as a screening test for the presence of PH. In addition, BNP enables an assessment of the relevance of PH and could serve as a useful prognostic parameter in chronic lung disease.

### **Alpha1-Antitrypsin Gene Polymorphisms Predisposing to Emphysema**

Alpha1-antitrypsin (AAT) is a plasma glycoprotein that inhibits neutrophil elastase, and individuals who inherit altered AAT genes resulting in deficiency of the protein are at high risk for COPD and liver cirrhosis. This deficiency can be detected by serum protein pattern studies. In the past, testing for the deficiency has been done retrospectively in patients with COPD or liver disease, but the introduction of a home-administered finger-stick blood spot test for AAT genotype enables affected families to construct pedigrees to enable them to identify children who are at risk for developing COPD in later life and should avoid exposure to dust and smoke (Strange et al. 2006).

## ***Biomarkers of Asthma***

Although the aim of management of patients with asthma is to control their symptoms and prevent exacerbations and morbidity of the disease, optimal management may require assessment and monitoring of biomarkers, i.e., objective measures of lung dysfunction and inflammation.

### **Comparison of Biomarkers of Asthma and COPD**

Airway inflammation is associated with an increased expression and release of inflammatory reactants that regulate processes of cell migration, activation, and degranulation. One study was done to quantify bronchial lavage (BAL) fluid and serum levels of IL-8, secretory leukocyte protease inhibitor (SLPI), soluble intracellular adhesion molecules-1 (sICAM-1), and sCD14, as surrogate markers of inflammatory and immune response in asthma and COPD patients with similar disease duration time (Hollander et al. 2007). Biomarkers were measured using commercially available ELISA kits. The findings show that of four measured biomarkers, only the BAL IL-8 was higher in COPD patients when compared to asthma.

### **Exhaled NO as a Biomarker of Asthma**

Airway hyperresponsiveness is the main feature of asthma and is defined as an increase in the ease and degree of airway narrowing in response to bronchoconstrictor stimuli. Inflammation plays a central role in the pathogenesis of asthma and much of it can be attributed to helper T-cell type 2 cytokine activation, the degree of which strongly correlates to disease severity. One of the inflammatory mediators in asthma is nitric oxide (NO). The exhaled NO level is elevated in asthma and can predict asthma exacerbation (Heinen et al. 2006). The average exhaled NO concentration is significantly increased in subjects with seasonal allergic asthma during the pollen season compared with before the season. It may be clinically more useful to compare exhaled NO values with a subject's previous values than to compare them with a population-based normal range.

Cough variant asthma (CVA) and atopic cough both present with bronchodilator-resistant non-productive cough but may be differentiated from other causes of chronic non-productive cough by measuring exhaled NO. Exhaled NO levels in patients with atopic cough are significantly lower than those in patients with CVA and bronchial asthma (Fujimura et al. 2008). There are no significant difference in the exhaled NO levels between patients with CVA and bronchial asthma.

A UK study findings show that it is feasible to measure bronchial flux NO concentration ( $\dot{V}NO$ ) and alveolar NO concentration ( $C_{alv}$ ) in 70% of children, with  $C_{alv}$  levels potentially reflecting alveolar inflammation in asthma (Paraskakis et al. 2006).  $C_{alv}$  and  $\dot{V}NO$  were measured from the fractional exhaled NO ( $FeNO_{50}$ ) at multiple exhalation flow rates in asthmatic children. Although  $FeNO_{50}$  and  $\dot{V}NO$  give essentially the same information,  $C_{alv}$  is higher in asthmatic children than in normal

children. This study also highlights the relationship between poor control of asthma and  $C_{alv}$  (a biomarker of alveolar inflammation) but further work is needed to confirm the relevance of this. Researchers at the University of Pittsburgh, Pennsylvania, have developed a novel nanosensor that can detect a possible asthma attack before it begins. The minute sensor can be fitted into a hand-held device, and when a person blows into the device, it measures the NO content of their breath. Use of this device would provide asthma sufferers with a simple and cost-effective way to monitor their asthma inflammation.

An explanation for increased levels of exhaled NO is non-enzymatic generation of NO from nitrite due to airway acidification in asthmatics. Reduced arginine availability may also contribute to lung injury by promoting formation of cytotoxic radicals such as peroxynitrite. As arginine levels decline, nitric oxide synthase (NOS) itself can begin to generate superoxide in lieu of NO, thereby favoring NO consumption via the generation of peroxynitrite that could induce lung injury. This reduction in bioavailability of NO via formation of species such as peroxynitrite could be further amplified by the rapid loss of SOD activity during the asthmatic response.

Plasma arginase activity declines significantly with treatment and improvement of symptoms. Additional studies are needed to determine whether measurements of plasma arginase activity will provide a useful biomarker for underlying metabolic disorder and efficacy of treatment for this disease. The arginase activity present in serum probably does not accurately reflect whole body arginase activity or that compartmentalized in the lungs, since the arginases are intracellular enzymes. Because arginase is induced in monocytes in response to helper T-cell type 2 cytokines, it is speculated that these cells are one likely source of the elevated arginase in serum, consistent with the localization of arginase expression within macrophages in the lungs.

Although exhaled NO is a clinically useful biomarker of eosinophilic airway inflammation in asthma, significant validation and investigation are required before exhaled breath condensate could be utilized for making decisions in clinical practice (Simpson and Wark 2008).

### **Cytokines as Biomarkers of Asthma Severity**

Severe asthma is characterized by elevated levels of proinflammatory cytokines and neutrophilic inflammation in the airways. Blood cytokines, biomarkers of systemic inflammation, may be a feature of increased inflammation in severe asthma. One study found that IL-8 and TNF- $\alpha$  levels were higher in severe asthmatics than in mild-moderate asthmatics or in controls and, in conjunction with augmented circulating neutrophils, suggest the involvement of neutrophil-derived cytokine pattern (Silvestri et al. 2006). Furthermore, in patients with severe asthma, TNF- $\alpha$  levels were positively correlated with both exhaled nitric oxide and circulating neutrophil counts. Cytokine levels were elevated even though the patients were on high-dose inhaled steroids. This finding might reflect the inability of these drugs to significantly suppress production of this cytokine by airway cellular sources including

epithelial cells and inflammatory cells. In patients with severe asthma there may be an imbalance between IL-8 production and the blocking capacity of IL-8 autoantibodies. The findings of this study may be clinically relevant and suggest that drugs that block TNF- $\alpha$  release or activity might represent a new treatment option in severe asthma.

### **Biomarker for Rhinovirus-Induced Asthma Exacerbation**

Clinical observations suggest that rhinovirus infection induces a specific inflammatory response in predisposed individuals that results in worsened asthmatic symptoms and increased airway inflammation. A study has shown that IFN- $\gamma$ -induced protein (IP)-10 is specifically released in acute virus-induced asthma and can be measured in the serum to predict a viral trigger of acute exacerbations (Wark et al. 2007). Primary bronchial epithelial cell models of rhinovirus infection were used to identify mediators of rhinovirus infection and responded to infection with rhinovirus-16 by releasing high levels of IP-10, RANTES, and IL-16, as well as smaller amounts of IL-8 and TNF- $\alpha$ . IP-10, perhaps in combination with TNF- $\alpha$ , might be a useful clinical marker to identify rhinovirus and other virus-induced acute asthma. Additional findings suggest that IP-10 or CXCR3 (an IP-10 receptor that is highly expressed in activated T cells) might have a role in worsening of airflow obstruction and airway inflammation and may therefore be potential therapeutic targets.

### **Biomarkers for Predicting Response to Corticosteroid Therapy**

International guidelines on the management of asthma support the early introduction of corticosteroids to control symptoms and to improve lung function by reducing airway inflammation. However, not all individuals respond to corticosteroids to the same extent and it would be desirable to be able to predict the response to corticosteroid treatment. Several biomarkers have been assessed following treatment with corticosteroids including measures of lung function, peripheral blood and sputum indices of inflammation, exhaled gases, and breath condensates. The most widely examined measures in predicting a response to corticosteroids are airway hyperresponsiveness, exhaled NO (eNO), and induced sputum. Of these, sputum eosinophilia has been demonstrated to be the best predictor of a short-term response to corticosteroids. More importantly, directing treatment at normalizing the sputum eosinophil count can substantially reduce severe exacerbations. The widespread utilization of sputum induction is hampered because the procedure is relatively labor intensive. The measurement of eNO is simpler, but incorporating the assessment of NO in an asthma management strategy has not led to a reduction in exacerbation rates. The challenge now is to either simplify the measurement of a sputum eosinophilia or to identify another inflammatory biomarker with a similar efficacy as the sputum eosinophil count in predicting both the short- and long-term responses to corticosteroids.

### **IgE as Guide to Dosing of Omalizumab for Asthma**

IgE plays a central role in the pathophysiology of asthma. The two essential phases in this pathophysiology are sensitization to allergen and clinical expression of symptoms on reexposure to the sensitizing allergen. Omalizumab (Xolair, Genentech) is a recombinant humanized IgG1 monoclonal anti-IgE antibody that binds to circulating IgE, regardless of allergen specificity, forming small, biologically inert IgE- and anti-IgE complexes without activating the complement cascade. An 89–99% reduction in free serum IgE (i.e., IgE not bound to omalizumab) occurs soon after the administration of omalizumab, and low levels persist throughout treatment with appropriate doses. A total serum IgE level should be measured in all patients who are being considered for treatment with omalizumab, because the dose of omalizumab is determined on the basis of the IgE level and body weight (Strunk and Bloomberg 2006). The dose is based on the estimated amount of the drug that is required to reduce circulating free IgE levels to less than 10 IU/mL.

### **Endothelin-1 in Exhaled Breath as Biomarker of Asthma**

Endothelins are proinflammatory, profibrotic, broncho- and vasoconstrictive peptides, which play an important role in the development of airway inflammation and remodeling in asthma. A study has evaluated the endothelin-1 (ET-1) levels in exhaled breath condensate (EBC) of asthmatics with different degree in asthma severity (Zietkowski et al. 2008). ET-1 concentrations in EBC of all asthmatic patients were significantly higher than in healthy volunteers. ET-1 levels were significantly higher in patients with unstable asthma than in the two groups with stable disease. Thus, measurements of ET-1 in EBC may provide another useful diagnostic tool for detecting and monitoring inflammation in patients with asthma. The release of ET-1 from bronchial epithelium through the influence of many inflammatory cells essential in asthma and interactions with other cytokines may play an important role in increase of airway inflammation, which is observed after postexercise bronchoconstriction in asthmatic patients.

### ***Biomarkers for Cystic Fibrosis***

Cystic fibrosis (CF) is the most common serious genetic disease among Caucasians in the United States. The disease results from a defective gene that affects multiple aspects of cellular function. Its most serious symptom is a build-up of thick, sticky mucus in the airways, which can lead to fatal lung infections. The usual method for screening and diagnosis is genotyping of cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations.

Antibody microarrays have been developed as a platform for identifying a CF-specific serum proteomic signature (Srivastava et al. 2006). Serum samples from CF patients are pooled and compared with equivalent pools of control sera in order to

identify patterns of protein expression unique to CF. The set of significantly differentially expressed proteins is enriched in protein mediators of inflammation from the NFkappaB signaling pathway, and in proteins that may be selectively expressed in CF-affected tissues such as lung and intestine. In several instances, the data from the antibody microarrays can be validated by quantitative analysis with Reverse Capture Protein Microarrays. In conclusion, antibody microarray technology is sensitive, quantitative, and robust and can be useful as a proteomic platform to discriminate between sera from CF and control patients. Extensive protein degradation and differentially expressed proteins have been identified in sputum as biomarkers of inflammation relating to pulmonary exacerbations of CF (Sloane et al. 2005).

### ***Biomarkers of Pulmonary Embolism***

Management of venous thromboembolism in the past was characterized by a high degree of complexity and lack of both efficacy and efficiency. The non-specific clinical signs of acute pulmonary embolism (PE) and the limitations of earlier imaging procedures led to the development of numerous sophisticated, multistep diagnostic algorithms which, however, have proved extremely difficult to implement in clinical practice. The diagnosis of potentially life-threatening PE is still missed in many patients who subsequently die of the disease without receiving appropriate treatment, while other patients unnecessarily undergo an invasive, time-consuming procedure due to a vague, poorly documented clinical suspicion.

The widespread use of D-dimer testing in the outpatient setting, and particularly the technical advances of multidetector-row CT scan, had an enormous impact on the strategy for approaching patients with suspected PE. D-dimer testing was originally developed in the diagnosis of disseminated intravascular coagulation, but later turned out to be useful in thromboembolic disorders. D-dimers are unique in that they are the breakdown products of a fibrin mesh that has been stabilized by factor XIII, which crosslinks the E-element to two D-elements. This is the final step in the generation of a thrombus. D-dimer ELISA assays rely on MAbs to bind to this specific protein fragment. D-dimer can also be used to monitor anticoagulation therapy for pulmonary embolism. Patients with an abnormal D-dimer level 1 month after the discontinuation of anticoagulation have a significant incidence of recurrent venous thromboembolism, which is reduced by the resumption of anticoagulation (Palareti et al. 2006). The optimal course of anticoagulation in patients with a normal D-dimer level has not been clearly established.

Combined use of brain natriuretic peptide (BNP) and cardiac troponin T (cTnT) may be useful in risk stratification of normotensive patients with acute pulmonary embolism. Patients with increased BNP and cTnT are at risk for adverse outcome. Future studies in larger numbers of patients are needed to confirm the usefulness of biomarkers in the clinical management of individual patients with pulmonary embolism. A study to compare the respective prognostic values of biomarkers in with non-massive PE as carried out to predict an adverse outcome



at 3 months (Vuilleumier et al. 2009). The combined outcome consisted in need for intensive care monitoring on admission, death, or hospitalization attributable either to PE-related complication (defined by PE/DVT relapse or major bleeding under anticoagulation) or to dyspnea with or without chest pain during follow-up. The outcome was met in 12% of patients. In univariate analysis, NT-proBNP level above 300 pg/mL was the strongest predictor of unfavorable outcome with a negative predictive value of 100%. At that cut-off, the true negative rate for NT-proBNP was 40%. In multivariate analysis, NT-proBNP was the only significant independent predictor. Therefore, NT-proBNP is a useful biomarker for risk stratification to identify low-risk patients with non-massive PE who could be treated in an outpatient setting.

## **Biomarkers in Obstetrics and Gynecology**

### ***Biomarkers for Preeclampsia***

Preeclampsia is an idiopathic multisystem disorder specific to human pregnancy occurring after the 20th week of gestation. There is a sudden and dangerous rise in blood pressure that can result in premature delivery, disability, or death for mother and fetus. The condition, which affects 5–8% of pregnancies worldwide, constitutes a medical emergency and often requires a Caesarean section delivery. The condition is estimated to cause 50,000–76,000 maternal deaths each year. Delivery of the placenta results in resolution of the condition, implicating the placenta as a central culprit in the pathogenesis of preeclampsia. In preeclampsia, an inadequate placental trophoblast invasion of the maternal uterine spiral arteries results in poor placental perfusion, leading to placental ischemia. This could result in release of factors into the maternal circulation that cause widespread activation or dysfunction of the maternal endothelium. Factors in the maternal circulation might induce oxidative stress and/or elicit an inflammatory response in the maternal endothelium, resulting in the altered expression of several genes involved in the regulation of vascular tone.

Currently no predictive test exists for preeclampsia. Routine laboratory tests such as liver function, proteinuria, and platelet count are neither accurate nor sensitive. Studies of gene polymorphisms have not revealed any definite evidence of genetic markers of preeclampsia. Polymorphisms of the adiponectin gene show a weak, but statistically significant, haplotype association with susceptibility to preeclampsia in Finnish women (Saarela et al. 2006). Several studies have investigated protein biomarkers of preeclampsia.

### **Protein Biomarker of Preeclampsia in Urine**

A protein biomarker in urine of pregnant women could serve as a screening/diagnostic tool for preeclampsia. Many proteins present in the serum and blood could provide a clue to preeclampsia, but only the relationship between them has

diagnostic significance. An algorithm is used to calculate the ratio for the presence or absence of three specific proteins that are normally secreted by human placenta; the ratio between two of the proteins correctly identifies women who have severe preeclampsia (Adachi et al. 2006). The proteins are vascular endothelial growth factor (VEGF), placental growth factor (PlGF), and their soluble VEGF receptor (sFlt-1). The ratio of sFlt-1 and PlGF has a high sensitivity (88%) and specificity (100%) for identifying severe preeclampsia and is more accurate than proteinuria alone.

A panel of biomarkers present in urine could distinguish pregnant women with preeclampsia from healthy pregnant women. Combined presence of two biomarkers, specific fragments of albumin and serpin-1, is highly characteristic for preeclampsia superimposed on chronic hypertension. The marker panel, assayed using Vermillion's SELDI-TOF technology, could classify the study subjects with 92% accuracy. Further development of a new test using these biomarkers could lead to earlier diagnosis and treatment of preeclampsia and help prevent premature births.

### **Protein Biomarkers of Preeclampsia in CSF**

One study used proteomic analysis of CSF to identify protein biomarkers characteristic of preeclampsia and related to its severity (Norwitz et al. 2005). Samples were subjected to proteomic analysis using surface-enhanced laser desorption/ionization time-of-flight (SELDI-TOF) mass spectroscopy. A discriminative proteomic biomarker profile was extracted by applying Mass Restricted analysis, and a Preeclampsia Proteomic Biomarker (PPB) score developed based on the presence or absence of four discriminatory protein peaks in individual CSF SELDI tracings. In-gel tryptic digests, Western blot analysis, on-chip immunoassays, ELISA, and spectral analysis were used to identify the biomarkers composing the PPB score. PPB score distinguished patients with a clinical diagnosis of severe preeclampsia (sPE) from mild preeclampsia (mPE) and normotensive controls. PPB scores were unaffected by parity, magnesium seizure prophylaxis, CSF leukocyte counts, and total protein content. Proteomic identification techniques matched the discriminatory protein peaks to the alpha- and beta-hemoglobin chains. ELISA confirmed that women diagnosed clinically with sPE had significantly higher CSF hemoglobin concentrations than women with mPE or CRL. Thus proteomic analysis of CSF can accurately distinguish sPE from both mPE and normotensive controls. Patients with sPE have nanomolar amounts of free hemoglobin in their CSF. Further studies are needed to confirm these observations and determine their physiologic implications.

### **Protein HtrA1 as Biomarker for Preeclampsia**

Protein HtrA1 is known to be involved in programmed cell death, cell change, and invasiveness, i.e., the ability of cells to invade and colonize new areas. This process can be physiological as in establishing growth of a placenta in the uterus during the first trimester. Invasion also can be pathological as in the cases of cancer.

Aberrant expression of developmentally regulated genes during placental development could affect fetal growth and contribute to preeclampsia. A study was designed to determine the expression of HtrA1 in placental tissues from control and preeclamptic pregnancies to determine the effect of HtrA1 expression in trophoblast cell migration and invasion (Ajayi et al. 2008). Higher expression of HtrA1 was detected in placental tissues collected from patients with early-onset preeclampsia, compared with those from gestational age-matched control samples. Higher expression of HtrA1 is associated with early-onset preeclampsia and may affect trophoblast cell migration and invasion.

This work is the first to link high levels of HtrA1 in third-trimester placental tissues with severe preeclampsia. Though preliminary, the findings may one day lead to development of a blood test to track HtrA1 levels to identify women at risk of preeclampsia. Although the initial results are really encouraging, it is too early to say if HtrA1 is a definite biomarker of preeclampsia. Because greater amounts of HtrA1 indicate greater placental distress and disease severity, developing a blood test to detect levels of HtrA1 may possibly serve as an early warning system that placental conditions are changing. The hope is that such a predictive test would allow physicians to manage preeclampsia on a non-emergency basis when it is less threatening for mother and fetus or possibly to devise therapies to stop the process or prevent it altogether.

### **sFlt1 and Soluble Endoglin as Biomarkers of Preeclampsia**

Maternal endothelial dysfunction mediated by excess placenta-derived soluble VEGF receptor 1 (sVEGFR1) or soluble fms-like tyrosine kinase (sFlt1) is emerging as a prominent component in disease pathogenesis. Increased levels of free sFlt-1 have been measured by immunoassay from serum and urine samples of preeclampsia patients (Buhimschi et al. 2006). However, for unknown reasons, a subset of preeclampsia patients will go on to experience severe preeclampsia – a group of dramatically escalated symptoms characterized by a sudden, massive rise in blood pressure, which can lead to the onset of seizures, as well as the development of fetal growth restriction and the HELLP (hemolysis, elevated liver enzymes and low platelets) syndrome, which indicates that the mother's liver and blood-clotting systems are not functioning properly, and the health of both mother and infant are in serious danger. Investigation of HELLP led to the search for another factor that acts jointly with sFlt1 to induce vascular damage and escalate the disease to its severe form.

A novel placenta-derived soluble TGF- $\beta$  co-receptor, endoglin (sEng), which is elevated in the sera of preeclamptic individuals, correlates with disease severity and falls after delivery (Venkatesha et al. 2006). sEng inhibits formation of capillary tubes in vitro and induces vascular permeability and hypertension in vivo. Its effects in pregnant rats are amplified by coadministration of sFlt-1, leading to severe preeclampsia including the HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome and restriction of fetal growth. sEng impairs binding of TGF- $\beta$  to its receptors and downstream signaling including effects on activation of eNOS

and vasodilation, suggesting that sEng leads to dysregulated TGF- $\beta$  signaling in the vasculature. These results suggest that sEng may act in concert with sFlt-1 to induce severe preeclampsia and can be considered a biomarker of this condition. This has important diagnostic and therapeutic implications for the management of this disease.

### **RNA Biomarkers**

Placental RNA analyzed in maternal plasma permits rapid screening of novel biomarkers including markers not accessible by antibody-based assays. This includes transcription factors, non-coding RNA, epigenetic features, as well as genes functionally or genetically linked with preeclampsia. By reviewing genes with placental expression in the Human SymAtlas and comparison with proven qualifiers, a large set of RNA biomarkers have been targeted for use in maternal plasma (Smets et al. 2006). These biomarkers qualify as novel RNA biomarkers for the presymptomatic detection in first trimester of pregnancy-associated diseases with placental origin and/or dysfunction such as pregnancy-induced hypertension without or with proteinuria (preeclampsia), and intrauterine growth restriction.

### ***Biomarkers of Premature Birth***

It is estimated that preterm labor complicates 6–10% of all pregnancies and is the most common cause of neonatal morbidity and mortality. Worldwide statistics reflect that there may be as many as 13 million preterm births annually, and this figure is predicted to increase. In the United States, an estimated \$820 million is spent on preterm hospitalization subsequently shown to have been unnecessary. These hospitalizations tax both the mother's health and health-care resources. Currently, there are no rapid or accurate tests that positively predict preterm labor.

By profiling specific proteins in amniotic fluid for inflammation, researchers at Yale School of Medicine (New Haven, CT) can quickly and accurately detect potentially dangerous infections in pregnant women and also predict the possibility of premature birth. Mass Restricted (MR) score is a specific proteomic profile used in the study. Presence of the biomarkers indicating inflammation in amniotic fluid can be established in 20–30 min. This test is much faster than the current method of testing microbiological cultures. If no biomarkers are present, then the pregnancy is uncomplicated. Proteins in a small sample of amniotic fluid were tested to find a link between the amniotic fluid glucose value, white blood cell count, and the outcome of the fetus. An MR score of three or four is highly predictive of adverse pregnancy outcome. The presence of two biomarkers for inflammation indicates the median time for delivery is 4 days. If all the biomarkers for inflammation are present, delivery time occurs within hours. Studies to test treatment were not possible before. The results of this test can be used to provide a rapid treatment to the mother and its baby.

Metabolome Inc. has identified specific metabolites or biomarkers in the amniotic fluid metabolome that enabled classification of patients at risk for preterm delivery. Women who present at 26 weeks of pregnancy with premature labor have three outcomes: (1) false labor with normal full-term pregnancy; (2) premature birth; and (3) bacterial infection leading to inflammation. A stereotypic pattern of metabolites was identified in different groups. These observations enable identification of metabolic pathways which are altered in preterm labor. A third blinded study to refine the algorithms in order to improve the precision is now being planned as well as studies in urine and plasma.

PerkinElmer, in collaboration with the University of Leicester (UK), is working on the discovery of biomarkers that predict preterm birth.

### ***Biomarkers of Oxidative Stress in Complicated Pregnancies***

Oxidative stress may contribute to the development of complications in pregnancy and antioxidant activity in both maternal and umbilical cord blood may be an indicator of oxygen radical activity. Various parameters of oxidative stress are measured in pregnancies with hypertension and preeclampsia, insulin-dependent diabetes mellitus, gestational diabetes mellitus, oligohydramnios and abruptio placentae, as well as a healthy control group. The results of such studies suggest that oxidative stress and subsequent lipid peroxidation accompany the complications of hypertension, preeclampsia, and diabetes mellitus in pregnancy. Maternal erythrocyte GST activity seems to be a sensitive indicator of oxidative stress before delivery. The same enzyme can be used in cord blood as a biomarker of oxidative stress upon a sudden increase in oxygenation during delivery. These multiparameter biomarkers can also be used in monitoring the efficiency of antioxidant supplementation in complicated pregnant women.

### ***Biomarkers of Premenstrual Dysphoric Disorder***

Premenstrual dysphoric disorder PMDD is a severe form of premenstrual syndrome that affects 5% of menstruating women. Symptoms of the disorder include marked depression, anxiety, tension, irritability, and moodiness. Considered a mood disorder, women affected by PMDD have a significantly reduced quality of life. While triggered by reproductive hormones, the cause of PMDD is unknown, and current treatment options range from nutritional supplements to prescription medicine. PMDD afflicts a significant portion of the female population, yet little is known about it, and it has no definitive cure other than menopause.

Metabolon scientists will compare samples from women with PMDD to samples from normal, healthy women under controlled hormonal conditions. Metabolon will analyze the data to identify biomarkers that indicate a metabolic difference between the two groups. Results from this study could potentially lead to more effective

treatments for the disorder. Metabolon's technology will identify biomarkers that can be used to develop new treatments for the disorder itself, not just the symptoms.

### ***Biomarkers of Endometriosis***

Endometriosis is a painful, chronic disease that occurs when endometrium (tissue that lines the uterus) is found outside the uterus, usually in the abdomen on the ovaries, fallopian tubes, and ligaments that support the uterus; the area between the vagina and rectum; the outer surface of the uterus; and the lining of the pelvic cavity. It affects over 5 million females in North America and millions more worldwide. This misplaced tissue develops into growths or lesions which respond to the menstrual cycle in the same way that the tissue of the uterine lining does: each month the tissue builds up, breaks down, and sheds. This results in internal bleeding, breakdown of the blood and tissue from the lesions, and inflammation. It can cause pain, infertility, scar tissue formation, adhesions, and bowel problems.

Diagnosis of endometriosis is surgical through laparoscopy, which is invasive, costly and associated with potential complications. A non-invasive test for diagnosis of endometriosis will focus the use of laparoscopy on women who are highly suspected of having endometriosis. Considerable efforts are being made to discover biomarkers of endometriosis for non-invasive diagnosis. One study has used DNA microarrays to look for biomarkers for endometriosis in peripheral blood lymphocytes in premenopausal women with or without endometriosis undergoing gynecological procedures (Flores et al. 2006). A gene selection program identified two genes, IL2RG and LOXL1, which were differentially expressed in peripheral blood lymphocytes of samples from endometriosis patients. These may provide important clues regarding the pathogenesis of this disease. Moreover, they could be considered potential targets for non-invasive diagnostic assays for endometriosis but need to be validated in a larger population. Interleukin-6 provides a promising serum biomarker for the non-surgical prediction of endometriosis (Othman et al. 2008). Although some of the biomarkers that were investigated showed a good specificity, none of them had high sensitivity. More multicenter studies on larger numbers of patients are required to identify the most useful biomarker.

### ***Fetal Biomarkers in Maternal Blood***

The discovery of fetal mRNA transcripts in the maternal circulation holds great promise for non-invasive prenatal diagnosis. To identify potential fetal biomarkers, RNA was isolated from peripheral or umbilical blood and hybridized to gene expression arrays (Maron et al. 2007). Gene expression, paired Student's *t*-test, and pathway analyses were performed. These identified fetal biomarkers included developmental genes, sensory perception genes, and genes involved in neonatal physiology. Transcripts were predominantly expressed or restricted to the fetus, the

embryo, or the neonate. Real-time RT-PCR amplification confirmed the presence of specific gene transcripts; SNP analysis demonstrated the presence of fetal transcripts in maternal antepartum blood. Comparison of whole blood and plasma samples from the same pregnant woman suggested that placental genes are more easily detected in plasma. These findings show that fetal and placental mRNA circulates in the blood of pregnant women. Transcriptional analysis of maternal whole blood identifies a unique set of biologically diverse fetal biomarkers and has a multitude of clinical applications.

## **Biomarkers for Genetic Disorders**

There are a large number of genetic disorders where molecular diagnostics are used along with biomarkers for screening and diagnosis. These are described in more details in the report on molecular diagnostics (Jain 2010a). A few examples will be described briefly here with focus on biomarkers.

### ***Biomarkers for Down's Syndrome***

Down's syndrome is a genetic disorder caused by the inheritance of three copies of the 21st chromosome. It is the most common congenital disorder with impairment of mental function; a large percentage of these individuals develop Alzheimer disease in the fifth decade of life. There is some controversy about the best approach to screening for Down's syndrome. The competing claims of advocates of different screening approaches have made it difficult for health planners, clinicians, or pregnant women to reach a balanced decision about what should be offered or chosen.

Serum tests used to screen for Down's syndrome include  $\beta$ -human chorionic gonadotrophin (hCG), alpha-fetoprotein (AFP), unconjugated oestriol (uE3), serum pregnancy associated plasma protein-A (PAPP-A), and dimeric inhibin A. ADAM12 is a novel serum marker with biological properties similar to PAPP-A.

The Serum, Urine and Ultrasound Screening Study (SURUSS) has advanced our knowledge of the efficacy and safety of antenatal screening for fetal Down's syndrome and placed choices on a firmer platform of evidence (Wald et al. 2004). The best performer was the integrated test, comprising ultrasound measurement of fetal nuchal translucency and assay of PAPP-A at 10 weeks, combined with quadruple tests of serum  $\alpha$ -fetoprotein, unconjugated oestriol, hCG, and inhibin A during the second trimester (after 14 weeks). This two-step package had a false-positive rate of only 0.9%. The best first trimester screening package was a combination of nuchal translucency scan, serum-free  $\beta$ -HCG, and pregnancy-associated plasma protein A, which had a false-positive rate of 4.3%. Second trimester quadruple testing alone had a false-positive rate of 6.2%.

Quadruple Marker Prenatal Screening Test (Alfigen Inc.) is a blood screening test done in the second trimester of pregnancy (between 15 and 20 weeks) to help detect an increased risk for Down's Syndrome, trisomy 18, and neural tube defects or abdominal wall defects. Occasionally, the test may also detect a risk for other chromosome abnormalities. This test measures the concentrations of four biochemical substances produced by the fetus and placenta, AFP, hCG, uE3, and dimeric inhibin A. The test values, together with maternal age, are then entered into a mathematical formula to determine the risk for the various abnormalities. By adding a fourth marker to the prenatal screening test, the detection rate for an elevated risk of Down's syndrome can be increased from 60 to 75%.

### ***Biomarkers for Muscular Dystrophy***

Duchenne and Becker muscular dystrophy (DMD and BMD) share clinical symptoms like muscle weakness and wasting but differ in clinical presentation and severity. Immunohistochemistry using antibodies to dystrophin is the pathological basis for the diagnosis of DMD and BMD. While the sarcolemma of DMD muscle is negative, BMD muscle generally shows variable labeling because of the translation of a partially functional dystrophin that is localized to the sarcolemma. In some cases this differentiation is not possible. In such instances immunolabeling with antibodies to the neuronal form of nitric oxide synthase (nNOS) can be useful in suspecting a dystrophinopathy with a mutation in the "hot-spot" rod domain and help to direct molecular analysis (Torelli et al. 2004). nNOS localizes to the sarcolemma of mature muscle fibers via several components of the dystrophin-associated protein complex including dystrophin but sarcolemmal nNOS is lost when dystrophin levels are very low or absent because of deletions in critical regions of the rod domain.

Gene expression profiling of hind limb muscles of mouse models of muscular dystrophies was shown to clearly discriminate between severely affected and mildly or non-affected mouse models (Turk et al. 2006). Dystrophin-deficient and sarcoglycan-deficient profiles were remarkably similar, sharing inflammatory and structural remodeling processes. These processes were also ongoing in dysferlin-deficient animals, although at lower levels, in agreement with the later age of onset of this muscular dystrophy. The inflammatory proteins Spp1 and S100a9 were upregulated in all models. This study has identified biomarker genes for which expression correlates with the severity of the disease. This comparative study is an important step toward the development of an expression profiling-based diagnostic approach for muscular dystrophies in humans.

### ***Biomarkers of Phenylketonuria***

Phenylketonuria (PKU) is a genetic disease affecting 1:10,000–14,000 live births. In this condition, phenylalanine hydroxylase (PAH) deficiency is inherited as an autosomal recessive trait and the associated hyperphenylalaninemia phenotype is highly



variable. Neurological abnormalities in phenylketonuria include tremor, clumsiness, epilepsy, spastic paraparesis, and intellectual impairment. Screening for PKU was introduced in the UK over 30 years ago and has proved successful in preventing severe mental retardation. Genotype-based prediction of the biochemical phenotype is now feasible in the majority of newborns with hyperphenylalaninemia, which may be useful for refining diagnosis and anticipating dietary requirements. Methods currently used to screen for PKU include spectrophotometry, fluorometry, immunoassay, and tandem mass spectrometry with electrospray ionization. More recently, developments in tandem mass spectrometry have made it technically possible to screen for several inborn errors of metabolism in a single analytical step. NeoLynx Screening Application-Manager (Micromass Inc.) is indicated for the quantitative measurement of phenylalanine and tyrosine in neonatal blood samples by tandem mass spectrometry – exclusively with Quattro micro/Quattro LC mass spectrometers from Micromass. Additionally, measurements of tyrosine can be used as an adjunct to the measurement of phenylalanine in reducing the number of false-positive results with Micromass' NeoLynx Screening Application-Manager.

### ***Biomarkers of Lysosomal Storage Disorders***

Although several therapies are available or in development for lysosomal storage disorders (LSDs), assessment of therapeutic efficacy is limited by the lack of biomarkers to assess disease progression and severity. This is particularly true for rare diseases such as LSDs, since natural history data from human populations are often lacking. Gene expression analysis in the acid sphingomyelinase-deficient mouse model (ASMKO) of Types A and B Niemann–Pick disease (NPD) has been used to identify novel serum biomarkers (Dhami et al. 2006). Microarray and real-time PCR analyses were used to compare mRNA expression in ASMKO and normal mice in two important sites of pathology, lung and brain, and from these data identified and validated several potential biomarkers. The cytokine MIP-1 $\alpha$  was markedly elevated in ASMKO mouse serum, and following enzyme replacement therapy (ERT) it was reduced to normal levels. Total iron levels were similarly elevated in ASMKO mice, reflective of the elevated ferritin light chain transcript, and decreased to normal after ERT. Serum growth hormone levels were also elevated in ASMKO mice and were reduced to normal after brain-directed gene therapy, but not ERT. These studies illustrate the value of gene expression analysis for the identification of biomarkers and provide new insight into the pathobiology of NPD.

The mucopolysaccharidoses (MPS) is another group of LSDs presenting with broad multisystem disease and a continuous range of phenotypes. Currently, there are no objective biomarkers of MPS disease that clearly reflect disease severity or therapeutic responsiveness. Formation of the heparin cofactor II-thrombin (HCII-T) complex, a well-known serine protease inhibitor (serpin)-serine protease complex, has been identified as an informative biomarker for MPS I by using proteomic studies in the murine MPS I model (Randall et al. 2006). HCII-T complex was also elevated in plasma from MPS I patients. The degree of HCII-T complex formation

appears to correlate with disease severity and is responsive to therapy. In addition to its role as a biomarker, the discovery of increased serpin–serine protease complex formation provides a valuable insight into possible pathophysiological mechanisms of MPS.

Gaucher's disease is the most common LSD. Gene defect leads to deficiency or decreased activity of glucocerebrosidase followed by the accumulation of glucosylceramide. Frequent manifestations are hepatosplenomegaly, anemia, skeletal and hematological abnormalities. Recently used enzyme replacement therapy (infucerase) seems to eliminate the need for bone marrow transplantation and has favorable effects on symptoms and outcome. Development of gene therapy (reintroduction of missing DNA sequence) offers the possibility of cure of the disease. The biochemical markers secreted by Gaucher's cells are numerous, but none of those identified to date has offered all the expected qualities of a biomarker. Chitotriosidase and chemokine CCL18 are the most useful markers to follow enzyme replacement therapy. The identification of new biomarkers in the near future should enable a clearer understanding of the pathophysiology of this complex disease, which involves numerous cell processes.

Fucosidosis, another LSD, is an autosomal recessive disorder resulting from a deficiency of  $\alpha$ -L-fucosidase, encoded by the FUCA1 gene, which leads to failure in the catabolism of glycoproteins and glycosphingolipids resulting in the accumulation of a range of fuco-oligosaccharides and sphingolipids in all tissues including brain and liver. Severely affected patients present within the first year of life with mental retardation, growth retardation, and abnormalities in various other organs. Most of the diagnostic procedures are either invasive or impractical. PCR can be useful only once the mutation is known. The most practical test is detection by mass spectrometry of the elevated oligosaccharides as a biomarker in urine.

Prenatal diagnosis is available for many LSDs using chorionic villus samples or amniocytes. Such diagnoses can be problematical if sample transport and culture are required prior to analysis. It is possible to identify useful biomarkers for the diagnosis of LSDs from amniotic fluid. Amniotic fluid samples from control and LSD affected pregnancies have been analyzed for the protein markers LAMP-1 and saposin C by ELISA, and for oligosaccharide and lipid metabolite biomarkers by electrospray ionization-tandem mass spectrometry (Ramsay et al. 2004). LSD samples included aspartylglucosaminuria, galactosialidosis, Gaucher disease, GM1 gangliosidosis, mucopolysaccharidosis types I, II, IIIC, IVA, VI, and VII, mucopolipidosis type II, multiple sulfatase deficiency, and sialidosis type II. Each disorder produced a unique signature metabolic profile of protein, oligosaccharide, and glycolipid biomarkers. Some metabolite elevations directly related to the disorder while others appeared unrelated to the primary defect. Many LSDs were clearly distinguishable from control populations by the second trimester and in one case in the first trimester. Samples from GM1 gangliosidosis and mucopolysaccharidosis type VII displayed a correlation between gestational age and amount of stored metabolite. These preliminary results provide proof of principal for the use of biomarkers contained in amniotic fluid as clinical tests for some of the more frequent LSDs, which cause hydrops fetalis.

## Biomarkers of Aging

Although several biomarkers of aging have been described, the search for molecular biomarkers of aging has begun only recently. Table 5.4 classifies biomarkers of aging.

**Table 5.4** Biomarkers of aging

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Physiological measurements
Core body temperature
Blood pressure
24-h energy expenditure
Endocrinological biomarkers
Dehydroepiandrosterone sulfate
Insulin levels
Genes as biomarkers
DNA damage
DNA methylation
Mitochondrial mutations
Advanced glycation end products (AGEs): e.g., carboxymethyl-lysine

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A wide range of biomarkers, reflecting activity in a number of biological systems (e.g., neuroendocrine, immune, cardiovascular, and metabolic), have been found to prospectively predict disability, morbidity, and mortality outcomes in older adult populations. Levels of these biomarkers, singly or in combination, may serve as an early warning system of risk for future adverse health outcomes. In one investigation, several biomarkers were examined as predictors of mortality occurrence over a 12-year period in a sample of men and women 70–79 years of age at enrollment into the study (Gruenewald et al. 2006). Biomarkers examined in analyses included markers of neuroendocrine functioning (epinephrine, norepinephrine, cortisol, and dehydroepiandrosterone), immune activity (C-reactive protein, fibrinogen, IL-6, and albumin), cardiovascular functioning (systolic and diastolic blood pressure), and metabolic activity [high-density lipoprotein (HDL) cholesterol, total to HDL-cholesterol ratio, and glycosylated hemoglobin]. Recursive partitioning techniques were used to identify a set of pathways composed of combinations of different biomarkers that were associated with a high risk of mortality over the 12-year period. Of the 13 biomarkers examined, almost all entered into one or more high-risk pathways although combinations of neuroendocrine and immune markers appeared frequently in high-risk male pathways, and systolic blood pressure was present in combination with other biomarkers in all high-risk female pathways. These findings illustrate the utility of recursive partitioning techniques in identifying biomarker combinations predictive of mortal outcomes in older adults, as well as the multiplicity of biological pathways to mortality in elderly populations.

Advanced glycation end products (AGEs) have been associated with cardiovascular mortality and impaired renal function in diabetes as well as in uremia in aging individuals (Semba et al. 2009). Synvista Therapeutics is investigating

carboxymethyl-lysine as a biomarker of diseases associated with aging. Once this relationship is understood, the company will develop a diagnostic test to follow its level in blood to better determine the utility of medications.

If effective anti-aging interventions are to be identified for human application, then the development of reliable and valid biomarkers of aging is essential for this progress. The molecular biomarkers of aging should more accurately predict the physiological age of an organism than the chronological age. The difficulties in establishing useful biomarkers include the biological variation between individuals that makes generalizations difficult; the overlapping of aging and disease processes; uncertainty regarding benign versus pathogenic age-related changes; and the point at which a process begins to damage to the organism.

### ***Study of Biomarkers of Aging in a Genetically Homogeneous Population***

According to UNESCO's Preservation of Parsi Zoroastrian Project, 31% of the Parsi population in India lives beyond the age of 60, compared to 7% nationally (<http://www.unescoparzor.com/>). A better understanding of the genetic causes of longevity could have a major impact on the Indian Government's health-care budget and drug companies' marketing efforts. Affymetrix signed an agreement with Avesthagen Ltd (Bangalore, India), whereby Affymetrix' microarray technology will be used for the AVESTAGENOME Project<sup>TM</sup>, which will explore the genetic basis of longevity and create a genetic, genealogic, and medical database of the Parsi-Zoroastrian population. The use of Affymetrix technology will enable researchers to correlate genes with longevity, as well as neurodegenerative conditions, breast cancer, diabetes, and other complex diseases that affect the Parsi community. The Parsi community was selected because of its longevity and its relatively genetically homogeneous population. This project takes a systems biology approach that encompasses not only genotyping but also expression profiling and transcriptomics. The genotyping phase of the project, which began in 2007, consisted of 10,000 samples in the first year. By the middle of 2008, the team had performed expression profiling and transcript mapping experiments across a subset of the samples. The project is expected to be completed before 2013. All of the genetic information for The AVESTAGENOME Project<sup>TM</sup> is being collected following informed consent. Data confidentiality is being maintained as in accordance with the Indian Council of Medical Research guidelines.

### ***Genes as Biomarkers of Aging***

Since aging is a genetically programmed, it is controlled by genes, but environmental and epigenetic influences predominate in the second half of life. Genes that are expressed in a wide range of tissues and exhibit an age-dependent, easily

quantifiable increase in their expression represent a possible molecular biomarker of aging. Genetic mutations affect many phenotypes in flies, worms, rodents, and humans which share several diseases or their equivalents, including cancer, neurodegeneration, and infectious disorders as well as their susceptibility to them. DNA methylation, a force in the regulation of gene expression, is also one of the biomarkers of genetic damage. The mitotic clock of aging is marked, if not guided, by telomeres, essential genetic elements stabilizing natural chromosomal ends.

### **Telomere Attrition as Aging Biomarker**

Telomeres are tandem-repeated hexamers at the ends of mammalian chromosomes. Telomere shortening is associated with cellular senescence and mean telomere length has emerged as a replicative clock within each population of cells and the tissues and organs they build up *in vitro* and, consequently, as a biomarker for biological aging *in vivo*. Chronological aging *per se* does not parallel biological aging, yet accurate and reliable biomarkers are lacking to distinguish between them. The question remains as to whether telomere dynamics is a determinant or merely a predictor of human biological age over and above chronological aging. Although several reports have suggested a link between telomere attrition and aging phenotypes and disorders, both reference values and a complete set of determinants are missing. In the aged telomere attrition is associated with higher mortality due to infections and cardiovascular diseases. Increased telomere erosion in peripheral blood leukocytes is associated with atherosclerosis, hypercholesterolemia, hypertension, and diabetes mellitus. Longer telomeres are associated with slower aging.

### **Mitochondrial Mutations as Biomarkers of Aging**

Biomarkers of aging have been identified in the skin (Eshaghian et al. 2006). Mitochondrial DNA (mtDNA) deletion mutations were observed in skin samples removed from patients having non-melanoma skin cancer. A panel of mtDNA deletions were found in the tumor-free skin that was adjacent to the tumors, but not in the tumors themselves. The tumor samples were more likely to have full-length mtDNA, with point mutations rather than significant deletions. The mitochondrial DNA mutations in the tumor-free skin correlated with the aging process. The newly identified deletion mutations will now go into "Mitomap," a database of all known human mitochondrial genome changes. Unraveling the molecular clues as to why aging cells function differently than young cells requires that we have molecular markers that can be tracked. It will be interesting to see if the mtDNA mutations reported as markers of aging in the skin are found in other tissues as well or found only in tissues exposed to ultraviolet light. Some theories of cellular aging center on mitochondria and decreased energetic capacity resulting from mtDNA mutations. This study supports the use of mtDNA mutations as biomarkers of photoaging in the

skin. The newly identified biomarkers will provide another tool for studying mitochondrial damage that contributes to aging and cancer, and for screening compounds for these conditions.

### ***Role of Bioinformatics in Search for Biomarkers of Aging***

System wide functional and structural changes caused by the aging process encourage the implementation of new bioinformatics search strategies for markers of aging. Combinatorial biomarkers should be particularly favored, as they can quantify processes on multiple levels of biological organization and overcome an otherwise limited ability to access heterogeneities in populations. An even more challenging but rational approach is the development of systems biology models to describe molecular pathways and key networks mechanistically as they relate to age. Such reverse engineered models not only indicate critical and diagnostic components (that is, potential biomarkers) but also should be able to predict the progression of aging through computer simulation (Kriete 2006).

### ***Effect of Calorie Restriction on Biomarkers of Longevity***

Prolonged calorie restriction (CR) increases life span in rodents. A randomized controlled trial, called the Comprehensive Assessment of the Long-Term Effects of Reducing Intake of Energy (CALERIE), was conducted to determine if prolonged CR affects biomarkers of longevity or markers of oxidative stress or reduces metabolic rate beyond that expected from reduced metabolic mass in humans (Heilbron et al. 2006). The findings suggest that two biomarkers of longevity (fasting insulin level and body temperature) are decreased by prolonged CR in humans and support the theory that metabolic rate is reduced beyond the level expected from reduced metabolic body mass. Subjects on CR had less oxidative damage to their DNA, thought to be a marker of aging at the biochemical and cellular level.

## **Biomarkers of Miscellaneous Disorders**

### ***Biomarkers of Inflammatory Bowel Disease***

Inflammatory bowel disease (IBD) is a spectrum of disorders that affect the gastrointestinal tract, the two major entities being Crohn's disease and ulcerative colitis. IBD is an enduring disease involving mostly young people, with symptoms of bloody diarrhea and abdominal cramps. Several antibodies have been associated with IBD. The two most comprehensively studied are autoantibodies to neutrophils (atypical perinuclear anti-neutrophil cytoplasmic antibodies) and anti-*Saccharomyces*

*cerevisiae* antibodies (ASCA). These antibodies are useful for diagnosing IBD, differentiating Crohn disease from ulcerative colitis, indeterminate colitis, monitoring disease, defining clinical phenotypes, predicting response to therapy, and as sub-clinical biomarkers (Bossuyt 2006). Pancreatic antibodies have been described in patients with Crohn's disease. The antigen has not been elucidated and the antibodies are detected by indirect immunofluorescence. There is evidence that the number and magnitude of immune responses to different microbial antigens (outer membrane porin C and ASCA) in a given patient are associated with the severity of the disease course, i.e., the greater the number of responses and greater their magnitude, the more severe the disease course. The interest in antimicrobial and antiglycan antibodies has recently been increased as they have shown to act as surrogate markers of complicated aggressive disease.

Correlation of serum biomarkers with genotypes and clinical phenotypes would enhance our understanding of the pathophysiology of IBD and lead to new tools for diagnosis and stratification of patients for clinical trials. Biomarkers are helpful in prioritizing further examinations, including endoscopy, and/or in the decision to start or intensify treatment in IBD. CRP has many advantages, but its short half-life makes this a particularly good biomarker in the detection and follow-up of disease activity in Crohn's disease. In contrast, ulcerative colitis (with the exception of severe colitis) has only a modest-to-absent CRP response despite active inflammation. As stools are easy accessible in IBD patients, fecal biomarkers hold a specific promise and recent studies even claim superiority of fecal biomarkers over serum markers. A number of neutrophil-derived proteins shedding in stools have been studied. Calprotectin and lactoferrin are probably the most promising given their abundance in granulocytes and their stability and resistance to degradation (Vermeire et al. 2007). Although calprotectin and lactoferrin are very sensitive markers to detect inflammation in the gastrointestinal tract, they are not specific for IBD and increased levels are also found in neoplasia, NSAID abuse, infections, and polyps. In children with abdominal symptoms and diarrhea, a positive test for calprotectin or lactoferrin may prioritize endoscopy.

### ***Biomarkers of Erectile Dysfunction***

Erectile dysfunction (ED) is a highly prevalent functional disorder and its incidence increases with advancing age. Population-based studies estimate the prevalence at nearly 50% of the male population over the age of 45 across all racial and socioeconomic groups. There are multiple risk factors and biomarkers for ED. Currently serum testosterone is considered the most reliable biomarker for establishing the presence of ED due to hypogonadism. ED is more commonly seen in men with various components of the metabolic syndrome and can be considered as a risk marker of the metabolic syndrome and its associated conditions such as diabetes and cardiovascular disease.

Demographic studies have consistently demonstrated that risk factors for ED generally mirror risk factors also predictive of coronary and vascular disease

secondary to endothelial dysfunction of the vascular tree. These risk factors include obesity, hyperlipidemia, hypertension, diabetes mellitus, smoking, sedentary lifestyle among others and in clinical studies these co-morbidities are much higher in the ED population than reported in the general population. Various studies support the concept that ED is a marker of cardiovascular disease. Furthermore, endothelial dysfunction is a biomarker of vascular disease. Flow-mediated reflex brachial artery dilatation has been described as a diagnostic test for endothelial dysfunction and a high correlation with erectile dysfunction, but it is not commonly done, difficult to standardize, costly and time consuming therefore is not an acceptable screening test or procedure.

Coronary endothelial dysfunction (CED) precedes atherosclerosis and is associated with cardiovascular events. Both CED and erectile dysfunction (ED) are partly mediated by impairment in the nitric oxide (NO) pathway. Although ED is associated with established coronary atherosclerosis, its relationship with CED is unknown. A study has investigated the association of CED with ED in men with early coronary atherosclerosis as well as the role of the endogenous nitric oxide synthase inhibitor, asymmetric dimethylarginine (ADMA), which has been shown to be an independent biomarker for cardiovascular disease (Elesber et al. 2006). The results showed that CED is independently associated with ED and plasma ADMA concentration in men with early coronary atherosclerosis, further supporting the role of the endothelium in systemic vascular diseases and the role of ADMA in the systemic manifestations of endothelial dysfunction.

Daily sildenafil for 4 weeks ameliorates endothelial function in patients with ED as assessed by reduction of endothelin-1 levels and other biomarkers of endothelial function – nitric oxide and cyclic guanosine monophosphate (Angelis et al. 2009). The clinical implications of this finding warrant further investigation.

The availability of a simple, cost-effective biomarker to identify men at risk would not only allow for earlier treatment but for earlier evaluation and intervention, which could potentially significantly improve cardiovascular health and prevent or postpone potentially serious life-threatening events.

### ***Biomarkers of Heat Stroke***

Heat stroke, also termed “sun stroke,” is characterized by hyperpyrexia with core body temperature greater than 40°C and neurologic dysfunction. Early detection and management is essential to improve chances of recovery and survival. There are no validated biomarkers for early detection of heat stroke but studies are in progress to identify these.

Heat-shock protein (Hsp) 72 concentration has been shown to be higher in the serum (eHsp72) of runners with symptoms of heat illness than in non-ill runners and is a function of the core temperature attained rather than the rate of heat storage (Amorim et al. 2008). Measurement of antibodies to Hsp may be useful in assessing how individuals are responding to abnormal heat stress within their living and working environment and may be used as biomarkers to evaluate their susceptibility to heat-induced diseases.



## ***Biomarkers of Pain***

Pain itself is a biomarker of many diseases but there is lack of validated biomarkers of chronic pain syndromes. There is need for such biomarkers to guide analgesic development. Currently functional magnetic resonance imaging (fMRI) is the only reliable biomarker of pain. Activation of brain areas involved in pain can be visualized in response to painful stimuli and action of analgesics can be assessed. fMRI has been used to objectively evaluate acupuncture for pain.

### **Biomarkers of Neuropathic Pain**

Although there are changes in the nervous system in neuropathic pain, it is difficult to identify biomarkers in blood and peripheral tissues. There is evidence of CNS involvement in neuropathic pain and movement disorders in patients with complex regional pain syndrome (CRPS). Elevated cerebrospinal fluid (CSF) levels of IL-1 $\beta$  and IL-6 have been reported in CRPS patients with and without movement disorders but other studies have failed to replicate these findings.

Use of proteomic technologies to study proteins that are involved into the pathogenesis of nerve injury and neuropathic pain might enable a better understanding of the pathophysiologic signaling pathways in this impairment and facilitate the discovery of specific biomarkers. Validation of histologic and other biomarkers will provide the foundation for research advances, and new clinical trial designs will allow better discrimination of beneficial treatments for neuropathic pain.

### **Brain Insular Glutamate as Biomarker of Fibromyalgia**

Fibromyalgia (FM) is a chronic widespread painful condition that is thought to arise from augmentation of central neural activity. Glutamate (Glu) is an excitatory neurotransmitter that functions in pain-processing pathways. A study was carried out to investigate the relationship between changing levels of Glu within the insula and changes in multiple pain domains in patients with FM (Harris et al. 2008). Proton magnetic resonance spectroscopy (H-MRS) and fMRI examinations were conducted before and after a non-pharmacologic intervention to reduce pain. During H-MRS, the anterior and posterior insular regions were examined separately using single-voxel spectroscopy. The levels of Glu and other metabolites were estimated relative to levels of creatine (Cr) (e.g., the Glu/Cr ratio). During fMRI, painful pressures were applied to the thumbnail to elicit neuronal activation. Experimental pressure-evoked pain thresholds and clinical pain ratings were also assessed prior to each imaging session. Both experimental pain and clinical pain were reduced following treatment. Changes from pre- to post-treatment in Glu/Cr were negatively correlated with changes in experimental pain thresholds and positively correlated with changes in clinical pain. It was concluded that changes in Glu levels within the insula are associated with changes in multiple pain domains in patients with FM. Thus, H-MRS data may serve as a useful biomarker and surrogate end point for clinical trials of FM.

## **Biomarkers of Visceral Pain**

There is a need for predictive biomarkers to test novel experimental medicines in functional gastrointestinal disorders. With visceral pain models, the large coefficient of variation in sensation end points in human studies precludes definitive conclusions such as go/no go decisions or dose selection for phase IIb or III studies, unless very large numbers of patients are evaluated in phase IIA pharmacodynamic studies. This renders such pharmacological studies ambitious or unachievable in a timely fashion. Moreover, the results of tests and clinical trials should be interpreted with greater knowledge of the drug pharmacokinetics, including the influence of CYP metabolism and potential drug interactions. Thus, it is important to identify valid biomarkers of visceral pain for the assessment of treatment response in pharmacodynamic studies.

At present, there is no clear evidence that there are effective biomarkers for visceral pain. The pharmacological agents that have been available for testing to date have been able to demonstrate only modest changes in these sensory end points. The exceptions are single studies of high-dose fentanyl and octreotide, which showed magnitudes of change that would be demonstrable with a reasonable number of participants, that is, at least 20. However, a study of fentanyl recorded the fact that ~70% of participants identified that they were on fentanyl and that participants reported alterations in performance. Moreover, it is conceivable that the local irritant effect of octreotide at the site of injection may have unblinded the study or caused a competing somatic pain that interfered with the appraisal of visceral sensation.

Rectal sensitivity and brain-imaging studies seem too laborious and impractical to be useful as pharmacodynamic models. It is still unclear whether the foci activated in the brain are specific to irritable bowel syndrome (IBS). Indeed, studies contrasting liminal, subliminal, and supraliminal stimuli of the rectum and an acoustic control stimulus show that activation of higher emotional centers may more closely reflect the psychological state than being a true center associated with functional gastrointestinal diseases (Andresen and Camilleri 2006). It is unclear whether the activated brain centers provide a true reflection or biomarker of IBS or visceral pain.

## ***Nasal Nitric Oxide as a Biomarker of Response to Rhinosinusitis Therapy***

The assessment of the response of chronic rhinosinusitis to therapy is difficult. CT scans cannot be used repeatedly. Therefore, methods such as symptom scores and endoscopy are employed instead. Nasal NO has been considered a potential biomarker for assessing the effect of treatment. A prospective randomized trial with nasal NO measurement was conducted on patients with chronic rhinosinusitis, who had failed initial medical therapy with douching and nasal corticosteroids and who then had abnormal CT scans Ragab et al. (2006). The patients were treated either medically or surgically, with follow-up at 6 and 12 months while still taking nasal

corticosteroids. Nasal NO was measured initially and at 6 and 12 months along with symptom scores, endoscopy, polyp grading, and saccharin clearance time. Results showed that initial absolute nasal NO levels correlated inversely with CT scan changes. The percentage rise in nasal NO seen on both medical and surgical treatment correlated with changes in symptom scores, saccharin clearance time, endoscopic changes, polyp grades and surgical scores at 6 months as well as at 12 months. Nasal NO, which is easily measured, provides a valuable non-invasive objective measure of the response of chronic rhinosinusitis to therapy. Topical nasal corticosteroids may be needed to reduce the contribution of nasal eNOS and allow that emanating from the sinuses to be measured. Currently the expense of the NO chemiluminescence analyzers would limit application of the method in clinical practice.

### ***Biomarkers Common to Multiple Diseases***

Some biomarkers are found in more than one disease and their evaluation requires correlation with clinical manifestations. Some examples are listed in Table 5.5.

**Table 5.5** Examples of biomarkers common to multiple diseases

Biomarker	Diseases
Chromogranin A	Neuroendocrine tumors, cardiovascular disease, sepsis
C-reactive protein (CRP)	Diabetes mellitus, sepsis, pulmonary diseases, acute myocardial infarction, renal dysfunction
Cystatin C	Myocardial infarction, renal failure, cancer, Alzheimer disease, amyotrophic lateral sclerosis, multiple sclerosis
Inflammation biomarkers	Most diseases with inflammation
Natriuretic peptide	Ischemic heart disease, infections
Nitric oxide	Asthma (in breath), acute respiratory distress syndrome (in urine), cardiovascular disease (in plasma)
Oxidative stress biomarkers	Most diseases with oxidative stress
Serum 100B protein	Traumatic brain injury, stroke, epilepsy (in CSF)
Tau protein	Alzheimer disease, Parkinson disease, Creutzfeldt-Jakob disease, AIDS encephalopathy, alcohol-induced organic brain disorders
TNF- $\alpha$	Rheumatoid arthritis (serum and synovial fluid), neuroinflammation, ischemic heart disease

## **Biomarkers and Nutrition**

### ***Biomarkers in Nutritional Epidemiology***

Modern epidemiology suggests a potential interactive association between diet, lifestyle, genetics, and the risk of many chronic diseases. As such, many epidemiologic studies attempt to consider assessment of dietary intake alongside genetic measures and other variables of interest. However, given the multifactorial complexities of dietary exposures, all dietary intake assessment methods are associated with measurement errors which affect dietary estimates and may obscure disease risk associations. For this reason, dietary biomarkers measured in biological specimens are being increasingly used as additional or substitute estimates of dietary intake and nutrient status. Genetic variation may influence dietary intake and nutrient metabolism and may affect the utility of a dietary biomarker to properly reflect dietary exposures. Although there are many functional dietary biomarkers that, if utilized appropriately, can be very informative, a better understanding of the interactions between diet and genes as potentially determining factors in the validity, application and interpretation of dietary biomarkers is necessary. Some important biomarkers are being applied in nutrition epidemiology to address some associated questions and limitations (Jenab et al. 2009). There is still a need to identify new dietary biomarkers. Nutritional metabolomics can be used as an analytical method to assess metabolic profiles as measures of dietary exposures and indicators of dietary patterns, dietary changes, or effectiveness of dietary interventions. Future studies should be integrate high-quality dietary intake information, measurements of dietary biomarkers, metabolic profiles of specific dietary patterns, genetics and novel statistical methods to provide important new insights into gene-diet-lifestyle-disease risk associations.

### ***Biomarkers of Nutritional Status***

Humans have individual differences in response to diet and nutrition should take into account differences in their genetics and metabolic needs. The available diagnostic biomarkers for disease may not be appropriate or adequate to distinguish the nutritional status of humans or form a basis for recommending appropriate diets for optimal metabolic health. Metabolic profiling by use of metabolomics is required for this purpose. There is a need for measuring metabolites to assess human metabolism prior to onset of disease. The current use of single biomarkers as indicators of disease will be replaced by comprehensive profiling of individual metabolites linked to an understanding of health and human metabolism as determined by metabolomics. Industrial and academic initiatives are currently developing the analytical and bioinformatic technologies needed to assemble the quantitative reference databases of metabolites as the metabolic analog of the human genome. With these in place, dietetics professionals will be able to assess both the current

health status of individuals and predict their health trajectories. This will facilitate integration of metabolism with the genetic and dietary variables that affect health and lead to personalized nutritional counseling. There is, however, a paucity of good studies on nutritional biomarkers.

Low serum concentrations of vitamins B6 and B12 and selenium are biomarkers that predict subsequent disability in activities of daily life in older women living in the community (Bartali et al. 2006). This is one of the key factors to be considered in the development of strategies aimed at preventing or delaying the disablement process.

### ***Biomarkers of Branched-Chain Amino Acid Status***

Branched-chain amino acids (BCAAs) are not synthesized in the body in humans, but they are crucial in protein and neurotransmitter synthesis. The protein anabolic role of BCAAs seems to be mediated not only by their important role as a promoter of the translation process (and possibly acting at the transcription level) but also by inhibition of protein degradation. Leucine may play a critical role in these signaling pathways. Supplementation with BCAAs spares lean body mass during weight loss, promotes wound healing, may decrease muscle wasting with aging, and may have beneficial effects in renal and liver disease. BCAA supplementation is extensively used in the athletic field with the assumption of improved performance and muscle mass. Measuring serum BCAAs has limited clinical utility beyond the controlled setting because levels are affected by a variety of clinical states, and optimal levels in these scenarios have not been completely elucidated. Diet, hormones, stress, aging, and renal or liver dysfunction affect BCAA levels and our understanding the biological effects of BCAAs may help to develop biomarkers of BCAA status.

### ***Biomarkers of Caloric Restriction***

Caloric restriction is associated with a decreased level of oxidative stress. Reactive oxygen species (ROS), generated predominantly in mitochondria, are attenuated by decreased caloric intake (Skrha 2009). On the other hand, antioxidative mechanisms are frequently accelerated by increased gene expression or activities of antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, paraoxonase, etc.). Measurement of biomarkers of oxidative stress in caloric restriction is therefore important in experimental as well as clinical studies. Estimation of ROS in tissues and fluids is typically performed by measurement of oxidant products (i.e., malondialdehyde, F-2-isoprostanes, nitrotyrosine) and biomarkers of antioxidant system (enzymes, glutathione, alpha-tocopherol, ascorbic acid, ubiquinon, etc.).

### ***Biomarkers of Malnutrition***

Malnutrition is a general term for a medical condition caused by an improper or inadequate diet and nutrition. Manifestations may be subclinical. Malnutrition is common in infants in the third world and is a major cause of infant mortality. Nucleotide intake and nutritional recovery has a notable effect on IGF-I, IGF-binding protein-3 (IGFBP-3), and other hormonal biomarkers (Vásquez-Garibay et al. 2006). This outcome could stimulate the catch-up growth of severely malnourished infants and toddlers during the nutritional recovery period.

Malnutrition is found even among the elderly in developed countries. Levels of serum lipids are influenced by malnutrition and inflammation. Total cholesterol, HDL, and LDL can be considered novel biomarkers of malnutrition and inflammation in geriatric patients (Hrnciarikova et al. 2009). A close relation has also been demonstrated between serum lipids and prealbumin. In healthy elderly subjects, plasma transthyretin and alpha 1-acid glycoprotein could be helpful in identifying elderly subjects at higher risk of death (Carriere et al. 2008).

### ***Proteomic Biomarkers and Nutrition***

Scientists at the Nestlé Research Centre (Lausanne, Switzerland) are employing proteomics to address questions of nutrition and health. Nestlé believes that foods and drinks affect individual consumers differently. A food may be well-tolerated by one individual cause but cause violent gastric discomfort in another. Food preference may be related to biomarkers. It is worthwhile to investigate genes that are activated by specific foods for enhancing health and wellness. Certain individuals are more predisposed than others to conditions like obesity or diabetes. If protein markers that indicate such predisposition can be identified before disease symptoms arise, dietary approaches could be devised for health promotion and disease prevention. Nestlé is now including genomics and proteomics approaches into consumer research to impart the health and wellness dimension and to more accurately address individual differences in terms of response to diet and food preference. The long-term deliverable of “Omics”-driven food research is personalized nutrition. Proteomics adapted and applied to the context of nutrition and health has the potential to deliver biomarkers for health and comfort, reveal early indicators of disease disposition, assist in differentiating dietary responders from non-responders, and, last but not least, discover bioactive, beneficial food components (Kussmann and Affolter 2006).

## **Biomarkers of Gene–Environmental Interactions in Human Disease**

Gene–environmental interactions play an important role in human disease, but they have not been studied systematically. The Genes, Environment, and Health Initiative

(GEI) of NIH in 2006 has supported research that will lead to the understanding of genetic contributions and gene–environment interactions in common disease. NIH GEI awarded a \$5.9 million grant to the Pacific Northwest National Laboratory (PNNL) that houses the Center for Novel Biomarkers of Response. Scientists at the center intend to create new exposure assessment tools to better understand the role of gene–environmental interactions in human disease. Development of these tools will enable precise measurements of personal exposure to environmental, chemical, and biological agents. Two of the most important risk factors for human morbidity and mortality – cigarette smoke and obesity – will be the primary targets of interest for PNNL scientists. Research will focus on biomarkers for systemic stress caused by mainstream and secondhand cigarette smoke, with obesity as a confounding physiological factor. This research is comprised of two basic elements. The genetic component will rely on newfound abilities to swiftly identify genetic differences between people with illnesses and those who are healthy, leading to a greater understanding of genetic contribution to the disease. The environmental biology component will focus on developing new technologies to accurately measure personal exposures with small, wearable nanobiosensors that can be used to assess environmental agents. The center will provide NIH NIEHS with a database of response biomarkers, as well as chemical substances for selected biomarkers that are tested and validated in humans and supported by parallel studies in mice. The researchers will also develop nanobio sensors for measurement of biomarkers at POC.

## **Future Role of Biomarkers in Health Care**

Questions are still being raised as to whether detecting diseases at their earliest stages actually improves health. Based on the information presented in various chapters of this report, it can be concluded that biomarkers will play an important role in future medicine. Biomarkers will have a considerable impact on diagnostics and will facilitate the development of personalized medicine. Role of biomarkers in the management of various diseases have also been discussed. Biomarkers are already playing an important role in management of cancer. Screening approaches have led to dramatic changes in the outcome of cervical cancer.

A promising area in the future application of biomarkers is that in which diseases can be diagnosed in their earliest stages in hopes of effective intervention. The new classes of sensitive imaging technology such as spiral CT can be made more specific through combination with a biomarker in blood, and disease relapse can be detected earlier through entirely new types of protein biomarkers.

One area not discussed so far is the role of biomarkers in preventive medicine and health education. Biomarker profile of a healthy individual may be used to guide individualized health counseling. Susceptibility to certain diseases may require modifications of the general preventive medicine advice. Future studies need to establish the effectiveness of such an approach.

## **Applications of Biomarkers Beyond Health Care**

There are several potential applications of biomarkers beyond drug development and human health care. Proteomic biomarkers have been used in stem cell research. Applications of biomarkers of infections can be extended to detection of biological agents used in bioterrorism and monitoring human exposure to environmental toxins.

### ***Combating Bioterrorism***

An integrated rapid, semiportable, prototype point microbial detection/identification system was proposed for clinical specimens, which is also capable of differentiating microbial bioterrorism attacks from threats or hoaxes by defining the pathogen (White et al. 2002). The system utilizes “flash” extraction/analytical system capable of detection/identification of microbes from environmental and clinical matrices. The system couples demonstrated technologies to provide quantitative analysis of lipid biomarkers of microbes including spores in a system with near-single-cell (amol/microl) sensitivity. Tandem mass spectrometry increases specificity by providing the molecular structure of neutral lipids, phospholipids, and derivatized spore-specific bacterial biomarker, 2,6-dipicolinic acid as well as the lipopolysaccharide-amide-linked hydroxy-fatty acids of Gram-negative bacteria. The extraction should take about an hour for each sample but multiple samples can be processed simultaneously. MALDI-TOF MS-specific biomarkers have been shown to be an effective tool for identifying microorganisms. Feasibility of this technique was demonstrated by detecting the obligate intracellular bacterium *Coxiella burnetii*, a category B bioterrorism agent (Shaw et al. 2004).

### ***Biomarkers for Monitoring Human Exposure to Environmental Toxins***

Establishing associations between environmental agents and disease presents challenges to both epidemiologists and toxicologists, particularly in cases of complex gene–environment interactions and when there is a long latency between exposure and disease. The epidemiologic value of a biomarker lies in its ability to predict backward toward exposure and forward toward risk of clinical outcome. In 1995, the World Health Organization recognized that biological markers can potentially improve the way in which exposure to environmental factors is assessed. However, only a few valid biological markers were available at that time, which could be effectively used in epidemiological studies and the assessment of risk.

The need for new approaches to assess DNA damage has been increasing due to the implications that different insults on genetic material may have on human health. In this context, the identification of how chemical agents with different mechanisms



of action (i.e., antineoplastic drugs) damage DNA provides a good model to investigate some cellular and molecular mechanisms underlying the basis of genetic toxicology. The nasal epithelium is the first barrier with which environmental pollutants interact, and for this reason this epithelium can be useful as a sentinel in order to assess the interactions between the environment and the living organisms. Taking these phenomena into account and using a simple, sensitive and rapid method such as the single-cell gel electrophoresis, we could obtain information and an initial approach on the DNA status. This assay in combination with other techniques that provide more information about other molecular parameters could give us a better view of the biological status of the living cell.

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Organophosphorus (OP) compounds are still among the most widely used insecticides, and their main mechanism of acute toxicity is associated with inhibition of acetylcholinesterase. Measurements of urine metabolites and of blood cholinesterase activity are established biomarkers of exposure to OPs and of early biological effects. In recent years, increasing attention has been given to biomarkers of susceptibility to OP toxicity. Polymorphisms of paraoxonase (PON1), a liver and serum enzyme that hydrolyzes a number of OP compounds, play a role in modulating the toxicity of OPs. It is important to determine PON1 status, which encompasses the PON1192Q/R polymorphism (that affects catalytic ability toward different substrates) and PON1 levels (which are modulated in part by a C-108T polymorphism) over straight genotyping. Epidemiological studies on OP-exposed workers that include assessment of PON1 status to validate in human populations the role of PON1 as a determinant of susceptibility to OPs, as indicated by animal studies, are needed. Documentation of exposure and of early health effects would be most relevant to increase the predictive value of the test.

### ***Application of Biomarkers in Animal Health***

Many of the advances in human health care are being applied to animal health. In fact, some of the new technologies were tested in experimental animals before they were introduced into human medicine.

Dogs suffer from many of the same disease as humans. The value of measuring blood levels of the myocardial protein cardiac troponin I (cTnI) was tested for the diagnosis of congenital and acquired heart disease in the dog and in the

evaluation of the severity of heart failure (Spratt et al. 2005). Serum samples obtained from healthy dogs and from dogs diagnosed with a variety of congenital and acquired heart conditions were assayed for cTnl concentration using an automated immunoassay method. Results were also analyzed according to the degree of heart failure as assessed using the International Small Animal Cardiac Health Council's scheme. Healthy dogs had very low or undetectable blood cTnl levels, as did dogs with congenital heart disease. However, cTnl levels were significantly elevated in dogs with acquired mitral valve disease, dilated cardiomyopathy, and pericardial effusion. Blood cTnl levels also varied with severity of heart failure. Measurement of blood cTnl levels may be a useful aid in the diagnosis of dogs with suspected heart disease and in indicating the severity of heart failure.